The hygiene hypothesis
and implications for home hygiene

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The International Scientific Forum on Home Hygiene (IFH)
THE HYGIENE HYPOTHESIS AND IMPLICATIONS FOR HOME HYGIENE

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INTRODUCTION BY THE INTERNATIONAL SCIENTIFIC FORUM ON HOME HYGIENE (IFH)

Over the past 2 centuries, sanitation, clean water, clean food and the promotion of hygiene practice have played crucial roles in reducing infectious disease. Following the introduction of antibiotics and vaccines in the 1930-1950s, it seemed possible that infectious disease might someday become a thing of the past. In the last 10 years however a range of events have underlined the need for renewed action. Global figures published in 1996 indicate that, out of 52 million deaths, 18 million were attributable to infectious and parasitic diseases. These figures combined with concerns about emerging and re-emerging pathogens, antibiotic resistance and so on mean that, after several decades of complacency, infectious disease prevention is most definitely back on the global health agenda.

Now, in the year 2004, the importance of hygiene is again being challenged. The ‘Hygiene Hypothesis’ contends that if we are not exposed to infectious agents the immune system becomes imbalanced, thereby increasing susceptibility to allergic diseases. Alongside this a populist view has emerged, that this key exposure no longer occurs because of improved household amenities, higher standards of personal cleanliness, and cleaner homes.

In response to our concerns that these concepts could have a detrimental impact on the public’s perception of infectious disease risks in the home and the importance of using hygiene measures to control such risks, IFH has commissioned the following report. This aims to provide a better understanding of the nature and extent of the link between microbial exposure and the development of the immune system, the implications it might have for hygiene, and how the problems presented by the hypothesis might be addressed.

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FOREWORD BY PROFESSOR STEPHEN HOLGATE

Allergic diseases of all types including rhinitis, asthma, atopic dermatitis, drug and food allergy and anaphylaxis are on the increase, but the underlying mechanisms responsible for this have yet to be found. Allergic disorders have strong genetic as well as environmental determinants. It is highly unlikely however that a change in genetic makeup could account for the trend, although it is of interest that the trend is highest amongst English speaking nations, suggesting that some genetic background is important. Of the many environmental determinants, it appears that allergen exposure per se is probably not as important as originally thought, although of course susceptible individuals must be exposed to a specific allergen in order to become sensitised.

Since the trend is particularly manifest in children, it seems likely that any environmental factor(s) is manifesting early in life and even pre-natally. Although the nature of the environmental factors which are involved has eluded definition, recent evidence supports the idea that defects in the ability of children of atopic parents to protect themselves against allergen sensitisation might be further enhanced in the absence of adequate programming by micro-organisms, or products from micro-organisms, present in the environment. This concept has been referred to as the ‘hygiene hypothesis’, but in many ways this is a misnomer, not least because the range of factors causing changes in microbial exposure that it considers are much wider than are commonly understood by the term ‘hygiene’

Indications are that bacterial, fungal and viral cell wall and nucleic acid products capable of stimulating Toll receptors as part of the innate immune response are fundamental in the early life origins of allergic disease, although the mechanisms involved are far from clear. However the idea that being too ‘clean’ is responsible for the immunological abnormalities that may be behind the changing trends in allergic disease is a gross oversimplification, and has led to misinterpretation of the concept. While much research effort is being focused on understanding the link between disordered immune responses and the trends in allergy, there has been little attempt to evaluate the validity or otherwise of the relationship to ‘hygiene’, and the adverse impact that uncritical acceptance of this hypothesis could have on public hygiene standards at a time when there is growing awareness of a need for improved rather than relaxed personal and community hygiene.

This report looks at both aspects of the problem. The thoroughness of the review is refreshing, as is the commonsense approach to hygiene which is adopted. The report makes recommendations for adoption of a hygiene strategy based on risk assessment and stresses the importance of not relaxing hygiene standards, but ensuring that they are reinforced to protect the public
from catastrophic infections, the consequences of which we are only too familiar. In the meantime, the study of cell and molecular biology combined with epidemiology and genetics should reveal how the immune response interacts with environmental factors and what aspects of the Westernised lifestyle are important in translating this into allergic disease. If the trend is to be reversed, research is needed to understand what factors direct an allergic response to a particular organ. Atopy, the genetic predisposition to develop allergy, may be present in up to 40% of a population but as yet a large number of these individuals remain asymptomatic.

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SUMMARY

When a disease, or group of diseases, rises rapidly without a specific explanation, it stimulates hypotheses and investigations to identify the causes, so that preventive measures can be devised. An example of this is the largely unexplained rise in the incidence and prevalence of atopic (allergic) disorders, such as asthma, hay fever, eczema and food allergies. This rapid rise has occurred over the last 30 years, predominantly in developed and industrialised countries.

The hygiene hypothesis as an explanation for increasing atopy was first postulated by an epidemiologist, Strachan, in 1989, who reported an inverse relationship between family size and development of atopic disorders. He proposed that the rise in allergic diseases could be explained if these were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings or acquired prenatally.

Subsequently, as the concept was further explored by other specialists in allergy and immunology, it evolved into the broader notion that a major causative factor in the increasing incidence of atopy in recent years is declining microbial exposure. A wide range of factors which might have resulted in altered microbial exposure have been examined, including the measures introduced to protect health, such as clean water and food, sanitation, antibiotics and vaccines, as well as incidental factors such as the move from farm to urban living.

Most recently, a further aspect of Strachan’s hypothesis has received increasing attention, particularly from the media, namely his proposition that “the reason why this key exposure no longer occurs, or occurs to an insufficient extent, is the trend not only towards smaller family sizes but also improved household amenities and higher standards of personal cleanliness – in effect, cleaner homes.”

Until recently, discussion of the hypothesis has been relatively compartmentalised, with most debate focusing on immunological models and the intensive epidemiological and other research to elucidate the nature of the possible link between atopy and microbial exposure. Research into other factors which could explain the atopy trends, such as changes in diet, obesity, other shifts in lifestyle and environmental pollution have continued in parallel. It is only now that infectious disease and hygiene specialists are entering the debate, concerned that publicising the concept that we might be ‘too clean’ could have a detrimental impact on the public’s perception of infectious disease risks in the home and the importance of using measures to control such risks. Another concern is that the data on microbial exposure and hygiene practice currently used to test the hypothesis rely too heavily on assumptions and proxy measures.

This review aims to bridge the different specialities by examining all aspects of the hypothesis, in order to consider the implications it might have for hygiene, particularly hygiene in the domestic setting. It seeks to do this by addressing two distinct questions:

• how clear is the evidence of a causal link between a decline in microbial exposure of some kind and the recent rises in atopic disease?

• to what extent might ‘better hygiene’, as distinct from other influences on microbial exposure, be a significant factor?
The first part of the review considers the epidemiological and immunological evidence relevant to the putative link between microbial exposure and the rise in atopic disease. Alternative hypotheses related to other elements of our changing environment and lifestyle that have been put forward to explain the trend are also examined. The following section discusses trends in infectious disease, including infections arising in the home environment and in other parts of local communities. It then draws on the growing evidence from home-based and other studies, to assess the extent to which trends in hygiene amenities, hygiene practice and hygiene behaviour might have significantly changed the extent and/or patterns of exposure to microbes. Other factors which might have altered our exposure to microbes, such as antibiotic treatments, vaccination or changes in the microbial content of foods are briefly reviewed. The review then goes on to consider the implications of the hygiene hypothesis for hygiene, the impact which reduced emphasis on hygiene might have on infectious disease control, and whether everyday hygiene might have a significant impact on our surrounding microbial flora.

Finally, the totality of the evidence is weighed in relation to the two key questions it has set out to address. The review concludes that:

• there is significant evidence suggesting a link between reduced microbial exposure and atopic disease, though in some areas the evidence is conflicting.

• further research is needed to elucidate the nature of any critical change in microbial exposure and how it might operate. Most particularly there is a need to understand whether it involves certain specific types or organisms, whether these are invasive strains, and what form the ‘invasion’ needs to take. There is also need to understand whether the protective effect of microbial exposure is only important at certain times of life (e.g. immediately after birth or in infancy).

• evidence on the role of hygiene indicates little or no justification for continuing to cite hygiene practices as a major influence on microbial exposure. In view of this the report proposes that the hypothesis should be renamed as the ‘microbial exposure hypothesis’ in order to prevent further misinterpretation.

• Of the alternative, ‘non-microbial’ explanations for the recent rapid rise in atopy, the dietary and exercise hypotheses remain promising in terms of explaining some of the epidemiological inconsistencies, while not affronting the knowledge base of either atopic or infection specialists. Since an ‘environmental’ or a ‘lifestyle’ factor must be responsible for the rapid change in atopic diseases, the evidence of changing diets would appear to meet criteria of causality and controlled intervention studies are awaited with great interest.

Although the review concludes overall that the relationship of the hypothesis to hygiene practice has not been proved, it lends strong support to recent initiatives which seek to improve our approach to hygiene practice. Trends in infection, and new data showing how infection is transmitted in the home, suggest that the traditional approach to hygiene is unfocussed, with insufficient regard to where the risk of infection transmission is highest. The report supports the concept that, regardless of whether the hygiene hypothesis is correct, there is a need to change the prevailing approach to hygiene practice, particularly in the home by adopting a ‘targeted’ or risk assessment approach focused on preventing the spread of infection. Such an approach seeks to maximise protection against the harmful effects of infectious disease and to limit its spread in the community, whilst retaining the beneficial effects which microbes may have on our human and natural environment.
INTRODUCTION AND SCOPE OF THIS REVIEW

When a disease, or group of diseases, rises rapidly without a specific explanation, it stimulates hypotheses and investigations to identify the cause, or causes, so that preventive measures can be devised. An example of this is the largely unexplained recent rise in the incidence and prevalence of atopic (allergic) disorders, such as asthma, hay fever, eczema and food allergies. This rapid rise has occurred over the last 30 years, predominantly in developed and industrialised countries. A more gradual increase has occurred over the last two centuries, particularly in the 20th century, in association with trends towards higher frequency of all chronic and non-infectious disorders and a downward trend in the developed world of mortality related to pandemics of infectious disease. Increase in asthma and other atopic disorders was initially accepted as an inevitable consequence of changing patterns of mortality and morbidity: a removal of traditional infectious scourges of ill health, rather than emergence of other diseases linked to, or possibly caused by, the absence of infectious disease.

More detailed investigation of the rise in atopic disorders prompted questions about causes in changes in lifestyle and the environment. Such questions were posed first by specialists in the treatment of atopic disorders and epidemiologists studying the trends. Immunologists researching the basis and regulation of immunity linked these questions to increasing research into the genetic and environmental determinants of a healthy immune system. The hygiene hypothesis as an explanation for the increase in atopy was postulated by an epidemiologist (Strachan 1989) but was quickly elaborated by immunologists, based on the emerging knowledge about immune system regulation. The hygiene hypothesis was based on the epidemiological evidence of an inverse relationship between family size and the development of atopic disorders. It states that the apparent rise in allergic diseases could be explained if these were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings or acquired prenatally. The evidence used to form the hypothesis was strongest for hay fever and eczema, but association of reduced exposure to microbes has since been proposed for all the atopic disorders, as well as some autoimmune diseases and other disorders involving possible immune dysregulation.

The idea implicit in Strachan’s proposition that hygiene amenities, hygiene practices and reduced opportunity for microbial exposure could in some way be detrimental, rather than beneficial, to health has inevitable implications for methods used to control infectious disease and for hygiene
practice, particularly in the home, since this is where key early exposures occur. Yet, until recently, discussion of the hygiene hypothesis has been restricted mainly to specialists in atopy and immunology. Specialists in infectious disease, including epidemiologists, have been more concerned in seeking to control emerging or increasing microbial disease, such as Campylobacter enteritis, cryptosporidiosis, legionellosis, viral gastroenteritis, tuberculosis and AIDS. Similarly, hygiene practice specialists have focused on the most effective means of controlling the risk of infectious disease. While concerns about safety and toxicity of hygiene procedures and products have been a part of this focus, the issue that hygiene practices themselves could have an impact on non-infectious disease, particularly on atopy, has only emerged as a result of the dissemination and discussion of the hygiene hypothesis, forming what may be termed the contemporary ‘hygiene debate’. Public attitudes to hygiene and disease trends are relevant to this debate: advances in medicine and science have led to high expectations of the ability to identify and prevent causes of disease – and to more concern about diseases that are apparently increasing, despite the great progress in scientific achievements.

Within the different disciplines, discussion is still compartmentalised and tends to ignore the practical implications of the hygiene hypothesis and other hypotheses put forward to explain the atopy trends, such as changes in diet, obesity, other shifts in lifestyle and environmental pollution. Strong views have been presented on both sides of the hygiene debate, some proponents of the hygiene hypothesis arguing for an urgent need to question the need for some aspects of hygiene or to increase types of microbial exposure that are posited as beneficial, while opponents point to the worldwide prevalence of infectious diseases and the problem of controlling them. If hygiene is in question, and vaccines or treatment ineffective or too expensive, then how can mortality and morbidity due to infectious disease be reduced? To date, there has been no synthesis of the academic and pragmatic issues concerned in this debate, together with no comprehensive account of the evidence on both sides. While there have been excellent reviews of the aetiology of asthma and atopy, and the immunological implications of the hypothesis, none have so far addressed the practical consequences for hygiene, nor whether trends in infection justify references to decreased burdens of microbial exposure, or of infectious disease.

This review aims to bridge the different specialties involved, by examining:

• the evidence for the rise in atopic disorders;
emerging knowledge about the functioning of the immune system;

• hypotheses about causation of atopy and other types of immune system dysfunction;

• epidemiological investigation of the causes of immune system dysfunction

• trends in infectious disease and microbial exposure;

• trends in hygiene practice.

The review begins (Section 2) by defining what is meant by atopic disorders, the immune system, microbial exposure, colonisation, infection and hygiene. This is necessary because different contributors to the hygiene debate have tended to ascribe different, or sometimes vague, interpretations to these terms. The synthesis of a unified hypothesis and a strategy for action cannot be accomplished without a clear and consistent approach to these concepts.

Research has helped identify different types of immune system dysregulation and clarify differences in ‘atopic’ and ‘non-atopic’ asthma, while epidemiological studies have revealed the need to be specific about types of atopy, as well as about different types of microbial exposure, such as colonisation, invasion, subclinical infection or clinically diagnosed infectious disease. Emerging understanding of immune system development is described briefly to show how this has contributed to the questions, and suggested answers, about how microbial exposure may influence both the regulation of the immune system and, in some instances, its dysregulation.

In Section 3, the review examines the trends in atopic disorders and the epidemiological evidence for the many possible factors involved. Factors which might affect microbial exposure or interaction, and fall under the current broad concept of the hygiene hypothesis, are examined. Other hypotheses put forward to explain the trend related to non-microbial factors in our changing environment and lifestyle are briefly described. Studies exploring the parallel idea of links between microbial exposure and auto-immune disease are also outlined.

In Section 4, infectious disease trends are described in terms of international patterns of mortality and morbidity, national trends within the United Kingdom and other parts of Europe; and also the evidence for infections arising in the home environment and other parts of local communities. The section then draws on the growing evidence base from home-based and other studies, to assess the extent to which trends in hygiene amenities,
hygiene practice and hygiene behaviour in the home and community might have changed the extent and/or patterns of exposure to microbes. Other factors which may have reduced or altered our exposure to microbes such as antibiotic treatments, vaccination or changes in the microbial content of foods are also evaluated.

In Section 5 the data from sections 3 and 4 are critically examined, and the balance of evidence assessed in relation to the two distinct questions at the heart of this review:

- is there a causal link between a decline in microbial exposure of some kind and the recent rises in atopic disease?
- to what extent might ‘better hygiene’, as distinct from other influences on microbial exposure, be a significant factor?

Several approaches are used to shed light on the first of these questions. This includes examining the epidemiological studies which suggest a relationship between prevalence of atopy and measures of infection and infection exposure and also the data which suggest a plausible immunological mechanism for the link. Since a putative cause must precede an observed effect, the temporal association between trends in infection and atopic disease is examined. A key question is whether a decrease in mortality from infectious diseases has been accompanied by a decrease in morbidity. Trends in atopy are also compared against trends in microbial exposure including exposure to harmful organisms and non-invasive micro-organisms, such as harmless species in the soil, water or food.

Evidence in relation to the second question, a relationship between atopy levels and unhygienic contact and/or hygiene practice, is taken from a number of sources. These include epidemiological studies of the relationship between the rise in atopy and proxy measures of cleanliness and hygiene; the temporal relationship between the rise in atopy and trends in hygiene practice; and studies of the influence of hygiene, as currently practiced in the home, on microbial contamination and microbial exposure. This section questions the key assumption that increased attention to cleanliness e.g. home cleaning to remove visible dirt, more frequent bathing etc is necessarily associated with reduced microbial exposure – in short, to what extent does ‘cleaning’ also deliver ‘hygiene’ i.e the prevention of infectious disease.

In the final section (section 6) the review considers the implications of the evidence on the hygiene hypothesis for the key role of hygiene, which
is infectious disease control. In particular the implication of any change in hygiene practice on infection and on environmental microbial biodiversity is assessed. The section evaluates whether there is a way, regardless of whether the hypothesis is correct, in which we might change the prevailing approach to hygiene practice, particularly in the home, in order to maximise protection against the harmful effects of infectious disease and limit its spread, whilst minimising the disturbance of the beneficial effects which microbes may have on our human and natural environment. The potential for adopting a targeted or risk appraisal approach to preventing the spread of infection is considered (Bloomfield 2002).

By all the standard demographic measures, people in developed and industrial countries are healthier and living longer than at any previous time. This stunning achievement is based not just on medical and scientific advance, but also on education, household income and other non-medical factors. Hygiene has always been a sign of advanced human civilisation, but there may have been too little emphasis on where and when it is most important for health. It may be more than a decade before resolution of the research issues raised by the hygiene hypothesis: meanwhile it is hoped that this review will help to promote more precise definitions of atopy and microbial exposure, as well as focusing on research and surveillance needs for a 21st century approach to infectious disease prevention.
This section introduces the hygiene hypothesis and some key concepts including the functioning of the immune system, the mechanisms involved in inappropriate immune responses and summarises the range of atopic and autoimmune diseases that have been linked with the hypothesis. This is followed by an outline of mechanisms of infection and different types of exposure, definitions of hygiene, lifestyle factors that may increase the risk of atopic disorders, and a summary of models illustrating postulated relationships in the hygiene hypothesis.

2.1 The hygiene hypothesis

**Box 2.1: Statement of the hygiene hypothesis**

The apparent rise [in the prevalence of allergic diseases]…. could be explained if allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally…. Over the past century declining family size, improved household amenities and higher standards of personal cleanliness have reduced opportunities for cross-infection in young families. This may have resulted in more widespread clinical expression of atopic disease.

(Strachan 1989)

The ‘hygiene hypothesis’ (Box 2.1) was conceived as an attempt to explain rising trends in atopic disease over the last 30 – 40 years, particularly in industrialised/developed countries. The expression of ‘atopic disease’ derives from a hierarchy of factors. Conventionally, this was viewed as originating in the genetic predisposition of the individual to atopy, and this is still seen to be a fundamental underlying factor. The extent to which potentially atopic individuals develop atopic disease is then determined by the extent of sensitisation to specific allergens and by the subsequent triggering of attacks following allergen exposure events.

The sharp rise in atopy has occurred over too short a time period for genetic changes to have significantly influenced the increase, pointing to environmental or lifestyle changes. Although some increase in exposure to allergens to sensitise and then trigger attacks has been observed, such as increased exposure to house dust mites in modern, centrally heated houses,
2. CONCEPTS AND DEFINITIONS OF THE HYGIENE HYPOTHESIS, ATOPIC DISORDERS AND AUTOIMMUNE
DISEASES, INFECTION/MICROBIAL EXPOSURE, LIFESTYLE FACTORS AND HYGIENE

these environmental changes do not adequately explain the rise. This has led to the idea that other factors must be operating to increase the proportion of the susceptible population likely to express clinical disease, i.e. increasing phenotypic expression of atopy in genetically predisposed individuals. As outlined in Figure 2.1a, a number of lifestyle changes, operating independently or in combination, have been considered as possible factors.

![Figure 2.1a: Model showing hierarchy of factors leading to expression of atopic disease](image)

[Note: genetic predisposition derives from the genotype, the encoding of genetic information in each individual's chromosomes. The phenotype is the expression of this encoding as an individual's characteristics, including interaction between the genotype and the environment.]

The hygiene hypothesis proposes reduced childhood infection and less opportunity for 'unhygienic' contact as key factors, although changes in the nature of microbial exposure in general have also been suggested in relation to this hypothesis. Other factors still under investigation include atmospheric pollution, changing diet, lack of exercise, increasing obesity and shifts in the age of motherhood. The possibility that the rising trend is simply an ascertainment effect, that is, related to increased awareness and diagnosis rather than to a real change in the incidence, has been largely discounted.

The hygiene hypothesis was proposed by Strachan in 1989 while working at the London School of Hygiene and Tropical Medicine. It was later expressed in immunological terms by Martinez and Holt (Holt 1994,
Martinez 1994, Martinez and Holt 1999). While Strachan based his hypothesis on the epidemiological evidence of an association of atopic disease, particularly hay fever and eczema, with smaller families and birth order, the suggestion that infection or a more microbe-rich environment may be beneficial had been raised previously (Godfrey 1975, Gerrard et al. 1976). Godfrey (1975) observed different Immunoglobulin E (IgE) levels (a marker for immune system response, particularly in atopy) between urban and rural communities in the Gambia, suggesting an influence from the different environmental exposures. Gerrard and colleagues (1976) further defined the difference as particularly relating to early exposure to helminths (specifically tapeworms) in a study of different ethnic groups in Saskatchewan (Canada). The study involved a comparison of atopic disease and serum IgE levels in a remote Metis (native North American) community with those of the white population in Saskatchewan. Asthma, eczema and urticaria were all more common in the white community. While both the white and Metis populations had a wide range of serum IgE, levels peaked at earlier ages in the Metis community, associated with infestation with fish tapeworms in early childhood and untreated viral and bacterial diseases. Gerrard et al. argued that the lower prevalence of atopic disorders could be genetic as well as environmental, but concluded that:

"...atopic diseases are, in part, the price paid by certain members of the white community for relative freedom from infectious and parasitic diseases."

The concept of early environmental influences on later disease also draws on the increasing interest in fetal programming, popularised as the ‘Barker hypothesis’ (Barker 1992). Although originally proposed in relation to early environmental determinants of cardiovascular disease, the Barker hypothesis has been linked to the hygiene hypothesis in regard to risks for childhood asthma (Tantisira and Weiss 2001). Both hypotheses are concerned with the consequences of stimuli or insults at critical, sensitive periods of early life, including the influence of malnutrition (Barker 1998) as well as other environmental interactions. The hygiene hypothesis represents a specified type of such environmental interactions, singling out the potential role of decreased microbial exposure.

The hygiene hypothesis is an evolving concept, but Strachan’s statement and the earlier suggestion by Gerrard et al. (1976) remain the basis for later modifications and interpretations. The hygiene hypothesis contends that if we are not exposed to infection the system becomes imbalanced in favour of allergic response thereby increasing susceptibility to atopy. The factors involved are represented in Figure 2.1b.
2. CONCEPTS AND DEFINITIONS OF THE HYGIENE HYPOTHESIS, ATOPIC DISORDERS AND AUTOIMMUNE DISEASES, INFECTION/MICROBIAL EXPOSURE, LIFESTYLE FACTORS AND HYGIENE

The hypothesis is defined partly by the rise in atopic disease that it seeks to explain, as well as subcomponents on exposures and its interpretation through laboratory and clinical research into immunology. Since reduced, or changed, microbial exposure of some kind is the central tenet of the hygiene hypothesis, there is a need to define what is meant by ‘unhygienic contact’ and clearer resolution on whether it applies to specific types of atopic disorders or to a non-specific general effect on the tendency to atopy. These points are discussed later in the review.

Figure 2.1b: Model showing hierarchy of predisposing factors for atopic disease, based on the hygiene hypothesis

By drawing attention specifically to hygiene, Strachan’s statement helped to crystallise a growing unease both about the artificiality of modern lifestyles, as well as to offer an explanation for emerging ideas about the development and regulation of the immune response. The immunological interpretation of the hygiene hypothesis is that exposure to micro-organisms is essential for the healthy maturation of the immune system. Whether this postulated necessary microbial exposure involves mild, severe or clinically unidentifiable infection is not yet determined; but it is held that without some kind of microbial stimulation, the immune system develops inappropriately or inadequately, leading to a rise in atopic disease and autoimmune disorders. Proponents of the hypothesis attribute this rise to trends in modern
industrialised countries: first, an assumption of decline in exposure to invasive microbes (pathogens) at key development stages; secondly, increasingly hygienic environments and purity of food and water, that further reduces exposure to micro-organisms.

2.2 The immune system: a brief outline

The function of the human immune system is to provide defence against invasion by disease-causing micro-organisms (pathogens), foreign bodies and toxins. For example, the visible signs of the immune system in action are the inflammatory response in an infected skin wound or after exposure to toxins from plants such as nettles. The system comprises two basic mechanisms:

1. **Lymphocyte** ‘white blood’ cells, produced by the bone marrow, spleen and peripheral lymphoid tissue. T-lymphocytes (T-helper cells) are involved in the cell-mediated response to invasion, particularly lymphocytes known as CD4 helper cells. These cells produce substances called cytokines, which serve to enhance or suppress the inflammatory response.

2. **Immunoglobulins (Ig)** produced by the liver (in fetus only) and B-lymphocytes. Babies are born with the mother’s immunoglobulin (IgG), giving passive immunity to any infections to which the mother was immune. Specific types of IgG represent antibodies against particular infections. Maternal immunoglobulin declines after birth and further production of IgG depends on stimulation by invasion, as well as upon appropriate development of the other parts of the immune system.

The system includes regulatory cells and also functions as two main pathways, **Th 1 and Th 2**, each involving different cytokines, including a range of substances called interleukins (Box 2.2a). The Th 1 and Th 2 pathways are mutually inhibitory, but also inter-related and governed by T-regulatory cells. There appears to be a ‘Yin-Yang’ relationship between the two (Figure 2.2a), so that synergism rather than competition is involved.
Box 2.2a: Interleukins produced by Th 1 and Th 2 cells

The Th 1 and Th 2 cells produce a different range of interleukins serving to either enhance or suppress the inflammatory response.

- The Th 1 pathway is characterised by the cell-mediated pro-inflammatory response: they also produce interferon γ (IFN-γ), a key part of the defence against viral infection. This pathway is also involved in certain autoimmune responses, where antibodies or immune lymphocytes are produced against substances naturally present in the body.

- The Th 2 pathway is involved in production of Immunoglobulin E (IgE), eosinophilia, atopy and airway hyper-responsiveness. Th 2 pro-inflammatory cytokines include a range of interleukins, such as interleukins 4, 5, 10 and 13. Interleukin 10 has a regulatory role in the anti-inflammatory response, counteracting Th 1-mediated microbiocidal action.
gens and to antigens produced by infectious organisms. Like other lymphocytes, the Th 2 cell is able to recognise specific allergens through binding to the T-cell receptor. This occurs after the allergen has been taken up by an antigen-presenting cell (APC) (Box 2.2b). The division of immune reactions into two major pathways (Th 1/Th 2) represents an oversimplification of the multifaceted immune system. There are no infectious agents found in nature that promote only a Th 1 or a Th 2 response (Wold 1998). However, the observation that Th 1 cytokines inhibit the development of Th 2 responses, and vice versa, is important in the understanding of how imbalance or dysregulation of the Th 1/Th 2 pathways may underlie development of atopic and autoimmune disease.

Figure 2.2b: The inter-relationships between T-cells in the immune response

**Box 2.2b: Recognition and processing of antigen presenting cells (APCs)**

Allergens are processed and re-expressed on the surface of the APC in association with major histocompatibility Class II molecules. It is currently thought that the APC plays a central role in the type of response generated, although the exact mechanism is not yet understood. Recognition of the allergen activates the Th 2 cell to secrete cytokines that mediate the immune response. Interleukins 10 and 4 (IL-10, IL-4) are typical of Th 2 cells. Under the right conditions, activation produces a clonal expansion of B-lymphocytes that can recognise the same allergen. In the presence of IL-4, these lymphocytes undergo immunoglobulin class switching to produce IgE.

The postulated pathways relating to the APC are shown diagrammatically below (after Settipane and Settipane, 2000).
2.3 The immune system in pregnancy and infancy

2.3.1 Early development of the immune system

At birth, the infant’s immune system is biased to make Th 2 responses (Koning et al. 1996, Holt et al. 1997, Jones et al. 2000) and is therefore particularly susceptible to develop allergic conditions. This is a consequence of the Th 2 immune environment during pregnancy, which is necessary to its viability by minimising the possibility of rejection of the growing fetus in utero (Gupta 1998). Maternal exposure to pathogens can alter the development: pathogen exposure may result in marination of the fetus by interleukin 12 in the amniotic fluid, stimulating development of Th 1 cells. The overall bias towards Th 2 response continues after birth until about the age of 2 years. Normal development involves activation of the Th 1 pathway to provide the appropriate balance: Th 1 responses develop gradually in response to the immunological challenges of life, such as infections. Humans appear to have evolved an immune system ready to deal with such challenges.

The fetus is capable of making immune responses as early as 15 to 16 weeks of gestation and is primed to the antigens in its environment by swallowing antigen present in the amniotic fluid. For example, in primitive societies early exposure to helminths would be expected. Babies born to parasitised mothers produce specific IgE to gastrointestinal helminth para-
sites such as *Ascaris lumbricoides*, as demonstrated by studies of cord blood samples, indicating that the exposure has stimulated production of IgE by fetal lymphocytes during pregnancy (King *et al.* 1998). Such infants do not acquire infection in early life with the strain of parasite carried by the mother. This sensitisation appears to also tune the immune system away from inappropriate allergic responses later in life (Cookson and Moffatt 1997). However, the neonate is not protected against infections by other parasites and the protection can be overwhelmed by recurrent infection. Helminth infection remains a major problem in developing countries, with a prevalence of up to 50%, particularly in children aged 3 to 8 years, often resulting in chronic debilitating disease (Chin 2000).

2.3.2 The ‘window’ for immune system development

“The period shortly before and after birth may be the key to understanding immunologic memory.”

(Zinkernagel 2001)

Most developmental stages in the fetus and newborn infant are subject to a narrow time scale, hence the devastating effects of teratogens, such as thalidomide, at particular times of fetal growth and differentiation. It would seem logical that the immune system differentiation is also timed to occur over a short period. The window for immune deviation to a type 1 or type 2 response may be limited to a short 3-4 week time period in late pregnancy/early neonatal period, possibly between 20 and 30 weeks of gestation. Without an intervention study, the timing and duration of this window remains uncertain, although it almost certainly occurs before 6 months of age and the ‘switch’ may occur as early as 3-4 weeks after birth. Research into the still mysterious trigger factors for the onset of labour suggest that an inflammatory response at the placental interface may be involved, implicating the pro-inflammatory, type 1 cytokines (e.g. IFN-γ). While the 2nd trimester may be a key sensitising point, the 3rd trimester is also important, as this is the stage when maternal IgG passes to the fetus. Thus, while the baby is born with a suppressed immune system, maternal antibodies play an important role in early defence against pathogens.

2.3.3 Genetic variation and atopic/autoimmune disease

Some autoimmune diseases improve during pregnancy, such as rheumatoid arthritis, but this appears to be related to histo-incompatibility of MHC haplotypes between mother and baby: the greater the difference, the more chance of improvement and *vice versa* (Warner 1999). The mechanistic basis for this is that a greater degree of immunosuppression is required of the
mother if there is a large genetic difference between mother and child, to avoid rejection of the fetus, and therefore the greater the magnitude of the type 2 bias and suppression of the type 1 immune response. This may also explain to some extent the observed effect on second-generation migrants gaining the disease patterns of the new host country; although this explanation assumes that the migrant population becomes less genetically homogeneous by inter-marriage with other groups. Studies on the influence of genetic diversity on immune system development suggest that populations with wide genetic diversity – such as in the UK – have greater susceptibility to immune system dysregulation and hence atopic disease (Warner 1999).

2.4 The role of gut lymphoid tissue and the possible immune system priming function of intestinal bacterial flora

2.4.1 Peripheral lymphoid organs and immune reactions

The peripheral lymphoid organs include the lymphoid tissue of the gut: Peyer’s Patches, intraepithelial lymphocytes (IEL) and associated nodes. The gastrointestinal tract is sterile at birth, but is rapidly colonised by bacteria from the environment. This indigenous microflora has an important role in stimulating the host immune system to respond to pathogen challenge and also inhibits colonisation by overt pathogens (disease-causing organisms) (Berg 1996). There has been increasing interest in mediation by gut lymphoid tissue of immune reactions and hence of atopic disease. Greater understanding of the function of the commensal organisms of the gut has led to more conservative antibiotic policies that cause less disruption to gut flora.

2.4.2 Gut colonisation and the hygiene hypothesis

Investigators of the hygiene hypothesis, such as Strachan (2000), Björkstén 1999 and Wold (1998) now favour the possibility that gut colonisation during and after birth may be the most critical element in the priming of the immune system. The important organisms are likely to include lactobacilli, enterobacteria and exposure to their products through the gut flora. Work on endotoxin receptors, such as the receptor for lipopolysaccharide (LPS) (CD14) on macrophages, suggests the importance of pattern recognition in immunity (Kurt Jones et al. 2000). Several species of bacteria produce toxins, categorised as exotoxins and endotoxins (Box 2.4).
Box 2.4 Exotoxins and endotoxins

**Exotoxins** are highly toxic polypeptides excreted by living bacteria, a few micrograms capable of killing animals. Examples include the exotoxin produced by the organism causing botulism, *Clostridium botulinum*, the organism responsible for tetanus, *Clostridium tetani* and the enterotoxin produced by *Vibrio cholerae*, the cause of cholera.

**Endotoxins** are lipopolysaccharide molecules in the outer layer of Gram-negative bacteria cell walls. These are released when the micro-organism disintegrates. Examples of endotoxin-producing Gram-negative organisms include many of those responsible for diarrhoeal or other gastrointestinal infectious disease, such as *Salmonella typhi* (cause of typhoid fever) and strains of *Escherichia coli*.

2.4.3 Diet and gut colonisation

The role of diet in early priming of the gut has only recently received more attention, although evidence of possible changes in intestinal flora in developed countries dates back at least to the 1960s, including a study of patients with asthma and rheumatoid arthritis (Månson and Colldahl 1965), persistence of Gram-positive bacteria in faeces (Gossling and Slack 1974) and the effect of feeding on infants’ faecal flora (Simhon et al. 1982). Probiotics (cultures of potentially beneficial bacteria of healthy gut microflora) enhance gut-specific IgA responses (Isolauri et al. 1993) that may be defective in children with food allergies (Kalliomäki et al. 2001). Evidence relating to the possible ‘gut priming’ role for endotoxins is reviewed in Section 3.

2.5 What is atopy? Introduction to atopic diseases

Atopic diseases are commonly known as allergic diseases, for example eczema, hay fever and asthma. The word ‘atopy’ was applied to these diseases by Coca in 1923, derived from the Greek meaning out of place, strange or odd: a suitable term for something that should not occur in a well regulated immune system. Atopy refers to the predisposition towards an excessive immune response, mediated by IgE antibodies, to a stimulus, such as an allergen (dust particle, pollen, animal dander) or antigen (micro-organism). Following the production of IgE specific for an allergen, it is secreted and binds to effector cells (e.g. mast cells, eosinophils) through specialised receptors. A response occurs when the allergen cross links to those IgE molecules. The resulting signal provokes mast cell degranulation and release of pharmacologically active mediators, which produce the symptoms of atopic disease. This inappropriate immune response is also known as immune system ‘dys-
regulation’ and the diseases/responses as immune system disorders. In the narrow sense, individuals are referred to as atopic when they show positive skin test reactions to certain environmental allergens, such as house dust mite or pollen, or possess serum IgE antibodies specific for those allergens. Thus IgE mediated reactions characterise the cluster of atopic diseases. Affected individuals are termed allergic to the agent in question. Eosinophilia, a rise in a type of white blood cell, is another diagnostic marker for atopic reactions and may occur in response to some infections, particularly infestation with helminths. Different allergens appear to have different effects on the cellular and molecular mechanisms of the immune system (Box 2.5). The possible cellular and molecular mechanisms for allergy are shown diagrammatically in Figure 2.5 (see also Box 2.2b, Section 2.2).

Box 2.5: The nature of the allergen in determining cellular or molecular effects

Many inhalant allergens (such as those from dust mites) have enzymic activity. Simultaneous exposure to allergen and proteolytic (breaking down protein) activity enhances sensitisation, possibly allowing greater access to dendritic (antigen processing) cells in the immune system, or interrupting critical molecules in IgE regulation e.g. CD23 (low-affinity receptor for IgE) or CD25 (interleukin-2 receptor) (Holgate 1999).

Figure 2.5: Proposed mechanisms of allergy (adapted from Holgate 1999)
Note to diagram: Dendritic cells direct the differentiation of naïve CD4+ T cells to the Th 2 phenotype, which has capacity to secrete cytokines encoded in the IL-4 gene cluster on chromosome 5q31-33. This polarization can be suppressed if T cells are driven along the Th 1 pathway by IL-12, producing increased interferon-γ (IFN-γ).

In the immunological interpretation of the hygiene hypothesis, it is suggested that exposure to bacteria or viruses at a critical time in childhood development enhances Th 1 at the expense of Th 2 polarization.

Allergic reactions range from mild to severe, the latter including anaphylactic shock. Whether a reaction is severe depends on factors such as the portal of entry of the antigen/allergen and the dose. Atopic disease includes asthma, allergic rhinoconjunctivitis (hay fever), atopic eczema and food allergies (Table 2.5). Food allergy is one of the first signs of atopy, which may be followed by atopic dermatitis and later by asthma and allergic rhinitis, the so-called ‘atopic march’ (Sicherer 2002).

Asthma is often considered separately from other atopic disorders, not least because it is generally the most severe, but also because there are both atopic and non-atopic forms and different theories of aetiology (cause). There are two overlapping syndromes of reversible airway obstruction in childhood: the first usually starts with an episode of bronchiolitis in the first year of life, followed by multiple episodes of viral induced wheezing. The second form is characterised by eosinophil-rich inflammation of the airways and occurs predominantly in children with a genetic predisposition, becoming apparent after 2 years of age, although some do not develop clinical disease until after the age of 5 years. By the age of 10 years, allergic asthma is the dominant form of the disease and 90% of patients have developed the disease by six years of age (Weiss 2001). The distinction between the two types is important because immune responses are highly relevant to allergic asthma, but not clearly related to bronchiolitis and wheezing illness before the age of 2 years (Platts-Mills et al. 2000). The first manifestation of atopy is usually eczema, which may coincide with development of food allergy in four out of five children with atopic eczema; asthma may follow, then allergic rhinitis (Barnetson and Rogers 2002).
### 2. Concepts and definitions of the hygiene hypothesis, atopic disorders and autoimmune diseases, infection/microbial exposure, lifestyle factors and hygiene

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical presentation</th>
<th>Known triggers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asthma</strong></td>
<td>State of recurrent and paroxysmal bouts of chest wheezing and breathlessness: associated with bronchial hyper-reactivity and acute, reversible obstruction of the bronchus. Severe bronchospasm can lead to status asthmaticus and requires acute medical care to relieve the associated exhaustion, dehydration, depleted oxygen, acidosis and cardiac arrhythmias. About half of all cases of asthma present in early childhood. The broad diagnosis of asthma includes several syndromes such as acute wheezy chest in young children (occurs in about 25% of children but most resolve by age 15 years). Also occurs as an acute exacerbation of chronic bronchitis (in about 25% of bronchitis sufferers, increases with age) or as a localised obstruction of the bronchus e.g. due to tumour or inhaled foreign body. About a third of asthmatics also suffer from hay fever and about 15% from eczema. GP attendance for emotional disorders is also more common.</td>
<td>In young children, acute wheezy chest may be associated with a triggering infection. Triggering agents include pollens, dust, house mites and animal danders (hair/feathers).</td>
</tr>
<tr>
<td><strong>Atopic eczema</strong></td>
<td>Inflammation of the skin associated with itching (scratch marks) and blisters (endogenous dermatitis); face usually first area affected in younger children; flexor aspects of elbows and knees in older children; infection complications common e.g. invasion by <em>Herpes Simplex Virus, Staphylococci</em> and <em>Streptococci</em>.</td>
<td>Wide variety of allergens e.g. latex.</td>
</tr>
<tr>
<td><strong>Hay fever</strong></td>
<td>State of hypersensitivity of upper respiratory tract to pollen: acute reaction on inhalation including sneezing, watery nasal discharge, irritation and inflammation of the eyes (conjunctivitis) and nasal irritation/blockage. Most cases start in childhood (90% cases by age 30). Tends to improve/lessen with age. Symptoms seasonal: during hot, humid, windy days. May be associated with chest wheezing in about a third of cases. About a third also have asthma; 25% have eczema and 5% urticaria. About a fifth have a family history. Another form of hay fever, vasomotor rhinitis, is not seasonal.</td>
<td>Commonly grass pollens, also tree pollens and moulds.</td>
</tr>
<tr>
<td><strong>Food allergy</strong></td>
<td>Adverse immunological response (hypersensitivity) to particular types of food, which commonly develops in childhood but may occur at any age. Broadly divided into allergies mediated by IgE antibodies (usually associated with acute onset after eating the food) and those that are not. May present as ‘pollen-food syndrome’ combined with seasonal allergic rhinitis (50% cases), where IgE antibodies produced to proteins homologous to those in particular fruits/vegetables. Food allergy produces symptoms in a range of organs (skin, respiratory tract, GI tract). Severe reactions (e.g. anaphylaxis) often involve multiple organ systems and may be precipitated by extremely low doses of allergen (typically, peanut, other nuts, seafood). Food allergy is one of the first manifestations of atopy: young children with food allergy and atopic dermatitis may later present with asthma, respiratory allergies and allergic rhinitis.</td>
<td>Mostly the protein component of foods: recently emerging food allergies include nuts, soya and natural food colourings.</td>
</tr>
<tr>
<td><strong>Urticaria</strong></td>
<td>Hypersensitivity of skin in response to allergen, mediated by histamine and serotonin (5-HT), with formation of wheals and blisters. May develop in response to absorption of allergen via gastrointestinal tract.</td>
<td>Allergens, but also some drugs.</td>
</tr>
</tbody>
</table>

Table 2.5: Atopic diseases
Since Th 2 cells have a central role in atopy, atopic diseases are designated Th 2 diseases. Th 1 cells can also recognise allergen and respond, under the right conditions, by producing cytokines (IFN-γ, IL-2) that direct the immune system to a Th 1 response. While atopy is associated with an apparent shift towards the Th 2 pathway, it is not a simple matter of ‘switching on Th 2’ or ‘switching off Th 1’. The inter-relationship between the two pathways means that atopic disease cannot be controlled by simply suppressing the Th 2 reactions: early therapeutic attempts to do this resulted in an inappropriate shift towards Th 1 and the development, in some cases, of auto-immune disease. Also, the failure to make the transition from Th 2 to Th 1 in early life probably occurs only in genetically susceptible individuals (Holt et al. 2000), with increasing evidence of varying response by different genotypes (Tantisira and Weiss 2001). For example, infants whose Th 2 lymphocytes produce IL-13 may be particularly prone to asthma (Spinozzi et al. 2001).

Atopic disease appears to have been rare before the 19th century (Emanuel 1988) and shows wide variation by country and ethnic group. The international variation in atopic disease has engendered much debate (Warner 1999). While atopic disease is generally more common in countries with higher standards of hygiene, the variations in particular types of atopic disease do not necessarily follow this pattern. African countries, for example, were among those with highest prevalence of atopic eczema in the ISAAC survey of 58 countries (ISAAC 1998). Similarly, countries with Anglo-Saxon cultures showed a very high prevalence of atopic disease, while other affluent countries, such as the Scandinavian ones, had a much lower prevalence, even though their hygiene standards are arguably at least as high.

Genetic predisposition appears to be relatively common in the population and it may involve genetic determinants of immune system regulation, as well as disposition towards particular responses. One of the mysteries of atopic disease is that despite a widespread genetic predisposition, a minority of individuals develop allergies, even where there is a strong family history of atopic disease. The mechanism that leads to presentation with a particular type of atopic disease, for example, hay fever rather than asthma, is also not well understood and some individuals may present with a combination of different types of atopy. In the UK ISAAC study, 43% of children with current wheeze also had one or more of the other atopic disorders (Austin et al. 1999).
2.6 Autoimmune diseases and other diseases in which immune dysfunction has been implicated

An immune reaction against one of the body’s own tissues is known as autoimmunity, causing chronic changes and clinical presentation of disease. While the mechanisms for this group of diseases are incompletely understood, they may be viewed as a chronic type of immune dysregulation: an end stage produced by chronic inappropriate reactions by the immune system. Researchers have therefore investigated whether immune imbalance, as postulated in interpretations of the hygiene hypothesis, might also explain observed rising trends in some autoimmune and other immune related diseases. However, people with atopic disorders do not have a higher risk of autoimmune diseases, suggesting that different mechanisms and risk factors are involved. An increasing range of diseases is now classified as ‘autoimmune’ (Table 2.6). The list in Table 2.6 is not exhaustive: for example, some chronic bowel disease, such as ulcerative colitis, may have an autoimmune aetiology. Reduced microbial exposure in early life has been proposed as a predisposing factor for some autoimmune diseases: conversely, some types of these diseases are suspected to have an infectious aetiology, such as juvenile diabetes following Coxsackie B virus infection (Pugh et al. 1993). Exposure both to specific infections, as well as to reduced microbial exposure, has been implicated in the aetiology of childhood acute lymphoblastic leukaemia (see 3.8.5). Most of the autoimmune diseases are exacerbated by infection, which may activate a predisposition to the disease and the aetiology and disease pathways are not fully understood.
2.7 Infection and microbial exposure

Strachan’s 1989 statement of the hygiene hypothesis refers to “infection in early childhood”. Careful assessment of trends in infection and of the different types and effects of microbial exposure is thus essential for any analysis of the hypothesis. As an example, the “unhygienic contact” that is suggested to limit levels of atopy, inevitably involves exposure to a wide range of different microbial species, including non-invasive, harmless microorganisms as well as invasive organisms (pathogens), or microbial by-products. Current interpretations of the hypothesis tend to reflect the possibility of the importance of non-invasive, as well as invasive, microbial exposures.

2.7.1 Definition of ‘infection’

The word ‘infection’ covers a wide range of meanings, from exposure with no discernible reaction to serious systemic disease. In general use the term refers to penetration of microbes (micro-organisms) beyond the skin.

### Table 2.6: Autoimmune diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ankylosing spondylitis</td>
<td>Progressive inflammatory joint disease, particularly involving back and pelvis.</td>
</tr>
<tr>
<td>Diabetes (infantile, insulin dependent)</td>
<td>Reduced availability of insulin, leading to inability to metabolise glucose: several types and multifactorial aetiology, including genetic predisposition and possible viral causes (e.g. mumps, Coxsackie B).</td>
</tr>
<tr>
<td>Lupus erythematosus</td>
<td>Progressive joint disease associated with inflammation of connective tissue, also involving skin, kidney, lungs and other organs.</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>Painless muscle weakness due to failure of neuromuscular transmission, most marked in face and eyes.</td>
</tr>
<tr>
<td>Polyarteritis nodosa</td>
<td>Progressive fibrosis of small/medium arteries, associated with tender nodules.</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>Progressive weakness and tenderness of muscles, particularly in proximal limbs, may be associated with skin rashes and joint involvement.</td>
</tr>
<tr>
<td>Polymyalgia rheumatica</td>
<td>Muscle pain, stiffness, may include inflammation of arteries (temporal arteritis).</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Chronic relapsing disease of joints associated with inflammation of joints and other tissues.</td>
</tr>
<tr>
<td>Thyroiditis</td>
<td>Characterised by presence of circulating thyroid antibodies: may present as over or under active thyroid gland or with goitre (Hashimoto’s disease).</td>
</tr>
</tbody>
</table>

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THE HYGIENE HYPOTHESIS AND IMPLICATIONS FOR HOME HYGIENE
barrier and membranes lining the orifices, with three possible results:

i. A typical attack of the illness associated with that particular micro-organism (including progression to severe disease and death for some types of infection): **clinical infection**.

ii. A mild disease with indefinite symptoms: **subclinical infection**.

iii. No illness at all: **asymptomatic infection**.

Clinical infection can also be subdivided into infection where the responsible micro-organism is confirmed by laboratory investigation, and infection resting on clinical diagnosis alone. In the latter, diagnosis is made on symptoms and signs that meet diagnostic criteria, for example, the presence of a combination of vomiting, diarrhoea, fever, nausea or abdominal pain in the case of a diagnosis of gastrointestinal infection. Case definitions with defined criteria of symptoms, signs, onset dates, duration of illness and results of microbiological investigation are essential for the epidemiological assessment of patterns of infectious disease. While serological tests provide evidence of microbial exposure, those taken months or years after exposure cannot distinguish between whether the exposure resulted in asymptomatic, subclinical or clinical infection, or whether raised levels of specific immunoglobulin (IgG) for a particular infection are due to vaccination.

Other important factors in the definition of infection are the mode of spread (e.g. airborne, foodborne, direct contact) and route of entry (e.g. oral, respiratory, skin, sexual). The carrier of a disease is known as a vector: this may be another human, an animal, or an object contaminated with the micro-organisms. An inanimate object serving as a vector is known as a fomite: a substance capable of absorbing or retaining viable micro-organisms and thus to spread disease. Important examples of fomites include contaminated bed linen, toys, carpets, curtains and woollen garments. Typhus fever, for example, is spread by such fomites, although also by person to person contact (**Box 2.7a**). While the control of typhus in Europe is a major triumph for hygiene practice, the history of this deadly infection also shows the importance of changes in lifestyle. The infection first emerged in recognisable epidemic form with the widespread availability of woollen clothing, particularly in cooler climates and seasons (Kiple and Ornelas 1997). Infrequently changed or washed clothing provided the ideal reservoir for the lice and their cargo of rickettsial organisms.
Box 2.7a: Typhus: example of vector/fomite borne disease

Typhus, a rickettsial infection, was a major cause of mortality in previous eras: the vector was the body louse (*Pediculus humanus*). The lice and their faeces readily survive in unwashed clothing or spread via contaminated dust in the environment and by being accidentally rubbed into skin abrasions. It thrives in overcrowded conditions and thus earned the names of jail fever, camp fever and ship fever. The control of typhus is directly linked to improved environmental conditions, laundry and facilities for personal hygiene: World War I produced the greatest lice-related mortality in the history of the world (Kiple and Ornelas, 1997) and it was also a major problem during World War II. It remains prevalent in developing countries, particularly in refugee camps and in impoverished communities.

2.7.2 ‘Microbe’ classification

Microbes (micro-organisms) can be classified into ‘pathogens’ that cause disease; ‘commensals’ found in the normal body flora (for example, in the mouth, gut and on the skin) and ‘saprophytes’ and other micro-organisms found in environmental sites such as soil or plants. This classification is of only limited value, since there are many examples of ‘commensals’ causing disease in vulnerable individuals and in certain circumstances, such as high density exposure. The invasiveness or ‘pathogenicity’ of a microbe depends on host as well as microbial factors. Some microbiologists therefore classify micro-organisms into three groups:

1. ‘conventional pathogens’ (e.g. species of *Salmonella*);
2. ‘conditional pathogens’ that rarely cause disease;
3. ‘opportunist pathogens’, which, as the term suggests, can take advantage of certain circumstances and reduced immunity (Shanson 1999, p9).

For most recognised routes of infection, colonisation may occur instead of obvious disease and this does not engender a measurable immune response (**Box 2.7b**). This applies particularly to colonisation of the skin, which provides an effective physical and chemical barrier to invasion: colonisation of the sweat ducts may occur but the eccrine secretions usually prevent further penetration (Hedström 1999). For example, staphylococci and streptococci may be carried on the skin but an immune response (or progress to invasive infection) may only occur after interruption of the integrity of the skin or in people with compromised immunity due to genetic or chronic disease (Jarvis 1996). Invasive species produce extracellular enzymes to aid skin or membrane penetration by breaking down tissues and fibrin (Ljungh and...
Wadström 1999), so the difference between colonisation and infection may also depend on species. Since colonisation, or other forms of benign microbial exposure, may be undetectable by conventional tests, it is impossible at the current stage of microbiological technique to determine whether microbial exposure at this level causes unrecognised interactions that may be involved in the development of the immune system.

Box 2.7b: Colonisation by micro-organisms

Colonisation is the presence of a micro-organism in or on a host, with growth and multiplication, but without any overt clinical expression or detected immune response at the time it is isolated. Normal colonisation in humans begins during the birth process and through subsequent contacts with the inanimate or animate environments until a delicately balanced ‘normal’ flora is established. For example, normal skin flora includes coagulase-negative Staphylococcus or *Staphylococcus aureus*; and *Candida albicans* is a normal coloniser of the gastrointestinal tract, vagina or perineal area. Colonisation can result in infection when the normal body defences are impaired, through underlying disease or alteration of the normal flora by antimicrobial therapy (Jarvis, 1996).

2.7.3 Factors that determine whether exposure to micro-organisms will result in disease

Several factors determine whether exposure to infectious organisms will result in colonisation, subclinical infection or infectious disease. These include impaired host defences, characteristics of the organism, such as virulence or invasiveness, the duration, frequency and conditions of exposure; and the route of entry.

2.7.3.1 Host susceptibility

a) Genetic determinants of susceptibility to infection

The likelihood that a given exposure to a pathogen will result in infectious disease in a specific individual, and the resulting severity of such disease, depends on: age; ethnic group; genetic components; nutritional status; presence of other disease. There is now strong evidence for a genetic component in susceptibility to tuberculosis, leprosy, malaria and *Helicobacter pylori* infection (Kwiatkowski 2000). A higher risk of death from infection was found in adopted children with a biological parent who had died prematurely from infection (Sorenson et al. 1988). A specific gene mutation affecting interferon γ receptors determines susceptibility to mycobacteria and *Salmonella* species, as demonstrated in a study on Maltese children who developed atypical fatal mycobacterial infections (Newport et al. 1996).
b) Vulnerability at extremes of age

In an aging society, with a larger proportion of very elderly people than any previous generation, plus growing proportions of people with chronic or debilitating disease, the host susceptibility factor is as least as important as infection routes. Age at infection is one of the most important determinants of disease morbidity and mortality (Miller and Gay 1997). The probability of a clinically apparent infection with poliomyelitis and hepatitis A increases with age: the likelihood of a fatal outcome from measles or pertussis is much higher in adults than in young children (Miller and Gay 1997). By contrast, measles infection in the first year of life is associated with a higher risk of subacute sclerosing panencephalitis in adults. Immunity to infection wanes with age, affecting both the likelihood of invasive infection and of serious consequences.

c) Vulnerability due to chronic disease, deprivation and homelessness

Chronic disease and poor nutrition impair the functioning of the immune system. In addition to the increased vulnerability in older people, poverty and homelessness are important determinants of higher susceptibility to infectious disease. Homeless people in developed countries suffer higher rates of respiratory infection, including tuberculosis, skin infections ranging from scabies to gas gangrene, ‘urban trench fever’ (a louse borne bacterial infection – *Bartonella quintana* –), typhus fever, bacteraemia; and, particularly in homeless intravenous drug users, HIV, hepatitis B and C infections (Raoult *et al*. 2001).

d) Virulence and the influence of dose-response/repeated exposure

Some micro-organisms are more invasive than others and more likely to produce infectious disease. For example, some saprophytic species of mycobacteria are not known to cause human disease, while species such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* are responsible for tuberculosis and leprosy, chronic and debilitating forms of infectious disease. Linked to both host factors and virulence, repeated exposure or an exceptionally high ‘dose’ of a particular microbial species may result in disease, where a lower level of exposure would only produce temporary colonisation or subclinical, non-progressive infection.

2.7.3.II Route of infection

In order to produce infection, organisms must gain entry to the body via a route that enables invasion of body tissue. The infection route, also known as portal of entry, may be via the respiratory tract, gastrointestinal tract, genitourinary tract or the skin (*Box 2.7c*). Most infectious diseases can spread by two or more alternative routes: for example, tuberculosis spreads
most commonly by airborne infection, but may also spread via ingestion of unpasteurised milk from infected animals or by direct skin contact. Similarly poliovirus is principally spread through the faecal-oral route, but pharyngeal spread may also occur. The route of infection has important implications in considering possible effects of improved hygiene, since some routes, such as respiratory spread and skin penetration, are less amenable to hygiene interventions. By contrast faecal/oral spread via the hands or via surfaces and fomites can be prevented by personal hygiene, such as handwashing, or environmental hygiene, such as cleaning contaminated surfaces. Route of transmission is therefore an important issue in the hygiene hypothesis.

Box 2.7c: Route of infection

The upper and (or occasionally lower) respiratory tract: The lymphoid tissue of the pharynx is the main localising site of entry. Once established in the tract, the infection may localise (as in whooping cough) or affect distant tissues via exotoxin production (as in diphtheria), or alternatively it may disseminate through the body (as in measles and chicken pox).

The gastrointestinal tract: Intestinal infections mainly localise in the lymphoid tissue of the intestines. It may establish as a local bowel infection (gastroenteritis, dysentery, endotoxin effects) or disseminate through the body (typhoid fever, brucellosis). Distant toxic effects are less frequent than via the respiratory portal, but botulism is an example of such effects.

Genito-urinary tract: via infected body fluids or direct contact e.g. Neisseria gonorrhoeae, HIV, Chlamydia spp.

Skin: infection, via five main routes: direct infection of skin surface (e.g. scabies and impetigo); penetration of intact skin (e.g. leptospirosis and erysipelas – streptococcal infection –); infection of broken skin (wound infection, burns sepsis, tetanus); penetration of skin by bites (malaria, typhus); and injection through the skin (Hepatitis B virus).
2.8 What is hygiene?

“*To the founders of the London School of Hygiene and Tropical Medicine, hygiene was the science of public health, the key weapon to prevent infectious diseases that still killed so many at home and abroad… Today’s scientists understand hygiene as a set of practices that prevent infection, while today’s consumers buy ‘hygiene’ products for reasons more connected to status, convenience and aesthetics than fear of infection.*”

(Press release for exhibition of hygiene and the art of public health, LSHTM 2002. www.lshtm.ac.uk/art/hygiene)

The definition and importance of hygiene has shifted over the last century, so that it is now both less clearly defined and less universally accepted as an ideal. The medicine of antiquity paid great attention to general health maintenance through regulation of diet, exercise, hygiene and lifestyle (Porter 1997). Hippocrates\(^1\), for example, wrote of the need to observe people’s habits, indulgences, water supplies and the environment in which they lived, in order to understand their diseases. Hygiene had a central role in this broad environmental approach, as did diet, exercise and exposure to fresh air. A current dictionary definition of hygiene still reflects the focus on health principles, practices and sanitary science (Box 2.8a).

**Box 2.8a: Definition of hygiene**

<table>
<thead>
<tr>
<th><strong>hygiene (n):</strong></th>
<th>(a) a study or a set of principles, of maintaining health (b) conditions or practices conducive to maintaining health (c) sanitary science.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxford English Reference Dictionary, 1995</strong></td>
<td></td>
</tr>
</tbody>
</table>

The medical and scientific definition of hygiene is narrower, focusing on the aim to prevent the spread of infectious diseases through “promotion of cleanliness” (Box 2.8b):

**Box 2.8b: Medical definition of hygiene**

**Hygiene:** the science of health and the study of ways of its preservation, particularly by widespread education and promotion of cleanliness. Especially valuable in developing countries, where it plays a vital role in helping to spread the limitation of infectious diseases.

(Black’s Medical Dictionary, 39th edition, 1999 edited by MacPherson)

\(^1\)Hippocrates (c460-359 BC): Greek physician and ‘father of medicine’, wrote of the role of the environment in shaping the health of a community in Airs, Waters and Places
While this contemporary definition specifies the particular importance of hygiene to developing countries, hygiene practitioners and infection specialists in both developed and developing parts of the world would also include the concept of regulation of procedures and practices, for example by monitoring water quality, improving sanitation, producing guidelines on good hygiene practice and regulating their application.

How does the concept and definition of hygiene apply to the hygiene hypothesis? First, it is notable that the definition is general and contains no specification of degrees of hygiene or its particular components. It is thus a word that can be used in a vague, non-specific sense, rather like the contemporary references to ‘stress’. It carries negative connotations, having acquired the concept that it can be ‘overdone’, as in some interpretations of the hygiene hypothesis. For assessing the implications of the hypothesis for hygiene practice, this poses a dilemma for the evidence-based approach: for example, how is a ‘high standard of personal cleanliness’ to be measured? In order to evaluate whether different levels of hygiene are favourable, or unfavourable, to immune system development, it is necessary to have baseline measurements of hygiene practice, for example, frequency, location, type of procedures, volume and type of any cleaning or disinfection product used. This is possible for water treatment, but presents practical difficulties for assessing home hygiene unless a cohort study can be conducted or a trial of different procedures – and even then with problems of observation, recall and social desirability bias regarding reported behaviours.

The concept of ‘unhygienic contact’, as specified in the hygiene hypothesis, is similarly difficult to define. This could mean, for example, not washing hands after defaecation or other activities involving exposure to potential pathogens – or living in a dirty environment where surfaces are contaminated with pathogens, or with close contact with animals. It may also imply absence of protective clothing, for example working without protective gloves and other barriers in a sewage-contaminated environment. Overcrowded home environments and dormitories have been shown to increase the risk of acquiring infection via the upper respiratory route (Glover 1920, Kaiser et al. 1974, Stanwell-Smith et al. 1994), but this applies to ‘hygiene’ in only a very broad sense and overcrowding does not appear in contemporary hygiene definitions. For the hygiene hypothesis to be proved, and certainly for any prospect of reversing atopy trends by adjusting hygiene, the type of unhygienic contact must be precisely defined. It is also essential to distinguish microbial exposures from hygiene exposures, since hygiene interventions offer better protection against some routes of microbial transmission, such as faecal-oral, than others. This is examined in more detail in Section 4.
2.9 Deliberate and incidental changes in microbial exposure

Many aspects of daily life have changed over the last century, particularly in industrialised, developed countries. This includes changes in microbial exposure. While the hygiene hypothesis initially focused on changes that may have occurred in the family and domestic environment, current discussions embrace a broader array of factors that may have influenced our exposure to microbes. While some changes have been the deliberate result of public health and medical interventions to combat infectious disease, such as improvements in sanitation and water quality, products and procedures to improve hygiene, and antibiotics and vaccines, others have only arisen as incidental effects of other lifestyle changes, for example the trend from rural, farm-based living towards urban dwelling, and to smaller family size. Table 2.9 reviews some of the factors that have been discussed in terms of the hygiene hypothesis. Some of these changes, such as water purification, improvements in sanitation and smaller family size, date back to the early part of the 20th century in the developed world. Others, such as the use of antibiotics and vaccines, changes in diet, urban dwelling (Box 2.9), are more recent or continuing trends.

Box 2.9. The global shift from rural to urban lifestyle

London was the first city in the world to reach a population of 1,000,000 and held the record as the most densely populated city until 1957. The migration of people towards cities and other conurbations is thus relatively recent.

An increasing proportion of the world’s population now lives in cities. During the last 30 years this has increased from 36.8% in 1970 to 45.4% in 1995. The urban population is expected to rise to 54.6% by 2015 (United Nations 1999a). This means that 3.9 billion of the projected 7 billion world population by 2015 (United Nations 1999b) will be urban dwellers.

About half of the world’s poorest people (420 million) live in urban settlements. Urban dwelling inevitably gives fewer opportunities for exposure to the countryside and also influences exercise and diet. In developed and affluent countries urban dwellers usually have improved amenities compared with a traditional rural environment, such as municipal treated water supplies and mains sewerage.
### Table 2.9: Changing factors influencing opportunities for microbial exposure over the last century

<table>
<thead>
<tr>
<th>Predisposing factors</th>
<th>Time period when likely to affect immune system</th>
<th>Influence on micro-organisms that may foster appropriate immune response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deliberate measures to avoid/reduce microbial exposure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleaner drinking and household water</td>
<td>Pregnancy, early childhood, adults</td>
<td>Mycobacteria &amp; other organisms present in untreated water.</td>
</tr>
<tr>
<td>Improved sanitation &amp; waste disposal</td>
<td>Pregnancy, early childhood, adults</td>
<td>Exposure to enteric organisms in sewage reduced.</td>
</tr>
<tr>
<td>Cleaner home environment</td>
<td>Pregnancy, early childhood, adults</td>
<td>General reduction in pathogens and less likelihood of 'intense' exposure.</td>
</tr>
<tr>
<td>Better personal hygiene</td>
<td>Pregnancy, early childhood, adults</td>
<td>General reduction in pathogens and less likelihood of 'intense' exposure.</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Early childhood and adults</td>
<td>Reduced risk of microbial exposure resulting in clinical disease but also prevents engendering of immune response to 'natural' pathogen exposure.</td>
</tr>
<tr>
<td>Treatment of infectious disease</td>
<td>Throughout life</td>
<td>E.g. successful antibiotic therapy limits intensity of infection and removes pathogens from circulation.</td>
</tr>
<tr>
<td>'Safer' food/more processed food</td>
<td>Throughout life</td>
<td>Less exposure to enterobacteria; also lower levels in processed/fast food of nutrients and vitamins essential to health immune system, e.g. Vitamin E, anti-oxidants.</td>
</tr>
<tr>
<td><strong>Incidental reductions or changes in microbial exposure, including 'lifestyle' trends and choices</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of rural exposure/increasing urban dwelling</td>
<td>Early childhood (and possibly later)</td>
<td>Reduced exposure to e.g. environmental Mycobacteria, enterobacteria.</td>
</tr>
<tr>
<td>Less outdoor exposure to sunlight/fresh air</td>
<td>Early childhood (and possibly later)</td>
<td>Less exposure to airborne microbes in natural environment.</td>
</tr>
<tr>
<td>Less exposure to animals in farms or as pets.</td>
<td>Early childhood</td>
<td>Wide range of micro-organisms.</td>
</tr>
<tr>
<td>Smaller families</td>
<td>Early childhood</td>
<td>Childhood infections, e.g. measles, Hepatitis A; birth order affects exposure to childhood infections.</td>
</tr>
<tr>
<td>Increased variety of food</td>
<td>Early childhood (and possibly later)</td>
<td>Does not involve reduced exposure, but likely to involve a different pattern of exposure (different species of microbes, etc).</td>
</tr>
</tbody>
</table>

Note (1): It is not yet agreed whether the ‘window of opportunity’ for priming the immune system is restricted to pregnancy and the first two years of life and there is some evidence of the importance of later exposures (see **Section 3**); the time periods in the table refer to generally agreed key points for exposure.
2.10 Models for the relationship between microbial exposure and atopic disease suggested by the hygiene hypothesis

2.10.1 Hypotheses and theories

Strachan’s statement in 1989 was a hypothesis, not a theory, but references to it in the literature often imply that it has the status of an established and proved theory. A hypothesis differs from a theory in several important respects and this distinction is worth exploring in the examination of the hygiene hypothesis. A hypothesis is a testable statement about a relationship, an educated guess requiring further observation of possible associations and, ideally, developed into a theory. A theory involves a set of such associations and their inter-relationships: it forms a logically derived network of associations between independent, dependent, moderating and intervening variables. The primary goal of a theory may be defined as answering the questions how, when and why, unlike the goal of description, which answers the question ‘what’ (Bacharach 1989). In other words, not only what variables are associated (as in a simple statistical test) but also the reason why they are important, the specific way they are inter-related and the conditions that govern the relationship (Campbell 1990, Greenwald and Pratkanis 1988).

2.10.2 Models based on the hygiene hypothesis

“This hypothesis depends upon two major immunological pathways”

(Settipane and Settipane, 2000)

One way of developing a hypothesis into a theory is to construct models based on its tenets, illustrating the possible relationships. The models are introduced here to provide a framework for evaluating the epidemiological evidence and later discussion in this review.

Most models described in the literature on the hygiene hypothesis start from the immunological perspective, examining how knowledge of the Th 1 and Th 2 pathways matches development of atopic disorders. The role of T regulatory cells may modify interpretations of the effect of infection exposure on the balance of Th 1 and Th 2 pathways, but infectious organisms are still a key part of the story. The idea that less exposure to infectious organisms might shift the balance towards Th 2 responses is based partly on what is known about the development of the immune system in the fetus and early infant life (Figure 2.10a; see also Section 2.3). The hygiene hypothesis offers an explanation of how the appropriate balance is achieved or disturbed. This model infers that evolution has biased fetal and early human development towards readiness for exposure to infectious agents, particularly pathogens.
likely to cause threatening disease. According to the hygiene hypothesis, if there is little or no exposure to infectious agents following birth, the bias towards Th 2 responses continues. The resulting imbalanced response may result in, for example, excessive eosinophil and IgE responses, both associated with the allergic reaction and thus with atopic disease.

Figure 2.10a: The postulated mechanism in the hygiene hypothesis

A model can also be constructed to show the specific factors cited in the hygiene hypothesis and the way they may influence a genetic predisposition to atopy towards manifestation of atopic disease, as shown in Figure 2.10b. This type of model attempts to illustrate the postulated sequence of causation/hierarchy of effects:

i. genetic predisposition to atopy (always present in about 50% of population, some ethnic variation);

ii. exposures that cause inappropriate dominance of the Th 2 pathway /imbalance of regulation of both pathways during early years of life (e.g. reduced microbial exposure due to small family/clean environment);

iii. sensitisation via exposure to specific allergens (dust, micro-organisms, feathers etc);

iv. manifestation of atopic disease.
Part of the problem in drawing models to show the interactions suggested by the hygiene hypothesis is the need to distinguish causal from triggering influences on atopy. To introduce more precision into the model, it is necessary to specify the type of microbial exposure envisaged. It is known that micro-organisms differ in their effect on the immune system and several specific organisms have been implicated as potentially beneficial, including helminths, mycobacteria, maternal *Escherichia coli* and some other enterobacteria, measles virus and *Hepatitis A virus*. Those with an apparent propensity to damage or ‘mis-shape’ the immune response include respiratory syncytial virus, coliforms (other than maternal *E. coli*), *Staphylococcus aureus* and the attenuated organisms in some vaccines. Some proponents of the hypothesis have suggested the need for serious, intense infection exposure, suggesting the need for clinical disease to stimulate the appropriate immune system development. Most proponents of the hypothesis take a more moderate line, referring, for example, to the general microbial exposure required to colonise the gut with a healthy balance of bacterial flora. Others have suggested that immunisation, particularly with live attenuated organisms, could take the place of ‘wild’ infection exposure and consequent illness. The generally tentative theories as to how much exposure or infection, and of what type, is needed to produce the required Th 1/Th 2 balance leaves a major question unanswered in the hygiene hypothesis: do we need to become ill with infection to develop appropriate immune systems? There is general agreement that it is probable that the ‘exposure’ needs to be very early in life, but not on the intensity or extent of such exposure. Cookson and Moffatt (1997), in their model to demonstrate the ‘advantage of infection’, imply that the infection needs to be at least capable of producing clinical disease.

In the Cookson and Moffat model, atopy is reciprocally related to immunity to tuberculosis (TB), as measured by delayed cutaneous hypersensitivity to a tuberculin test. If an individual has predominantly Th 2 producing T cells, the Th 2 phenotype interacts with environmental allergens to produce atopic disease. Infections are shown as potentially altering the balance between Th 1 and Th 2 phenotypes. Here, Th 1/Th 2 balance is maintained by a combination of overcrowding and poor sanitation, stimulating Th 1, while the polyclonal IgE produced in response to helminth infestation dampens the Th 2 response and thus is postulated to stimulate a healthy Th 1/Th 2 balance. Pollution is represented as aggravating existing asthma, while aero-allergens, such as the house dust mite, pollens and animal danders, provide the specific sensitisation (trigger), acting as intervening or moderating variables.
Cookson and Moffatt argue that the genetic predisposition towards asthma is widely distributed in populations and that therefore that “the probable cause of the epidemic must lie in the environment”, rather than a new allergenic factor. Their proposition is that we have evolved to deal with overcrowding and poor sanitation, and that the immune system requires the stimulation of pathogenic organisms for healthy development, a view echoed by Von Hertzen:

“The lack or scarcity of intense, systemic infections in early life has been postulated to increase susceptibility of becoming sensitised to otherwise harmless allergens in later life.”

(Von Hertzen, 2000)
One of the apparent assumptions in the model shown in Figure 2.10b is that there is a healthy balance in environmental hygiene, just as there is a healthy balance in Th 1/Th 2. Thus this interpretation of the hygiene hypothesis introduces the concept that hygiene can be measured and regulated, drawing on the systems approach, but as previously discussed, degrees of hygiene are difficult to define and this particularly applies to the hygiene elements specified in the model (overcrowding and poor sanitation). It would appear that the focus in this type of model shifts from a hypothesis largely concerned with infection exposure to the vaguer area of hygiene exposure.

2.11 Models incorporating other factors

As noted earlier, the hygiene hypothesis is only one, if currently the most popular, of the ideas about factors that may be influencing the rise in atopic disease by increasing phenotypic expression. The discussion includes the contemporary focus on changing lifestyle and deliberate choices. Lifestyle refers to the particular way of life of a person or group and includes a wide range of habits and activities: it is also often extended to cover changing features of demography, occupation, diet and hazards to which people are exposed. Several such factors have been implicated as increasing susceptibility to atopic disorders, for example, cigarette smoking, the increased pace and chaos of modern life characterised by the term ‘stress’, and the wider range of chemicals and pollutants in the environment. Table 2.11 lists some of the other factors that have been considered.
### Table 2.11: 'Non-microbial' factors suggested in the aetiology of atopic/immune dysfunction disorders

<table>
<thead>
<tr>
<th>Possible predisposing factors</th>
<th>Time period when likely to have effect</th>
<th>Influence on susceptibility to infection and/or to atopic disorders</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>Early childhood (passive exposure) &amp; adults</td>
<td>May increase opportunities for colonisation or infection via drying of mucosae in pharynx</td>
<td>?Could bias immune responses via increased infection with particular pathogens.</td>
</tr>
<tr>
<td>Changing diet</td>
<td>Pregnancy, early childhood, adults</td>
<td>Increasing susceptibility possibly also causal</td>
<td>Alteration in early priming of gut flora and maintenance of flora that ensures healthy immune response.</td>
</tr>
<tr>
<td>‘Stress’</td>
<td>Pregnancy, early childhood, adults</td>
<td>?Increased susceptibility to invasion by pathogens</td>
<td>Poorly defined concept but ? effect on immune system development/function.</td>
</tr>
<tr>
<td>Less exercise (particularly outdoor exercise)</td>
<td>Early childhood and adult life</td>
<td>Less ‘resistance’ rather than less exposure (decline in physical activity possibly causes loss of a lung-specific protective effect)</td>
<td>A generally impaired development and response system.</td>
</tr>
<tr>
<td>Less exposure to sunlight/fresh air</td>
<td>Early childhood and possibly throughout life</td>
<td>Less exposure to airborne micro-organisms, also less ‘resistance’ as with exercise effect</td>
<td>As above.</td>
</tr>
<tr>
<td>Obesity</td>
<td>As above</td>
<td>Increased susceptibility to asthma</td>
<td>As above.</td>
</tr>
<tr>
<td>Low-level ozone pollution</td>
<td>As above</td>
<td>Increased susceptibility to asthma</td>
<td>Toxic effect on immune system.</td>
</tr>
<tr>
<td>Greater exposure to synthetic chemicals pollutants</td>
<td>As above</td>
<td>Removal of micro-organisms that might favour healthy immune system biodiversity and presence of non-invasive micro-organisms that help to ‘train’ immune system</td>
<td>Failure to boost/shape Th 1 and balanced Th 1/Th 2 response development/reduced.</td>
</tr>
</tbody>
</table>
These ‘non-microbial’ factors may be sufficient on their own, or combined with each other to explain the trends and ‘hygiene’ changes may not be involved at all. Equally, some of these factors could be operating in concert with microbial factors, as outlined in Figure 2.11. The rapidly evolving understanding of gene-environment interaction points increasingly to multifactorial aetiology of disease and an extremely complex interaction between genotype, the encoding we are born with, and phenotype, the way the genes are expressed in an individual’s characteristics and in the diseases they develop throughout life. The restricted range of factors postulated in the hygiene hypothesis has helped to focus research on these factors, but the focus on ‘hygiene’ as a possible explanation for increasing levels of atopic disorders, may also have drawn attention away from other factors that may influence immune system development. It seems likely that improved understanding of genetic interaction with exposures will help to explain how different combinations of factors are important for disease risk in individuals at various points during their life.

Figure 2.11: Model of predisposing factors for atopic disease, including hygiene hypothesis and other factors
If the addition of new variables and relationships helps to address some of the inconsistencies, it would also suggest that the hygiene hypothesis should be renamed. One approach would be to encompass the broader concept of changed exposure to micro-organisms rather than just ‘hygiene’, for example as a ‘microbial hypothesis’, to take account of the wider range of factors in the current debate. If evidence points to the importance of non-microbial factors, the hypothesis would need to be approached as an even broader ‘lifestyle hypothesis’. This will be discussed further after evaluation of the epidemiological evidence on atopic disorders and infection. The strengths and weaknesses of these models will also be explored in more detail.

2.12 Assessing the hygiene hypothesis and the possible implications for home hygiene

Identifying the real reasons for the rising trends in atopic disease, and more importantly working out how the trends may be reversed, is thus a challenging problem. Even if we leave aside the possible contributions from genetic changes, and from changed exposure to sensitising and triggering factors, the following question are just some of those that need to be answered in regard to the influence of microbial exposure or other factors on the rise in atopic disease, by altering phenotypic expression at the population level:

i. What are the key essential factors?

ii. If not acting independently, how are the factors related to each other?

iii. When do the factors act and what are the time-relationships for any inter-relationship (e.g. timing of microbial and other exposures)?

iv. Why do particular effects occur after exposure to various factors? (what are the operational measures needed to examine these, and what additional research is needed?)

v. Are yet more variables needed to explain relationships and effects?

As regards microbial exposure, which is the broader focus of this review,

vi. To what extent does the evidence support the hypothesis that changed microbial exposure is a factor in the rise in atopic and related diseases in recent decades?

vii. Insofar as the evidence implicates microbial exposure, is there an ‘optimum’ exposure to micro-organisms, in terms of number and type that can be shown to be beneficial for immune system development?
viii. Does the exposure have to be “intense and systemic” (Von Hertzen 2000) and thus to involve risks of the exposure causing harmful disease; or are very low doses sufficient?

ix. To what extent might hygiene within the domestic setting be a contributory factor?

Finally, as regards hygiene in the domestic setting, and the possible implications, which are the specific aims of this review:

x. To what extent does the available evidence support the hypothesis that hygiene in the home is a factor? This will need to address, inter alia:

• To what extent is hygiene in the domestic setting likely to reduce or alter exposure to micro-organisms?

• To what extent does this happen, generally, or for specific groups of people, or at specific times etc. Is this happening in a way that is inconsistent with our evolutionary relationship with the environment?

• At what point, or degree of exposure, might hygiene become a hazard to health?

xi. What would be the implications for cross infection in the home and other parts of the community of modifying, particularly of reducing, hygiene practices?

xii. How might we modify hygiene practice to protect against infectious diseases whilst disturbing the normal functioning and balance of the immune system to the least extent?

After centuries of trying to improve hygiene and sanitation, with the assumption of beneficial effects on the public health, these questions pose difficulties for public health interventions and health promotion messages, as discussed later in this review.
3.1 The hygiene hypothesis

In this section, recent trends in the incidence and prevalence of asthma, other atopic diseases and autoimmune disease are reviewed. Most importantly, the section reviews the evidence in support, or otherwise, of the hygiene hypothesis, i.e. that the causative factor in the recent steep rise in atopy, and in some other immune system disorders, is a decline in the extent to which we are exposed to microbes, in turn postulated to be needed for the development of a balanced immune system that responds appropriately to allergens and pathogens. The immune system response and the aetiology of inappropriate allergic/autoimmune responses were outlined in Section 2. As noted earlier, although the hygiene hypothesis is currently the most popular explanation, it is only one of the mechanisms that have been proposed to explain the increased phenotypic expression of atopic disease. These other mechanisms/factors are also reviewed.

Ideally, a hypothesis that depends on comparing the health impact of different types of exposure should be tested by an intervention trial. In testing the relationship between atopy and microbial and/or allergen exposure there are practical as well as ethical difficulties. A micro-organism/allergen-free environment is impossible to achieve outside a laboratory-controlled setting. Prospective trials comparing the impact of hygienic or dirty environments would also raise difficulties, such as how to define the degree of dirtiness, how to prevent harmful effects of such environments; and the cost of the required long duration of follow-up. Given these constraints, much of the evidence for and against the hygiene hypothesis, relative to other hypotheses, derives from case-control or cohort studies, such as those comparing children from deprived environments where the assumption is made that they have greater or lesser exposure to microbes. In most studies, microbial exposure, or lack of it, is defined through proxy measures, such as the economic status of the parents or family size. This assumes that it is harder to maintain hygiene and eliminate cross contamination in larger or poorer families. Use of surrogate markers has introduced an inherent vagueness about the relationship between microbial exposure and occurrence of atopic disease. This vagueness is compounded by the use of retrospective measure of exposures in case control studies, raising issues such as inaccurate recall (recall bias) of the extent or nature of exposures. Although prospective studies, for example those following up cohorts of infants, present more reliable evidence of exposures, in most studies
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the evidence is restricted to frequency of infections (indicating exposure to pathogenic rather than direct measures of exposure to both pathogenic and other microbes).

3.2 Trends in atopic disease

3.2.1 Overview of trends

“Asthma is increasing in prevalence and intensity and is manifesting as persistent disease at earlier ages than ever before”

(Holt and Sly 2000)

Atopic disease is common in the industrialised world and has a significant impact on both quality of life and health costs. The rapid increase in allergic asthma and other atopic disorders started between 1960 and 1970 with progressive rise during the 1980s and 90s. Asthma, for example, increased by about 1% a year from around 1980 (Warner 1999). Among children 5-18 years of age, the increase has predominantly been among allergic individuals (Platts-Mills et al. 2000) and recent studies in the UK confirm that atopic, rather than non-atopic, asthma is responsible for much of the rise (Russell and Helms 1997, Upton et al. 2000). Upton et al. reported a twofold increase of adult atopic asthma (defined as asthma in association with hay fever), based on two epidemiological studies conducted 20 years apart in Scotland (1972-6 and 1996): non-atopic asthma prevalence did not change over this period. Nowak et al. (1996) found that atopy in males in West Germany had risen progressively in each decade since the 1950s. Upton et al. (2000) and Nystad et al. (1998) suggest that children without other atopic diseases are more likely to be diagnosed with asthma, than those with other atopic disorders such as hay fever. The reported rise in atopic disorders includes food allergies: 12% of respondents to the European Community Respiratory Health Survey reported either food allergy or food intolerance, with variation between the 15 countries in this survey: the lowest prevalence was in Spain (4.6%) and the highest in Australia (19.1%) (Woods et al. 2001). Differences in cultural perceptions, as well as in eating patterns, may influence self-reported food allergies: in a study of 20,000 people in the UK, 20% of adults reported allergies or intolerance to foods, but the overall prevalence was estimated to be only 1.8%, based on oral food challenges conducted in a small subset of study participants (Young et al. 1994).

3.2.2 Surveillance issues for population studies of trends

In assessing trends, it is important to note whether these relate only to new cases (incidence), or whether those still suffering from the disease are included in figures (prevalence). The case definition criteria for population studies are also important, for example whether the case counts for asthma are
based on measures of airway hyper-responsiveness (AHR), serological markers of atopy or questionnaire surveys in which case definitions are based on self-reported symptoms (Peat et al. 2001); self-reported estimates, for example of food allergies, appear to be much higher than those measured by objective tests (Sicherer 2002). Because of the reported difference in trends for atopic and non-atopic asthma, studies that do not differentiate between these types are difficult to interpret. Two recent reports question whether the rise in asthma is continuing in developed countries (Fleming et al. 2000, Ronchetti et al. 2001).

3.2.3 Within country and international differences in trends

Evaluation of trends is further complicated by rises in both atopy and asthma reported from studies in developing countries, although both incidence and prevalence remains much lower in the developing regions of the world (ISAAC 1998). Since variation is documented between areas within countries, as well as between countries, location of the study and other characteristics of the study population are essential factors for evaluation of trends. Nevertheless, Janson et al. (2001) conclude on behalf of the European Community Respiratory Health Survey that the geographical variations in prevalence are true and most likely due to environmental factors, rather than to differences in ascertainment and treatment. Von Mutius et al. (1998) reported increasing prevalence of asthma and allergic diseases among children in former East Germany. Their questionnaire-based survey showed that hay fever and atopic sensitisation both significantly increased between 1991-2 and 1995-6, raising the issue of ‘Western living’ influences on children, since previous studies had shown lower rates in East Germany compared with West Germany. A recent study in Greenland, comparing serological markers for specific IgE against a range of inhalant allergens in people aged 15-80 in 1987 and 1998, reported an overall increase from 10% to 19%: while the observed increase was largest in 15-19 year olds (from 4% to 20%), the rise in atopy found in all age groups led the authors to conclude that “the potential risk factors that caused the increase in atopy operate in adulthood as well as in childhood” (Krause et al. 2002). This finding also suggests that the ‘window of opportunity’ for development of either a well-balanced immune system, or one vulnerable to immune disorders, may be much longer than previously thought. In terms of the postulated environmental influences on atopy trends, the authors also commented that:

“Greenland has undergone major changes, including rapid urbanisation and a transition from a traditional hunting and fishing society to a modern society in which most people work in trade, administration and services.”

(Krause et al. 2002)
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A survey by ISAAC (International Study of Asthma and Allergies in Childhood) published in 1998 showed that the prevalence of asthma ranged from 2-3% in developing countries to 20-40% of the responding population of 13-14-year olds in industrialised countries, depending on the questionnaire used (written or video) (Beasley et al. 1998, ISAAC 1998). The prevalence of other atopic disorders varied over a similarly wide range, with countries showing similar, but not identical rankings. There are 20-fold to 60-fold differences in the prevalence of symptoms of asthma, allergic rhinitis and atopic eczema between ISAAC centres in different parts of the world, with high levels of eczema in some countries with low asthma rates (Africa, Scandinavia) and high prevalence of allergic rhinoconjunctivitis with low asthma levels (Warner 1999). The large global variation in asthma and allergy has prompted a second phase of ISAAC (International Study of Asthma and Allergies in Childhood): a recent report from this study (Priftanji et al. 2001) described large differences in exercise-induced bronchial reactivity between children in Albania (0.8%) and the UK (5.4%). These two countries represented extremes of the worldwide distribution of asthma (Albania low and UK high) in the first phase of ISAAC. Intriguingly, the frequency of allergic sensitisation, as measured by skin-prick tests, did not differ significantly between the two countries. The authors comment that a difference in prevalence of allergic sensitisation would have been expected, based on evidence of an east-west variation in prevalence around the Baltic Sea and within Germany (Pearce et al. 1999):

“Our findings … add to evidence that challenges commonly held concepts of a deterministic association between atopy and asthma. Elucidation of the factors that protect Albanian children (with or without allergy) from development of asthma could point to new opportunities for prevention of respiratory morbidity in countries, such as the UK, with a high prevalence of the disease.”

(Priftanji et al. 2001, p1427)

Their findings are relevant to assessment of the epidemiological studies on atopic asthma and other atopic disorders, since measures of atopy in blood or skin-prick tests have formed the mainstay of many studies in this field.

3.2.4 Trends in UK and other European countries

A comparison of two British birth cohorts (1958 and 1970) showed an increase from 3.1% to 6.4% in eczema and from 12% to 23.3% in hay fever at age 16 years. The proportion of people in the UK with atopic disease was quoted as 25% in the mid-1980s (Rubenstein and Wayne 1985) and most subsequent surveys have shown a steep rise in prevalence of atopic disease over the last 20-30 years. Asthma is now the most commonly identified chronic childhood disease in Britain, affecting one in eight children and associated
with 30,000 annual hospital admissions (National Asthma Campaign 2002). However, some epidemiologists have questioned whether the data used are robust enough (Magnus and Jaakkola 1997). A recent survey of GP consultations in the UK (Fleming et al. 2000) suggests that the incidence of new consultations for asthma is now falling, with declining reported episodes of asthma and acute bronchitis since 1993. The survey was based on the long term Weekly Returns Service of the Royal College of General Practitioners, involving 92 participating practices and approximately 680,000 people across England and Wales. The authors could not explain the decline, but it did not appear to be explained by changes in health care usage or diagnostic preference of doctors. Cross-sectional studies of children in Rome, Italy (Ronchetti et al. 2001), have also indicated that the trend in childhood asthma may be changing: a threefold rise of prevalence occurred between 1974 and 1992, but a third questionnaire-based survey in 1998 showed a stable trend for children born after 1985. No increase in asthma was found for the period 1992-98, although the results of the three surveys suggested that the association with a family history had strengthened between 1974 and 1998. Ronchetti et al. concluded “the progressive induction of asthma symptoms in genetically predisposed subjects has probably come to an end”. The possible recent decline in the incidence of asthma should be distinguished from reports on prevalence, since asthma is a chronic disease: the findings strongly suggest decline in incidence, but a high prevalence is likely to continue for several decades and longer time series are needed to evaluate both incidence and prevalence trends.

3.3 Aetiology of atopic disease and the rise in atopic disease: overview

While reduced (or changed) microbial exposure and its influence on susceptibility to atopic disease (the ‘hygiene hypothesis’) is viewed as one of the most credible explanations for the sharp rise and also the variation in prevalence of atopic diseases (Warner 1999), the hypothesis remains unproved. Many other hypotheses involving other causal factors continue to be considered and their plausibility assessed.

Since the principal focus of this particular review is on understanding the role of the microbial exposure and its impact on the balanced functioning of the immune system in the rise in atopic disease (i.e. the hygiene hypothesis) relative to other possible explanations, we start in sub-sections 3.4 and 3.5 by focussing on:

• An evaluation of the strength of the epidemiological and other evidence relating microbial exposure and its impact on the balanced functioning of
the immune system to incidence and prevalence of atopic disease. This includes:

- Direct measures of specific infectious diseases, exposure to specific pathogens or other micro-organisms, or microbial constituents such as endotoxins.

- Proxy measures of microbial exposure based on measures of family size, socio-economic group and poverty, rural versus urban environments, presumed ‘unhygienic’ environments e.g. nursery school attendance, or occupation involving exposure to high levels of pathogens/other microbes.

- Factors influencing the body’s response to infection: i.e. studies reporting an association with prophylaxis (breastfeeding/immunisation/probiotics) or antibiotic treatment of infection; also those that relate to alteration of the host response to infection, such as extremes of age and chronic disease.

For the purpose of this report these will be referred to as ‘microbial factors’.

Although the epidemiological evidence for some of the above microbial factors indicates that altered patterns of microbial exposure may be the cause of the apparent protection against atopy, for some of the factors, such as socio-economic group and poverty, there is quite likely to be an alternative explanation. In sections 3.4 and 3.5 these alternatives are also discussed.

As outlined in Section 2, the hygiene hypothesis is only one of the mechanisms that have been proposed to explain the increases in atopic and other allergic diseases. In subsections 3.6 these various alternatives are described. For the purpose of this report, they will be referred to as non-microbial factors. The various ‘non-microbial’ factors addressed in subsection 3.6 are not easily classified, consisting as they do of many quite different, and, in some instances, competing, ideas. The putative mechanisms of impact on atopy involved are also both wide-ranging and less well defined. Broadly the range of factors considered in these sections includes:

- Factors such as diet, obesity and body mass index, exercise and stress, (generally referred to as lifestyle factors) which may affect the development of a balanced immune system that responds appropriately to allergens and pathogens.

1Note: microbes such as fungi [and higher organisms such as house dust mites] are included here as ‘non-microbial’ factors because the postulated mechanism of impact on atopy is via allergen exposure rather than microbial exposure.
Factors which either directly sensitise the immune system and subsequently trigger an immune response; or those that affect such a response. The latter category includes environmental tobacco smoke and indoor or outdoor air pollution.

Subsection 3.6 also briefly reviews other issues that apply to the search for an explanation for the rise in atopy, including:

- Evidence of the direct effect of environmental exposures on the development and function of tissues, rather than on the inflammatory response.

- Migration studies and other genetic research that indicates either stronger evidence of genetically determined aberrant signalling, or possible population increases in genetic predisposition to atopy.

Whilst in constructing this report, the subdivision into ‘microbial’ and ‘non-microbial’ factors seems to us to offer the best structure for assessing the ‘hygiene hypothesis’ in context, i.e. in relation to the various other factors which may impact on atopic disease, this should not be taken to imply that these factors are necessarily mutually independent or exclusive. On the contrary, there are many interlinking or possible co-factors that make assessment of the evidence difficult and require great caution in interpretation.

It is thus possible that some of the factors classified here as ‘non-microbial’ may affect microbial exposure (and microbial susceptibility), but in a manner that is not immediately obvious. For example, genetic predisposition to atopic disorders may be associated with increased susceptibility to infectious disease, in that an inappropriate immune response may be involved in both. In addition, interest is growing in the role of genetic and environmental interaction.

There is also an increasing emphasis on determining the different aetiologies involved in asthma, compared with other forms of atopic disorder. In asthma, the abnormal development of lung tissue, including thickening of the basal epithelium and increased smooth muscle cells, may relate to genetically determined aberrant signalling, suggesting that environmental exposures may directly affect the development and function of the tissues, rather than the inflammatory response.

Other examples of factors that may affect immune responses to infection, as well as allergens, include malnutrition or an imbalanced diet, factors loosely grouped as physical or mental ‘stress’, and socio-economic hardship. Both obesity and lack of exercise have been implicated in increasing susceptibility to infection as well as to asthma. Since it is known that some pathogens
or types of infection, for example respiratory infection, can trigger asthma
attacks, there is also the problem of distinguishing causal from sensitising or
triggering effects. Finally, it is important to distinguish proxy measures of
microbial exposure, such as family size, socio-economic status and day nurs-
ery attendance, from objective measures, such as serological evidence of
infection or a recorded clinical diagnosis. In studies using proxy measures of
exposure, it is possible that these measures may be a ‘marker’ for some other
non-microbial cause of the rise in atopy or other immune system dysfunction.
Thus, while each factor is discussed in the context that seems most appropri-
ate given the studies and data available, and the hypotheses that have been
advanced, these factors should not be viewed as being unequivocally assigna-
table to one only of the above categories.

In the following sections each of these factors is reviewed, and the
possible link with atopic disease and the mechanisms by which the effect
might be mediated is assessed. Since this review focuses on the hygiene
hypothesis, the factors specified in Strachan’s 1989 statement of the hypothesis
will be considered first: family size; childhood infection; ‘unhygienic contact’
assumed from exposure to dirty environments, close proximity/overcrowd-
ing and attendance at day nurseries; and lack of household amenities such as
clean water, sanitation. Most of these factors fall into the category of ‘proxy’
exposures.

3.4 Direct and proxy measures of microbial exposure

3.4.1 Family size and structure and microbial exposure

Large families were the order of the day in previous generations, but
dropped during the 20th century in developed countries due to a number of
factors, including availability of contraception and declining infection-related
mortality. Differential occurrence of allergic disease in siblings in smaller
families led to the first statement of the hypothesis (Strachan 1989), based on
evidence of a strongly significant inverse association with the number of sib-
lings within a family and expression of atopy as hay fever or eczema.

An inverse association between atopy and family size has been found
in studies of hay fever, skin prick positivity and specific IgE (Strachan et al.
a protective effect for asthma of three or more older siblings in a question-
aire survey of parents of young children (aged 3-5 years) in New South
Wales, Australia: asthma was defined as history of a clinical diagnosis, cough
or wheeze in the previous 12 months. A case control study of clinically diag-
nosed asthma compared with healthy controls also showed a small family
size effect (Infante-Rivard et al. 2001): for cases diagnosed between 3 and 4 years of age, the odds ratio for asthma was 0.54 (95% confidence interval 0.36,0.80) for 1 sibling and 0.49 for 2 or more (95% CI 0.40,0.87).

The protective effect in large families is also associated with a stronger apparent protective effect by older siblings (Strachan 1996) and for brothers compared with sisters (Svanes et al.1999, Strachan et al. 1997a). Bodner et al. (1998), in a cross-sectional study of Aberdeen school children, found an inverse relationship between number of siblings and prevalence of atopic disease, but also conflicting findings regarding the influence of sibship on the type of atopic disease: a larger number of older siblings decreased the probability of hay fever and eczema, but the risk of asthma was reduced by the presence of younger siblings. Kurmaus et al. (2001) suggest that the sibling effect originates in utero: in a study of 981 newborn babies in the Isle of Wight, England, they found that levels of cord blood IgE reduce with increasing birth order. This could explain why younger siblings have less later atopy and that “the negative association of infections and atopic manifestation is not causal but more likely to be spurious” (Kurmaus et al. 2001).

Strachan et al. (1997b) studied the incidence of allergy in children of 11,042 pregnant women enrolled into the Avon longitudinal study of pregnancy and childhood (ALSPAC) study. While showing an inverse relationship between the number of brothers in a family and inhalant allergy, the study did not show significant trends for overall family size. A recent study (Wickens et al. 1999) compared relative changes in family size in New Zealand/England and Wales with reported levels of asthma and hay fever between 1961 and 1991. Changes in family size did not explain much of the reported increase in either atopic disease, particularly in England and Wales: whereas Wickens et al. estimated that declining family size could explain 4% of the increase in atopic disease, they found that it could account for only 1% of the rise. Seaton and Devereux (2000) investigated family size, childhood infection and subsequent atopy in a cohort of people followed up since a primary school study in 1964: membership of a large family reduced risks of hay fever and eczema, but there was no significant protection against asthma. Furthermore, the effect of a large family was not explained by infections the child had suffered: by contrast, the larger the number of infections, the greater the likelihood of later asthma, with the exception of a modest protective effect of measles. Devereux et al. (2002) report a further study of Th (T helper) cell proliferative responses in cord blood samples from a cohort of 2000 births, including comparison with birth order, maternal smoking and maternal dietary intake. The magnitude of the Th cell responses to allergens decreased with birth order and high maternal vitamin E intake, but increased...
with a family history of atopy or maternal smoking. They conclude that birth order, diet and smoking are the risk factors in the maternal environment that influence subsequent atopy.

Family size as a proxy for infection exposure is fraught with epidemiological problems, not least in the fact that there are now relatively few large families in industrialised countries. Genetic predisposition to allergy, for example an atopic father or mother, also complicates the research: Mattes et al. (1998) found a sibship relationship only in the children of atopic fathers: in the European study by Svanes et al. (1999), family size influence on prevalence of specific IgE was restricted to the children with no parental history of allergy. Commenting on these findings, Strachan (2000) postulated that genetic predisposition to allergy might “overwhelm” environmental influences on allergic sensitisation. Another possible explanation is that the inconsistencies re: sibship order relate to the complex relationships, as yet in completely understood, involved in the maternal-foetal influence on immunological development in successive pregnancies.

The epidemiological studies show that family size and sibling ranking/gender are related to the incidence of atopic disease, but do not explain clearly how this relationship operates. The key issue, from the perspective of the hygiene hypothesis, is whether large family size is associated with greater infection exposure. Family size undoubtedly influences the potential for case-to-case spread of infection by both aerosol and other routes, but it cannot be assumed that a large family inevitably causes increased spread of infection, or poor hygienic conditions: much depends on socio-economic factors such as overcrowding, sharing a bedroom, bed-sharing or awareness of how to prevent infections. The only way to resolve this is by studying documented infection exposure in large versus small families, and the subsequent incidence of atopic disease. Von Mutius (1998) has suggested that a gene-environment interaction may be involved, indicating a need to study all pregnancies, including miscarriages. Strachan (2000) concludes that whereas the inverse association between family size and allergic disease has been confirmed by several (while not all) studies, further clarification is needed on the modifying influences of household structure, such as birth order, sibling gender and parental age. The possible influence of maternal age is considered under non-microbial factors later in this section.

3.4.2 Infection and microbial exposure

As discussed in Section 2, infection is the process of an organism entering an individual, but it does not follow that an infectious disease will
result. Surveillance systems, such as statutorily notifiable infections and laboratory reporting networks, usually record only those cases serious enough to require treatment. Thus there are very few precise estimates of the frequency of most minor or asymptomatic cases of infection. It follows that past exposure to infectious organisms is also difficult to evaluate directly, because factors such as the ratio of asymptomatic to symptomatic infected individuals and the severity of the disease, will determine what is recorded. The accuracy and relevance of the data will be influenced accordingly by three factors:

- The ability to distinguish between exposure, colonisation and resulting infectious disease (and corresponding influences on case definitions);
- Under-ascertainment of the true incidence of infectious disease or subclinical infection;
- The ability to confirm clinical diagnosis of an infectious disease by microbiological tests.

3.4.2.1 Foodborne disease, faecal oral transfer and microbial exposure

Hepatitis A

The most consistent evidence for an inverse relationship between exposure to a specific pathogen and atopy is shown by Hepatitis A virus (HAV), an infection associated with large family size and low socio-economic status (Strachan 2000). Early subclinical infection with HAV is suggested by the strong positive association between seropositivity and age in European countries, such as France (Dubois et al. 1992) and the UK (Gay et al. 1994). Matricardi and colleagues (1997) found that the prevalence of high aeroallergen specific IgE was halved in Italian military students with evidence of previous HAV infection. In contrast to the evidence supporting a protective effect of large families or birth order (3.4.1), the finding was independent of age, sibship size, birth order, area of residence and parental education. Strachan (2000) comments that unmeasured socio-economic confounding could account for some of the association with HAV. In another study by Matricardi (1999), seropositivity for HAV was measured in the general population: seropositivity was associated with a 40% reduction in atopy. A 37% relative reduction in atopy in seropositive individuals was reported from a case control study in Aberdeen (Bodner et al. 2000).

A recent retrospective case control study in Italy examined the evidence of previous infection and later incidence of atopy in male air force cadets (Matricardi et al. 2000). The study included 240 cadets with atopy and 240 non-atopic controls: atopic participants had significantly lower serologi-
levels of antibodies to *Toxoplasma gondii*, *Helicobacter pylori*, as well as Hepatitis A Virus, although independent effects of particular infections were not assessed. The investigators concluded that early exposure to orofaecal and foodborne microbes protect against respiratory allergy and that:

“Hygiene and a westernised, semi-sterile diet may facilitate atopy by influencing the overall pattern of commensals and pathogens that stimulate the gut associated lymphoid tissue.”

An important question is whether HAV is directly involved in immune system ‘priming’ or regulation, or is merely a marker for poor orofaecal hygiene and exposure to other gastrointestinal organisms. Matricardi suggests the latter, emphasising the importance of general (i.e. non-specific) exposure to infections during the ‘window’ period of immune system development in early life (Matricardi 1997, Matricardi and Bonini 2000a). Matricardi and Bonini (2000b) have also postulated the role of frequent infections in a ‘traditional lifestyle’, leading to a high microbial turnover rate and more efficient stimulation of the immune system during critical stages of development. They comment:

“If we knew how high microbial turnover ‘educates’ our immune system, perhaps we could learn to mimic its action without giving up our hygienic lifestyle.”

(Matricardi and Bonini 2000, p1509)

Endotoxin exposure

Exposure to endotoxins would be expected to decline in conditions of clean water supplies and high standards of domestic hygiene. Accordingly, evidence of reduced endotoxin exposure has been cited in support of the hygiene hypothesis. In particular, variation in endotoxin exposure has been implicated as the important underlying difference between farming and urban environments (Riedler et al. 2001). Whether this is aerosolised exposure, for example, from animal dung, or increased exposure to Gram-negative organisms and their products in food, has not been determined. A possible ‘gut priming’ role for endotoxins is suggested by studies comparing infants/children from different populations and environments (Sepp et al. 1997, Bjorksten et al. 1999, Wold 1998, Bennet et al. 1991, Braun-Fahrlander et al. 2002).

Gereda et al. (2000) found that higher levels of bacterial lipopolysaccharide exposure (endotoxin) in house dust were associated with less allergen sensitisation in infants aged 9-24 months, as measured by skin prick testing. In a comparison of farming and non-farming families in Southern Germany and Switzerland, endotoxin concentrations were significantly higher in
indoor settled dust from kitchen floors and children’s mattresses in farming than in non farming families (von Mutius et al. 2000). This study did not include measures of atopy, but prompted a larger study of the atopy and endotoxin exposure in the homes of over 800 children aged six to thirteen from farming and non-farming households in Germany, Austria and Switzerland (Braun-Fahrlander et al. 2002). In this study, endotoxin levels from the children’s mattresses were compared with questionnaire data on asthma and hay fever and with tests for atopic sensitisation and peripheral blood leucocytes in blood samples: they found an inverse relationship between endotoxin in mattress dust and occurrence of hay fever, atopic asthma and atopic sensitisation. Cytokine production by leucocytes (for example IFN-\(\gamma\), IL-10, IL-12) was also inversely related to endotoxin concentrations in the bedding, an indication of down regulation of Th 2 responses [as shown diagrammatically in Figure 2.5, Section 2] in the children exposed to higher endotoxin levels.

Endotoxin exposure provides a possible explanation for the strong epidemiological evidence indicating a protective effect against asthma by farming environments, where higher endotoxin levels are generally found. However, a number of other factors need to be considered. Endotoxins have also been shown to both exacerbate existing asthma and induce new asthma in adults. For example, Rizzo et al. (1999) suggest that inhaled endotoxins may increase existing airway inflammation and therefore increase the risk of asthma in children. A birth cohort study of 499 infants with a familial predisposition to asthma or allergy showed that early exposure to indoor endotoxin increased the risk of repeated wheeze during the first year of life (Park et al. 2001). Animal studies, for example on rats (Tulic et al. 2000), have demonstrated that significant amounts of airborne allergens, including micro-organisms and endotoxins/other bacterial lipopolysaccharides, are ingested and therefore could affect the immune system via the gastrointestinal tract.

Douwes et al. (2002) sounded a note of caution about the potential role of endotoxins in immune priming. They argue that the central issue in the evidence is the distinction between primary and secondary causation, commenting also that the evidence for a protective role of endotoxins is particularly equivocal regarding asthma: Douwes et al. (2002) conclude that although a protective effect by endotoxins is plausible, there are three points of difficulty in this conclusion:

1. A protective effect has been established only for atopy (mainly specific pollen sensitisation) and hay fever;
2. The prevalence of asthma appears to be only marginally reduced in children with frequent contact with animals;

3. Very high levels of exposure to endotoxin occur during livestock farming, to the extent that this is a recognised occupational health hazard in farmers (Schenker et al. 1998). It is therefore not surprising that endotoxins can be readily found in the dust from mattresses and other sources in farming households: it should not be necessarily concluded that there is a causal relationship between this ubiquitous farm exposure and measures of atopy or other health indices in children.

Continuing research may clarify both the role of endotoxins and the range of possible protective factors in a farming environment (discussed further in 3.4.3).

Intestinal parasites

Intestinal infestation with helminths has been suggested to protect against atopy (Cookson and Moffat 1997) by promoting a Th1 bias. Yemanberhan et al. (1997) found that self-reported wheeze was lower in rural subsistence areas of Ethiopia than in the urban population: a follow up nested case control study in the same population suggested that high degrees of parasite infection, particularly hookworm, prevented asthma symptoms in atopic individuals (Scrivener et al. 2001). It was also suggested that this higher prevalence of wheeze in urban areas could be attributed to increased house dust mite (HDM) exposure. Although sensitisation to HDM was common in the rural areas, it was not associated with wheeze. Ultimately however, neither reduced parasite infestation nor allergen exposure could explain the three fold excess of wheeze in the urban compared with rural areas, after adjusting for these factors.

Campylobacter and Helicobacter pylori infection

*Campylobacter* and *Helicobacter pylori* occur naturally in the environment and the prevalence of infections associated with these organisms is known to be associated with factors also considered as protective factors for atopy, i.e. untreated drinking water (Klein et al. 1991), exposure to animals, poor levels of environmental sanitation and poverty (Vanagunas 1998). Therefore, their possible role in protection against atopy has been considered.

Rates of confirmed Helicobacter infection appear to be much higher in developing countries. Also, serological evidence of exposure to *Helicobacter pylori* has been reported to be higher in Italian air force recruits, associated with an inverse relationship with later atopic disorder (Matricardi et al. 2000).
Evidence that these organisms can interact directly with the immune system comes from the observation that *H. pylori* may also be associated with the sudden infant death syndrome (SIDS): evidence of the infection was found in 25 of 34 cases of such infant death, compared with only one of eight controls, a highly significant result, although involving small numbers of cases and a very small number of controls (Kerr *et al.* 2000) (Box 3.4a). On balance, apart from the seroprevalence study by Matricardi *et al.* (2000), there is little evidence to suggest a link between increased exposure to *Helicobacter pylori* and *Campylobacter* and reduced susceptibility to atopy.

**Box 3.4a: Sudden infant syndrome and *Helicobacter pylori***

The usual timing of death in SIDS, between 2 and 4 months, suggests an infectious aetiology, as this is the age when infants are most vulnerable to infection. A possible association of *H. pylori* with SIDS is also supported by the profusion of immunological mediators produced during *H. pylori* infection and consequent marked inflammatory response involving IL-1, IL-3, IL-4, IL-6, IL-8, tumour necrosis factor α and interferon γ (Kerr *et al.* 2000). The natural route of transmission for this micro-organism is believed to be via gastric juice as a consequence of childhood vomiting (Axon, 1995). It is also spread via saliva and on feeding bottles. These routes of transmission are more likely in conditions of overcrowding and poor sanitation.

### 3.4.2.1 Respiratory and other non-gastrointestinal diseases and microbial exposure

Respiratory infections, and infections spread by the respiratory route, have been studied in relation to the hygiene hypothesis. Those of particular interest include measles, mycobacteria (TB and non-invasive strains), respiratory syncytial virus; and the common viral respiratory infections for which only a clinical diagnosis is usually available.

**Measles**

An inverse relationship between wild measles infection and atopy has been cited as evidence for the hygiene hypothesis, with particular emphasis on results of the longitudinal study of children in Guinea-Bissau following an epidemic of wild measles (Shaheen *et al.* 1996). Sensitisation to seven common allergens in children who had had measles was approximately half that recorded for others who had received measles vaccination after the epidemic. An important feature of this ‘natural experiment’ was the high child fatality (25%). Thus, one possible explanation of the subsequent study result is that a reduced propensity to atopy could have had a selective survival value in the population. Measles undoubtedly influences the immune system, inducing an
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immune paresis (such as lymphopaenia, decrease in white blood cells) for at least two months after infection in young children (Okada et al. 2000). Measles infection is also associated with increased levels of IL-4 (interleukin-4) which could theoretically promote allergic reactions by increased switching to the IgE isotype (Bager et al. 2002). In the Guinea-Bissau study, the average age of wild measles infection was 3 years, later than the currently postulated ‘window’ for the immune system effects (see 2.3.2). Exposure to wild measles infection can be fatal, especially in developing countries and therefore one explanation for such findings could be that the children who survived were also those more likely to develop a protective (type 1) immune response to avert later atopy.

No evidence of a protective effect from wild measles infection was found in the 1970 British birth cohort (Golding and Peters 1986), or in a Finnish survey of children exposed to measles in the early 1980s (Paunio et al. 2000). Bodner et al. (1998) found conflicting results in a study on the influence of early childhood infection: measles appeared to protect against asthma, while varicella, mumps and rubella were associated with increased risk of atopic disease. Atopic disease also correlated with the number of recorded episodes of infection before the age of 3 years. In a study of 6000 children born in 1970, Lewis and Britton (1998) reported that both measles infection and measles vaccination appeared to reduce the risk of atopy, although the effects were strongly confounded by birth order. A recent cohort study suggested that measles exposure is more likely to be harmful than protective in terms of the risk of atopy (Bager et al. 2002) (Box 3.4b).

**Box 3.4b: Cohort study on effect of measles and other childhood infections**

Bager et al. (2002) studied 889 pregnant women aged 17-44 with documented histories of exposure to measles, rubella, varicella or mumps in childhood. 29% of this group were classified as having atopy based on serum IgE against 11 common inhalant allergens. A history of measles infection in the first year of life was associated with a 3.4 fold increase in the odds ratio of atopy (95% CI 1.47-7.68), compared with women who had not had measles. Measles before the age of seven years was also associated with an increased risk of atopy. Infection with rubella, varicella and mumps were not separately associated with later risk of atopy but the cumulative number of infections in the first two years of life increased the risk of atopy.
Mycobacteria

“The possibility that microbial products, as educators of immunity, might be thoughtfully developed to limit atopic disorder in many people is intriguing. What a sweet irony it would be if the products of M. tuberculosis, or its cousins, could be used to control asthma.”

(Hopkin 2000)

Current interest in the relationship between atopy and exposure to non-gastrointestinal organisms includes the possible protective role of some species of mycobacteria. Mycobacteria are widely distributed in the environment, including commensal, opportunist and pathogenic strains, the latter causing tuberculosis in humans and animals. These microorganisms are of particular interest to immunologists because of their ability to elicit strong protective Th 1 immune responses: interaction of mycobacterial lipoproteins with macrophage bound Toll-like receptors (TLRs), leading to synthesis of IL-12, Th 1 switching and secretion of interferon gamma (IFN-γ) and tumour necrosis factor (TNF-α) (Brightbill et al. 1999). These cytokines have been shown to repress Th 2 immune mechanisms: this has led to theory that exposure to mycobacteria could also repress atopy (Hopkin 2000).

A study of 867 children in Japan (Shirakawa et al. 1997) suggested a protective effect of natural exposure to the mycobacteria that cause TB, Mycobacterium tuberculosis. The children were aged 6 or 12 years at the time of routine tuberculin tests prior to BCG vaccination: positive tuberculin responses in the 12-year olds were not associated with any of the measures of atopy. The authors interpreted this as evidence of a beneficial effect of previous exposure to environmental mycobacteria or to tuberculosis. Others suggested that they could alternatively be explained by the known predominance to Th 1 responses associated with tuberculin reactivity and to Th 2 responses in atopy. Thus, the inverse relationship could be due to the Th 1/Th 2 host balance rather than to any causal effect between infection and atopy (Silverman 1997). Strachan (2000) observed that host differences could not be the explanation in the Japanese study, as a family history of allergic disease was equally common in the comparison groups. Nevertheless, genetic determinants of response to tuberculin seem to be important: reduced tuberculin reactivity may be predictive of subsequent development of atopic disease, rather than exposure to tuberculosis being protective. Also, while the results reported by Shirakawa et al. (1997) support the idea that M. tuberculosis promotes development of protective Th 1 mediated immunity, the benefits may be restricted to healthy subjects who do not develop tuberculous disease. Patients with TB often have impaired Th 1 responses which make them more vulnerable to other infections (Hopkin 2000).
Other retrospective analyses of BCG immunisation programmes in relation to atopy have produced conflicting results: Alm et al. (1997) found comparable rates of atopic disorder between children who had been immunised with BCG at the age of 6 months and a control group who had not. Omenaas et al. (2000) also found no evidence of a relationship between tuberculin reactivity and atopy in BCG vaccinated adults: these authors suggest that the relationship may hold only when mycobacterial exposure occurs early in life. Aaby et al. (1998) found lower levels of atopy, as indicated by allergen skin prick testing, in infants in Guinea-Bissau who had received BCG in the first week after birth.

As for natural exposure to pathogenic mycobacteria, a trend to less atopy in countries with high TB prevalence would be expected if such exposure protects against atopy: von Mutius et al. (2000) conclude that this is the case, from the results of their international ecological study in which tuberculosis notification rates were matched to the prevalence of atopic symptoms. The study included nearly a quarter of a million children within the ISAAC study frame: an increase in notifications of 25/100,000 population was associated with a decrease in ever having wheeze of 4.7%. Where TB rates are high it can be assumed that there is widespread natural exposure to *M. tuberculosis*, supporting the theory that the healthy individuals in those communities have enhanced Th 1 responses and consequently less atopy.

Whether non-tuberculous mycobacteria in soil and water could also have the protective effect postulated for pathogenic strains is still unknown, but the idea that exposure via the respiratory tract could be beneficial to the immune system led Holt (1994) to propose a vaccine strategy to replace more dangerous wild exposures of respiratory organisms. Rook and Stanford (1998) postulated that mycobacteria, in particular, have a key role in priming the immune system and that exposure to mainly innocuous mycobacteria in soil and water has been greatly reduced by water treatment and sanitation in Western urban environments. The use of non-invasive strains of mycobacteria in vaccines to treat or prevent immune dysfunction is discussed in 3.5.1.

**Respiratory syncytial and other respiratory viruses**

Infection with *Respiratory syncytial virus* (RSV), rose during the 1980s, but is mostly implicated in triggering asthma rather than protecting against it (Noma et al. 1996, Stein et al. 1999, Pershagen 2000). RSV infection causes bronchiolitis, mainly in children, and children with a tendency to atopy may be more susceptible to its effects, particularly where there is a family history of atopy (Trefny et al. 2000). RSV-bronchiolitis causes severe morbidity and long-term pulmonary abnormalities in some cases.
Mothers are less at risk from RSV infection, with maternal infection rarely presenting as severe clinical illness, but adverse effects have been reported, for example an association with spontaneous abortion (miscarriage) (Irvine et al. 1990), or maternal death in association with concomitant staphylococcal infection (Carfrae et al. 1982).

Recent investigation of the immunological mechanisms involved in RSV infection provides clues to a possible beneficial effect when RSV results in mild or subclinical infection (Box 3.4c). Seroprevalence for RSV is high in young children: for example, a study in Brazil reported antibodies in 90% of children by 3 years of age (Cox et al. 1998), including a large proportion with no clinical or only mild illness.

**Box 3.4c: Immunological mechanisms and RSV infection**

Two surface proteins of the Respiratory Syncytial Virus, F (fusion) and G (attachment), have differing effects: F tends to act on the Th 1 pathway while G acts on the Th 2 pathway. A study indicated a possible mechanism for the action of the F-protein, namely through interaction with CD14, the Toll-like receptor, which mediates the action of LPS on innate immunity (Kurt-Jones et al. 2000).

Babies born to mothers infected with RSV have protection against this infection and also higher levels of interferon γ, which has a key role in Th 1 responses. They may also have less predisposition to atopic disease as reflected in incidence later in childhood (Lewis 1998).

Investigators are now interested in the possibility that RSV may be an important primer of the immune system during the second half of pregnancy or in early infancy (Lewis 1998). The evidence for a protective effect is supported by theories based on immunological mechanisms (Lewis 1998) rather than epidemiological studies: Carlsen et al. (1987) reported no significant differences in family history of atopy in a cohort of 51 infants requiring hospital treatment for bronchiolitis, of which 31 cases were due to RSV. In comparison with 24 controls followed for the same time period, the infants with bronchiolitis came from more crowded homes. While it is impossible to conclude on present evidence that asymptomatic (non-clinical) RSV infection protects against asthma, the severe morbidity often associated with this infection argues against a beneficial role.

Although early evidence suggested that respiratory infections could exacerbate existing asthma, in 1995, Martinez concluded that there was no evidence that common viral respiratory infections during early life had any
impact on subsequent development of asthma. Recent studies have however focused on the possibility that viral respiratory infections could have a protective or immune system ‘priming’ role (Holt et al. 1997, Berstad and Brandtzaeg 2000, Illi et al. 2001, Riedler et al. 2001).

3.4.2.III Malaria and microbial exposure

A recent study in Gabon found that children with a positive atopic skin reaction had a history of less infections and lower incidence of malaria than children who tested negative (Lell et al. 2001). Children with a high exposure to *P. falciparum* malaria also appeared to have a lower risk of a positive skin test for atopy. Lell et al. postulated that since immuno-suppression is a feature of malaria attacks, the protective effect against allergic disease may be due to a counterbalancing effect on pro-inflammatory immune reactions. Their results could help to explain the lower prevalence of most forms of atopy in developing countries, but the influence of a survival effect (children who survive malaria may have less genetic susceptibility both to infection and to atopy) needs to be excluded before drawing conclusions about the association between parasitic infection and atopy.

3.4.2.IV Non-specific or combined effects of respiratory infections and the total burden of infection

Matricardi (1997) favours the concept that the overall load of microorganisms, including both foodborne and airborne organisms, contains the key to immune system priming and reduced susceptibility to atopy. This concept is also supported by Martinez (1999, 2001). High microbial turnover has been suggested as a solution to inconsistencies in the hygiene hypothesis (Matricardi and Bonini 2000a), repeated microbial exposure providing the beneficial stimulation of the immature immune system, shaping it towards the Th 1 phenotype. A recent cohort study (Illi et al. 2001) produced evidence of a protective relationship: the total burden of infections was inversely related to subsequent development of asthma, with specific associations for repeated runny nose and herpes-like infections, but not bacterial, fungal or gastrointestinal infections. Matricardi and Bonini (2000a) found that the protective effect was confined to infections other than those affecting the lower respiratory tract and no effect was found for other types of infections.

In contrast, an increased rather than reduced risk of atopy following a combination of early infections has been reported by Bodner et al. (1998) and by Bager et al. (2002). In the study by Bodner et al. the increased risk followed rubella, varicella and mumps in the first 3 years of life; while Bager et al. reported an increased risk following measles, rubella, varicella and mumps in first 2 years.
The possibility of a synergistic effect of respiratory viruses and allergens, such as house dust mite and pollen, in triggering atopic disease has also been proposed. Recently, Green et al. (2002) found evidence of a combined effect of allergens and respiratory viruses in triggering acute asthma attacks requiring hospital admission. If this applies to secondary causation (exacerbation of existing asthma), then it is a possible explanation for primary causation. The evidence to date has not resolved whether a combination of respiratory viruses is protective, whether the composition of specific viruses in any such combination is important, or whether co-factors such as allergens are involved.

3.4.3 ‘Unhygienic contact’: microbial exposures estimated by proxy in studies on rural environments, farms, day nurseries and households

3.4.3.1 Farm and other rural exposure

“It is clear that farming per se is not always linked to a reduced risk of allergy… Nevertheless, the apparent protective effect of growing up on a farm may well provide clues which help to focus on the likely causal factors of allergic disease.”

(Lewis 2000)

The link between farm environments and reduced susceptibility to atopy is a particularly intriguing theme in the epidemiological evidence. Hay fever gained its name from a belief that the symptoms were caused by grass pollen and other rural exposures (Emanuel 1988), but even early investigators in the 19th century, such as Dr Charles Blackley, recognised that this was a possible misnomer: the disease appeared to be more common in the middle class children of urban families, than in the pollen-rich environment of the country. More recent demographic studies have demonstrated that the specific protective environment for hay fever is the farm, with only marginal differences in comparisons of general rural and urban environments (Wuthrich et al. 1986). This protective effect is supported by several studies reporting a reduced incidence of allergic rhinitis (hay fever) in the children of farmers, as compared with other rural dwellers (Braun-Fahrlander et al. 1999, Gassner-Bachman and Wuthrich 2000, von Ehrenstein et al. 2000, Riedler et al. 2001).

The presence of a pig in the kitchen appeared to protect against atopy in a longitudinal study of children in Guinea-Bissau (Shaheen et al. 1996). Pets have also been reported to be associated with a lower risk of allergy (Svanes et al. 1999), but avoidance of pets by allergic families may partly explain this finding (Strachan 2000). Contact with animals is a feature of farming life: von Ehrenstein et al. (2000) and Riedler et al. (2001) reported greater animal contact in non-allergic children. The cross-sectional survey undertaken by Riedler et al.
(2001) including rural areas of Austria, Germany and Switzerland, comprising mainly small family-run farms. Exposure to stables before the age of 1 year was associated with a statistically significant reduction in subsequent atopic and non-atopic asthma, compared with those whose parents did not report such exposure. Consumption of farm milk (presumably raw/unpasteurised) was also protective and children from farms were less likely to be sensitised against grass pollen although they had far higher levels of sensitisation to farm allergens, including cow epithelium and storage mites, than non-farming children. Maziak (2002) commented that, if horse stables and farm milk are as protective as is claimed by Riedler et al., it is surprising that the frequency of eczema did not differ among groups with different farming exposures.

The evidence thus favours endotoxin exposure as the predominant ‘unhygienic’ contact associated with farm environments. In the survey by Riedler et al. (2001), reporting reduction in atopic and non-atopic asthma associated with exposure to stables before the age of 1 year, the authors cited identification of higher concentrations of endotoxin (lipopolysaccharide) in dust from kitchen floors and children’s mattresses in farming families, compared with non-farming rural residents. A recent European study showed that children’s mattresses on farms contained significantly higher levels of endotoxin than those of children with no farm animal contact (Braun-Fahrlander et al. 2002): endotoxin concentrations were highest in stables but were also high in dust from kitchen floors. While farm children had less hay fever, atopic eczema and atopic sensitisation than control children, non-atopic wheeze was not significantly associated with endotoxin levels.

In the 16-year serological and questionnaire survey reported by Gassner-Bachman and Wuthrich (2000), a statistically significant increase in the incidence and severity of hay fever and asthma, and of seroprevalence of sensitisation, was found in rural children with no direct contact with agriculture: by contrast, farming children had lower clinical atopic disease and lower levels of seroprevalence to a wide range of allergens, including those to which they had high exposure. A quantifiable inverse dose-response relationship was identified, with children who had intermittent farm contact showing intermediate results. Gassner-Bachman and Wuthrich concluded that this apparent immunological adaptation to anthropozoonotic allergens was due to greater and continual stimulation of the Th 1 immune response in farming environments. Similarly, Leynaert et al. (2001) suggest that “environmental factors encountered in childhood” is the most feasible explanation for their finding that adults who had lived on a farm as a child had lower levels of cat sensitisation and a lower risk of nasal symptoms in the presence of pollen, compared with those from other childhood environments. This association
held after statistical adjustment for possible confounders such as pet exposure, number of siblings and severe respiratory illness in childhood. Their study included data from 6,251 adults aged 20-44 who participated in the European Community Respiratory Health Survey. The association between farm exposure and allergy was mostly seen in the younger age group: the authors suggest that this could be due to a decline of the protective effect over time. However, an alternative possibility is that a generational effect is operating, with another common exposure applying to the younger cohort. Another point to note is that most of the farm studies rely on serum measures of atopy or allergen skin prick tests: the recent results from ISAAC (Priftanji et al. 2002, discussed in Section 3.2.3) suggest that this may be an unreliable way of testing for differences in atopy and asthma between population groups. Leynaert et al. (2001) found no consistent association between living on a farm in childhood and the risk of asthma or wheeze: a similar proportion had ‘atopic asthma’ in the farm and non-farm groups.

In examining such evidence, the possible protective role of other protective exposures in these environments must be considered. Farming families are likely to differ from other rural dwellers in several respects, including:

- diet, both in terms of freshness of food and variety;
- greater exposure to infection via animals at an early age;
- greater exposure to chemical pesticides and herbicides;
- greater opportunities for outdoor physical activity from an early age, particularly in small farms;
- socio-economic differences with other rural families and also with urban dwellers, although these include both greater and lesser affluence depending on the region and other factors such as size of farm and the local/national agricultural economy;
- differences in water supply (for example a higher frequency of private wells and untreated water) and in sanitation;
- greater genetic homogeneity, particularly in traditional farming areas.

It therefore follows that the protective effect seen in children of farming families may not be linked to infection exposure in the sense implied in the hygiene hypothesis, that is, that increased microbial exposure is protective. Farm children may be exposed to a different range of microbes: this range could be the important factor, rather than increased levels of specific microbes. It is also possible that the reduced risk of atopy seen in farming
compared with non-farming families could be due to lifestyle differences that are quite independent of hygiene and microbial exposure.

Strachan (2000) and others suggest that the key issue may be the effects of a farming environment on early programming of intestinal microflora. The differences in intestinal bacterial flora, between farm and urban children, appear to be one of the most fruitful areas for research into the influences on atopic disease, including the possible influence of a more ‘organic’ diet, for example, a diet containing a high proportion of probiotic foods. Lewis (2000), in a detailed review of studies on farming and animal exposure, postulated that if unpasteurised milk is consumed more often by farmers and their families, then diet may contribute to an increased level of pathogen exposure. However, Lewis also noted that there was inconsistent evidence for farming families having less asthma and asthma-like symptoms, and that there was no evidence for lower levels of eczema.

Other evidence, not specifically connected to farming families also suggests the importance of intestinal flora as a factor in atopy. Bjorksten et al. (1999) found that the intestinal flora of allergic children differed from those with no allergies: aerobic bacteria, coliforms and Staphylococcus aureus were more common in the flora of allergic children. The non-allergic children had a greater prevalence of Lactobacilli and Bifidobacteria in their gut flora. Sepp et al. (1997) studied the intestinal microflora of healthy infants from families in Sweden and Estonia, countries with broadly similar genetic populations but marked variation in the prevalence of atopy (high in Sweden and low in Estonia). Lactobacilli and Eubacteria were more common in the Estonian infants. The authors concluded that reduced microbial exposure in Sweden caused the differences, based on assumptions about levels of hygiene and overcrowding. It could be argued that this type of study relates also to differences between societies that have moved away from the small scale farming lifestyle and those still characterised by this lifestyle, with associated exposure factors that may explain differences in atopy. Bennett et al. (1991) studied Ethiopian and Swedish neonates, finding that differences at birth were lost by 2 weeks of age, except for a persistence of Lactobacilli in Ethiopian infants. Adlerberth et al. (1991) found a more rapid colonisation rate and greater heterogeneity of intestinal microflora in Pakistani infants, in comparison with those in Sweden.

The strongest evidence of reduced atopy in farming families is for hay fever, for which the explanation may be reduced risk of pollen sensitisation, a finding similar to that of the Ethiopian by Yemaneberhan, Lewis and colleagues in 1997.
Regarding the developing world, where allergic diseases are just emerging, Lewis suggested that farm studies may provide the most accessible source of answers about the relative influence of diet, pollen and other postulated risk factors on atopy, since they provide a model in which differences in exposure are comparatively limited (Lewis 2000, p156).

3.4.3.1 Close contact: bed sharing and day nurseries

Children in large families are more likely to share bedrooms. In the European Community Respiratory Health Survey (Svanes et al. 1999), sharing a bedroom as a child had a protective effect on the subsequent risk of atopic disease. As Strachan (2000) observes, this accords with the hygiene hypothesis, in providing more opportunity for exposure to infection.

A higher frequency of common colds was reported for children in large day care facilities in a prospective study in the USA (Tucson Children's Respiratory Study) (Ball et al. 2000): these children had more frequent colds at year two and less colds at the ages 6-11, than those cared for at home. There was no evident protective effect against colds by the time they had reached 13 years of age, and no protective effect was observed for children in small day care facilities (1-5 unrelated children). The authors observed that “toddlers and preschool aged children are ideal transmitters of infectious agents”, a point well established in the field of infectious disease epidemiology.

Results of studies of protection against asthma/atopy for children with a history of early exposure to large day care facilities are inconsistent, with some studies reporting a reduced risk of atopy (von Mutius et al. 1992, Krämer et al. 1999) and others showing no protective effect (Backman et al. 1984, Infante-Rivard et al. 2001). Using data from the Tucson Children’s Respiratory Study, Ball et al. (2000) reported that asthma was less frequent in children with 1 or more siblings at home or who had attended day care during the first 6 months of life. However, children with more exposure to other children at home or in day care were more likely to have frequent wheezing at the age of two, although they had a decreased risk of this from the age of 6 to 13 years. In studies on playgroups in East and West Germany, children exposed to more deprived, more overcrowded and possibly less sanitary environments in East Germany had lower levels of atopic disease than their West German counterparts (von Mutius et al. 1992). Exposure in playgroups occurred after the age of 6 months, suggesting a longer ‘window’ for immune system development, although comparisons may be biased by different perinatal exposures. These findings need to be clarified by further study, for example, comparisons between nursery attenders and non-attenders, with inclusion of family size and size of nursery, as well as hygiene.
measures, in the analysis. A small study by Krämer et al. (1999) also found that early enrolment in a day nursery appeared to be protective.

Backman et al. (1984) found no protective effect of day care for atopy in a larger study in Finland. Increased risk of transient childhood asthma associated with day care attendance was reported for Canadian children (Infante-Rivard et al. 2001), although factors such as short duration of breast feeding contributed to the raised risk; and the risk was decreased for persistent cases (six year follow up) with a history of day care attendance.

3.4.3. III Occupational microbial exposure

Several health related occupations, such as medicine and nursing, involve exposure to pathogens, although they also involve work in environments with high standards of hygiene, at least in theory. Two types of occupation are particularly relevant, namely farm workers and waste water/sewage workers.

Farm workers

Farmers have a higher risk of occupational allergic disease and there is no epidemiological evidence that working on a farm per se is associated with a reduced risk of adult atopy. All the available evidence about the protective effects of exposure to farm environments (as discussed in 3.4.3.I) relates to early childhood exposure.

Evidence of the possible beneficial effects of microbial exposure in early childhood must be balanced by the documented evidence of occupational health damage associated with endotoxins and other organic dust in farm environments (DoPico 1986, Rylander 1986). The harmful effect on lung function of long-term exposure to endotoxins, as measured in dust, has been reported for pig farmers (Vogelzang et al. 1998), with a mean decline in lung function tests over a 3-year period of observation. Since endotoxin exposure appears to be harmful later in life for farm work, such evidence raises questions about timing and dose-response effects of the postulated beneficial level of exposure.

Waste water workers

Although there is some evidence that waste water workers have more exposure to endotoxins and pathogenic microbes, there is no epidemiological evidence that this increased microbial exposure is associated with a protective effect against atopic disease. The available data indicate that wastewater treatment workers have a relatively low risk of acquiring infectious disease,
despite frequent exposure to sewage, rich in micro-organisms (Mulloy 2001, Rylander 1999, Khuder et al. 1998), although garbage workers experience a higher prevalence of chest tightness and toxic alveolitis than the general population, associated with exposure to allergenic organic dusts (Sigsgaard 1999). An increased prevalence of respiratory symptoms and declining lung function has also been reported in association with the ‘organic dust’ exposures experienced by waste management/garbage recycling workers (Sigsgaard 1999), although the results for respiratory symptoms are less consistent finding than those for gastrointestinal symptoms (Khuder et al. 1998), or for higher levels of headache and fatigue (Mulloy 2001). Sewage treatment workers have a high occupational exposure to endotoxins, possibly explaining observations of inflammation both in airways and the intestinal tract (Rylander 1999).

Exposures such as boiled sewage sludge are not found in typical rural, or urban, environments and such occupational hazards in later life do not exclude the possibility of benefit from low exposure in early life. Protective clothing and hygiene precautions have reduced the occupational incidence of disease from most sewage-related organisms (such as Leptospira icterohaemorrhagiae, Hepatitis A virus and Clostridium tetani).

3.4.3.IV Household hygiene and microbial exposure

A popular view, in relation to the hygiene hypothesis, is that the microbial exposure required for the development of a balanced immune system (i.e., one that responds appropriately to allergens and pathogens) has been reduced as a result of the more ‘rigorous’ hygiene practices which we have deliberately introduced and then developed, either to protect ourselves against infectious disease or to create a more aesthetically pleasing environment in which to live. The limited evidence for a link between hygiene as a proxy measure of microbial exposure and the risk of atopy is discussed in this section.

Household amenities and water supply, and microbial exposure

No studies were identified for this review concerning an association between household amenities (such as bathrooms, showers, indoor toilets, treated water supplies and mains sewerage) and the risk of acquiring atopic disorder, although this has been investigated in regard to the risk of developing acute appendicitis and inflammatory bowel disease. Studies by Coggon et al. (1991) and Barker et al. (1988a) implicated household amenities and water quality in the aetiology of appendicitis in the predominantly rural environment of Anglesey, North Wales. Barker et al. (1988b) had found an association, independent of social class, between bathrooms and higher occurrence of appen-
dicectomy, and postulated that acute appendicitis is associated with Western hygiene, particularly as appendicitis is rare in developing countries. A postal questionnaire study in Anglesey showed that people born into households with piped water, hot water systems and bathrooms had a generally reduced risk of needing appendix removal (Coggon et al. 1991). An association with later appendicectomy was confirmed only for people who moved to homes lacking these amenities. The authors concluded that, after infancy, household amenities protect against appendicitis, although “reduction in domestic crowding” was still suggested as the explanation for the rise in appendicitis in Anglesey after the Second World War rather than access to household amenities. Studies on the role of household amenities in inflammatory bowel disease are considered in 3.7.3.

Hygiene practices in relation to microbial exposure

While the concept of ‘unhygienic’ contact (or lack of it) has proved difficult either to define, study, or quantify, in a controlled investigation, the same could be said for what constitutes excessive hygiene in the home, or how this might rate as a proxy measure of microbial exposure:

“Little is known about hygienic practices in the home or norms of cleanliness in the United Kingdom today and virtually no information is available on the social, demographic, or lifestyle factors influencing hygiene practices.”

(Sherriff et al. 2002a)

In two recent studies, Sherriff and her colleagues (2002a/b) have attempted to address this lack by devising hygiene scores for reported frequency of personal hygiene practices, and examining statistical associations with reported levels of wheezing and atopic eczema. Hygiene scores for children aged 15 months were derived from data in the Avon Longitudinal Study of Parents and Children (ALSPAC), a prospective study of 14541 pregnancies and the surviving children. In self completed questionnaires for 10970 of these children, parents were asked how often in a normal day they wiped the child’s face and hands, whether hands were wiped before meals and how often the child was given a bath or shower. The cumulative infant hygiene score derived from these responses was examined statistically in relation to socio-economic and perinatal factors, as well as to reported wheezing and eczema. These data suggested a ‘cleanliness norm’ for the majority of the children comprising washing face and hands 3-4 times a day, hands cleaned before meals and a bath or shower once a day. The hygiene scores ranged from 2 (least hygienic) to 14 (most hygienic), although only 43 (0.4%) of the children had the highest hygienic score. A hygiene score of ≥10 was inde-
pendently associated with maternal smoking during pregnancy, low maternal educational achievement, living in local authority housing and increased use of chemical household products (the latter based on a score derived from reported use of disinfectant, bleach and aerosols for cleaning in the home). In the univariate (single factor) analysis, high hygiene scores were also associated with higher maternal parity (2 or more children), short or no duration of breastfeeding, overcrowded accommodation and little contact with dogs or other furry pets. In their follow up study on the relationship of the cleanliness data to atopy, Sherriff et al. (2002b) reported that high hygiene scores for children aged 15 months were independently associated with increased risk of wheezing and eczema, when this group of children reached 30 and 42 months of age. Study mothers with a history of eczema were less likely to have children with a high hygiene score than non-atopic mothers, possibly indicating that knowledge of factors that exacerbate eczema influenced frequency of child bathing: no such association was found for mothers with a history of asthma. Measures of these types of atopy were based on questionnaire responses asking about symptoms.

While the parental responders in this well established ALSPAC cohort of children would be accustomed to answering questions, the association of high hygiene scores with social disadvantage (low educational attainment and overcrowded living conditions) and maternal smoking seems somewhat surprising and raises the possibility of an association between social disadvantage and a tendency to exaggerate measures of personal cleanliness, particularly where this relates to childcare. Alternatively, it is possible that these social factors strongly influence hygiene practice: for example, in younger, poorer or less educated mothers. Hygiene practice, too, may be inappropriate, such as applied too zealously or use of cleaning products not linked to understanding of risk factors. “Some studies (e.g. Poyser et al. 2002) report that asthma prevalence and severity are both associated with social disadvantage. The findings from the ALSPAC study may thus reflect a non-causal association between asthma and hygiene practices.”

Use of household cleaning products

A comparison of soap and detergent consumption in different countries with corresponding data on prevalence of atopic disease (Pickup 2003) showed no evidence of a relationship. For example, plots of per capita consumption of soap, detergents and cleaning products in 1994 for 12 European countries against reported prevalence of asthma, hay fever and eczema (ISAAC, 1998) showed no correlation. Nor was any correlation apparent between individual diseases and individual product types such as fabric
washing detergents, dishwashing detergents, toilet soaps and hard surface cleaners. Consumption of household bleach, a highly effective, broad spectrum germicide that is widely used by consumers, varies greatly across Europe but again shows no correlation with prevalence of atopic disease. Bleach consumption per capita is highest in Spain and other countries of southern Europe, which have relatively low incidence of atopy, whereas in Scandinavia, where bleach use is very limited, some 30 times lower than in Spain, atopy rates are relatively high. Insights into the possibility that reduced microbial exposure may have occurred, as the result of increased cleaning product use, can only be gained by comparing temporal trends. This aspect is considered along with other trends in hygiene practice in Section 4.

It has been suggested that, while hygiene practices may have reduced microbial exposure, there may also be a harmful effect from a generally increased ‘burden of product exposure’. A potentially harmful role of increased use of ‘chemicals’ in relation to atopy has been suggested in analyses of the long-term longitudinal cohort study of children born in Avon (ALSPAC 2001, Sheriff et al. 2002b), although this is difficult to interpret without precise data on types of cleaning agents/disinfectants used.

While no other specific studies have been conducted to investigate the possibility of a link between the extent of the use of cleaning products and the risk of atopy, one specific product type implicated as a possible cause of atopy, particularly eczema, is fabric washing products containing proteolytic enzymes. These were introduced in the UK in the late 1960s (UKCPI 2002). Detergents have been shown to exacerbate existing eczema and enzymes themselves are well-documented occupational respiratory allergens, (Cullinan et al. 2001, Flindt 1996, Poulsen et al. 2000, Tripathi and Grammer 2001). The properties of enzymes as respiratory allergens were identified very early in their use and led to stringent measures to restrict workforce exposure. While a few instances of consumer sensitisation were noted soon after the introduction of such products in the late 1960s to early 1970s (Zetterstrom and Wide 1974, Zetterstrom 1977, Pepys et al. 1973, Belin et al. 1970, Bernstein 1972), none have been reported since encapsulated enzyme preparations were introduced. Extensive studies of respiratory effects of enzymes (reviewed by the US National Academy of Sciences National Research Council, 1971) and sensitisation to enzymes (Pepys et al. 1973) have demonstrated only a very small risk from such products. Several recent studies, including instances of exaggerated use and misuse, have confirmed these findings (Cormier et al. 1997, Sekkat et al. 1995). Such a low risk is not consistent with a significant role in the increasing prevalence of atopic disease over the last 20-30 years.
Detergents are irritant to the skin, but there is currently no evidence that the enzymes increase such irritant responses.

Occupationally, cleaners were found to have the highest period prevalence of hand eczema in a large questionnaire survey in Sweden (Meding 1990) but any separate influence of water, detergents, dust, dry dirt and unspecified chemicals could not be distinguished. It is, however, notable that the UK and Ireland remain the only European countries where a significant portion (25-30%) of fabric washing products do not contain enzymes (the so-called ‘non-biological products): yet these two countries have higher rates of atopy than almost all their European neighbours (ISAAC 1998). More systematic studies have also failed to reveal a relationship between exposure to enzymes (in detergents) and development of eczema (White et al. 1985, Andersen et al. 1998). Lee et al. (2002) found products containing proteolytic enzymes to be less irritant than their enzyme-free equivalents.

3.5 Factors influencing the body’s response to infection

3.5.1 Immunisation/vaccination

Vaccination involves the administration of attenuated or killed microorganisms or selected components from them in order to induce an immune response that protects against the disease. If the hygiene hypothesis is correct, this might be expected to influence susceptibility to atopic disease by a mechanism similar to that postulated for living microbes, namely by inducing development of a balanced immune system that responds appropriately to allergens and pathogens.

Epidemiological studies provide no consistent support for either a beneficial or adverse effect of vaccination/immunisation on atopic tendency. In countries with high vaccine coverage, caution is needed in the interpretation of the results of such studies: families who choose not to immunise their children are unusual and possibly include fewer allergic parents (Strachan 2000). Lewis and Britton (1998) reported that vaccination could both increase the risk of atopy and also protect against it, depending on the population examined. As noted regarding the Guinea-Bissau study, measles vaccination was implicated as possibly increasing the tendency to atopy (Shaheen et al. 1996), but this finding is not supported by other epidemiological studies. Haus et al. (1988) found that pertussis (whooping cough) immunisation could produce a specific IgE response in man. In an analysis of pertussis immunisation and subsequent self-reported wheezing in the ALSPAC cohort, Henderson et al. (1999) found no evidence of an association. Similarly, Nilsson et al. (1998) found no convincing evidence of an effect on atopy in a
randomised controlled trial of pertussis vaccine in Sweden. Vaccination was reported to be associated with a two-fold increase in asthma in a large US study comparing vaccinated with unvaccinated children (Hurwitz et al. 2000).

Mycobacterial vaccines have been particularly studied with regard to their effect on immune system disorders. The vaccine used to protect against tuberculosis (BCG) contains live attenuated mycobacteria. Mycobacteria are of particular relevance to discussion of the hygiene hypothesis, since it has been suggested that early exposure to mycobacteria with low or no pathogenicity may protect against later atopic disease (Rook and Stanford 1998). Several studies have reported that BCG has no protective effect against immune disorders (Omenaas et al. 2000, Alm et al. 1997, Strannegard et al. 1998, von Hertzen et al. 1999). At present there is therefore very little epidemiological support for the theory that either BCG vaccination, or active tuberculosis, reduces the human risk of atopy, despite the promising initial results of mycobacterial vaccines in the treatment of some diseases associated with immune dysregulation. The best evidence comes from animal studies, for example, suppression of allergen induced eosinophilia in mice by infection with *Mycobacterium bovis* (Erb et al. 1998). Based on animal evidence of the possible benefits of immunostimulatory DNA sequences, *M. vaccae* lipopolysaccharides, *Lactobacilli* spp. and oral bacterial extracts, Matricardi and Bonini (2000b) argue that there are strong therapeutic possibilities in using microbial stimuli to educate the developing mucosal immune system:

“Although such a strategy is far beyond our present potential, it may in principle revert the epidemic trend of atopy and allergic asthma without jeopardizing the fight against infectious diseases.”

They acknowledge that this form of programmed ‘immunoeducation’ is currently infeasible and that “poor hygiene will never cure asthma”, but it remains an active field of research.

3.5.2 Antibiotic therapy

Antibiotics are commonly prescribed for early episodes of chest and ear infection, raising the possibility that antibiotic treatment may be involved in reduced infection exposure and/or modification of the immune response. The relationship with later asthma or other atopic disease is difficult to disentangle from the possible confounders (Mattes and Karmaus 1999), such as whether the key exposure is the infection or the antibiotic. In a large study of German children, von Mutius et al. (1999) found that six or more courses of antibiotics in the first year of life were associated with a later excess of hay fever and eczema, but skin sensitisation tests (skin prick tests) did not indicate
an increase in the prevalence of atopy. Similarly, Farooqi and Hopkin (1998) reported that any course of an antibiotic before the age of 2 years in Oxfordshire residents was associated with a doubling of the risk of hay fever and eczema, particularly if the antibiotics contained cephalosporins and macrolides. The antibiotic effect could be linked to the influence on the bacterial colonisation of the gut in early years of development (Bjorksten 1999).

### 3.5.3 Breastfeeding

“Breastfeeding is more important than anything else in the prevention of infantile gastroenteritis… Human breast milk has properties aimed specifically at the prevention of intestinal infection.”

(Christie 1980, p191)

The value of breastfeeding in preventing infection in infancy is long established (Christie 1980, Hanson et al. 2001): a recent study claimed that 32% of infant deaths in Latin America could be prevented by exclusive breastfeeding for 3 months and partial breastfeeding throughout the remainder of infancy (Betrán et al. 2001). The protective effect of breastfeeding is mediated by transfer of maternal antibodies, as well as by constituents affecting the infant’s gut. While protecting against intestinal pathogens (Okuda et al. 2001, Mahmud et al. 2001) and respiratory infections (Bulkow et al. 2002, Arifeen et al. 2001), breastfeeding may also transfer active infections such as HIV and Hepatitis C from mother to baby (Jones 2001). Of particular relevance to this review, the reported benefits of breastfeeding include prevention of later asthma or atopic disorders.

Breastfeeding has been reported to be protective against asthma in a questionnaire study of parents of 974 children aged 3-5 years in Australia (Haby et al. 2001); against atopic disease in a 17-year prospective follow up study (Saarinen and Kajosaari 1995) and in a 12-month prospective study (Merrett et al. 1988); and protective against food allergies in a 15-year follow up study (Gruskay 1982). The duration of breastfeeding appears to be an important factor, with little or no protective effect for short periods of breastfeeding, for example for less than 3 months (Ronmark et al. 1999). Breastfeeding for more than 12 months was associated with lower levels of subsequent atopy in the longitudinal study in Guinea-Bissau (Shaheen et al. 1996); breastfeeding for at least 4 months was protective in an Australian cohort study (Oddy 1999). A dose-response effect of breastfeeding was also indicated in a retrospective longitudinal study of children aged 1-2 years in Canada: a higher risk of asthma was found in children who had been breastfed for up to nine months, than in those breastfed for longer. The trend to shorter periods of breastfeeding, for example due to women working
outside the home, was also indicated in this study: 44% of the children were breastfed for only two months.

Breastfeeding was one of several factors affecting prevalence and severity of allergic conditions (wheeze, rhinitis, hay fever, eczema) in an ISAAC questionnaire-based study in Malta (Montefort et al. 2002) but passive smoking, gender, family history of pets, soft furnishings and living next to busy roads were also identified as risk factors. Husby (2001) has argued that breastfeeding may be more important than any other factor in the development of clinical tolerance to allergens, particularly because of the importance of ‘oral tolerance’ mediated by lymphoid tissue associated with the gastrointestinal tract. This concurs with evidence of protection by exclusive breastfeeding against subsequent cow’s milk allergy (Host et al. 1988), although a recent study found no increased risk of later atopy from early (brief) exposure to cows’ milk (de Jong et al. 2002).

Halken and Host (2001) comment on the need for controlled prospective studies of the role of breastfeeding in protecting against allergies, noting the tendency for generally favourable speculation about the benefits of breastfeeding, based mainly on small retrospective/cross sectional studies. Detailed data on time trends for breastfeeding and its duration are also required to determine its role in immunological ‘priming’. Miller (2001) similarly comments on the limitations of retrospective surveys, finding only moderate concordance between maternal reports and medical records. Miller compared different data sources for asthma and risk factors in the USA: while black race, male gender and pre-term birth were found to be risk factors for asthma regardless of data source, breastfeeding was significantly inversely associated with asthma only on the basis of maternal reports. Results of a longitudinal study of six year old children recruited at birth suggest that any association between breastfeeding and subsequent asthma is dependent on presence or absence of maternal asthma and co-existence of atopy (Wright et al. 2001), although exclusive breastfeeding significantly reduced the risk of recurrent wheeze in the first 2 years of life regardless of maternal history and atopy.

Other investigators have commented on the difficulty of distinguishing breastfeeding effects from other factors. In some surveys, controlling for breastfeeding, or adjusting for it in the statistical analysis, may have obscured its importance. The body mass index (BMI) appears to be an important variable in interpreting results: in a prevalence survey of asthma and atopy by von Mutius et al. (2001), asthma and atopy rose significantly with increasing quartiles of the BMI, remaining significant after adjustment for breastfeeding and other factors. A meta-analysis of
prospective studies on breastfeeding and asthma suggested a small overall protective role of breastfeeding [summary odds ratio 0.7 (95% CI 0.60-0.81)], although this was also related to a combination of immunomodulatory factors (breast-milk quality, avoidance of allergens and other factors such as family history of atopy). Thus the mechanism of the protective effect is not understood. An effect on susceptibility to allergic disease could be independent of the influence of microbial exposure and microbial invasion, although this has not yet been specifically researched. The influence of breastfeeding may also be related to factors such as whether breastfeeding is exclusive or combined with early exposure to cows’ milk, duration of breastfeeding and genetic predisposition towards atopy.

3.5.4 Probiotics

Probiotics (microbial cell preparations or components of microbial cells claimed to have a beneficial effect on health) represent a type of microbial exposure, which may be relevant to the hygiene hypothesis. Probiotics commonly comprise lactic acid bacteria, such as lactobacillus or bifidobacterium. To be effective they need to survive passage through the acidic environment of the stomach and to colonise the intestine. Probiotics would be expected to work by priming or maintaining normal gut flora and preserving intestinal mucosal integrity, so effects on a variety of immune related conditions would be expected, including the response to infection. A study in mice showed suppression of antigen-specific IgE following injections with heat-killed Lactobacillus plantarum (Murosaki et al. 1998). Oral feeding with Lactobacillus casei prevented onset of insulin-diabetes mellitus in non-obese diabetic mice, who would normally be expected to progress to insulin dependency (Matsuzaki et al. 1997). Such evidence has been cited by proponents of the hygiene hypothesis, in support of the use of probiotics to replace the beneficial microbial exposure absent in contemporary Western environments and modern diets. Probiotics may be more protective against eczema (Kalliomäki et al. 2001) than against other forms of atopic disorder, although this finding may relate also to the duration of follow up in their study, since eczema tends to present earlier than asthma. The study by Kalliomäki and colleagues in Finland was a randomised placebo-controlled trial of Lactobacillus rhamnosus in families with a history of atopic disease, with a 24 month follow up. Probiotics have also been reported to prevent diarrhoea in children in Peru (Oberhelman et al. 1999) or at least to reduce its severity (Hatakka et al. 2001, Majamaa et al. 1995). Hatakka and colleagues (2001) reported that respiratory infections were also reduced in duration and associated complications. In a recent editorial, Wanke (2001) suggested that we do not yet have sufficient information to routinely recommend probiotics, but
that accumulating data suggest that they may have a role in preventing both respiratory and diarrhoeal diseases in children at increased risk of such infections, such as those in day care facilities. The role of probiotics is considered further in relation to changes in diet later in this section.

3.6 Non-microbial factors and the risk of developing atopic disease

In this section we examine various other factors which could be associated with increased risk of atopy. For each of these factors a link to atopy is plausible, and for some of them there is some epidemiological evidence of a link.

As discussed earlier (Section 3.3), the classification of these factors as ‘non-microbial’ is by no means clear-cut, since some have the potential to directly or indirectly influence microbial exposure. Though some of the microbial factors could contribute to the rising trend in atopy by increasing phenotypic expression albeit by a different mechanism, for many of them there is little to indicate the mechanism/s by which they may affect the risk of atopy. Alternatively the factor may exert an effect on atopy by an entirely different mechanism/s such as:

- a direct action on the tissues to produce a physiological response, for example, a narrowing of the airways of the lung;
- a direct sensitising/triggering action on the immune system to produce an allergic response;
- depriving the body of the nutrients needed for healthy immune system development.

In the following subsections, the various factors are discussed and the evidence for a link to the development of atopy reviewed. Where available, data indicating the possible mechanism/s by which an effect on atopy could be exerted, is given. Since in many cases there is no clear understanding of the causal mechanism, no attempt has been made to ‘classify’ or order the factors in subsections 3.6.1-3.6.11.

3.6.1 Anthroposophic lifestyle

One of the assumptions in the ‘changing lifestyle’ aetiological explanation for atopic disease is that modern, industrialised and affluent life tends to reduce exposure to pathogens and increase exposure to a wide range of processed food and synthetic products. Within industrialised countries, some groups have tried to maintain a more traditional lifestyle, for example, the approach advocated by Rudolf Steiner, which includes an organic food diet.
The anthroposophic lifestyle of Steiner followers has been implicated in preventing atopic disease (Alm et al. 1999). The problem is identifying which of several factors may be important in the anthroposophic lifestyle, which includes a radically different diet, refusal of childhood vaccination and limited use of antibiotics (Strachan 1999). Alm et al. 1999 reported that atopy was infrequent in families following these principles, concluding that a combination of natural measles infection, other childhood infections and dietary habits was involved in the protective effect. Matricardi 1999, commenting on these findings, proposed that the key factor was: “faecal contamination of the environment, together with unhygienic food handling”. Strachan (1999) emphasised the importance of timing of exposures to specific allergens, which may be more important than the chronological age proposed in the ‘window of opportunity’ theory of immune system development.

3.6.2 The role of non-microbial allergens and pollution

During the early phase of the rapid rise in atopy, the possibility of an increase in known environmental sensitisers or triggers was considered. These included non-microbial allergens such as pollen, house dust mites and air pollutants. Inconsistent results from epidemiological studies reduced support for a causal role, but evidence of the effect of very early exposures to allergens and pollutants has renewed interest that some could be involved in dysfunctional development of the immune system.

3.6.3 House dust mites, other insect allergens, fungi and moulds

Existing atopic disease is exacerbated by exposure to house dust mites, such as Dermatophagoides farinae and D. pteronyssinus and cockroaches. In a cross sectional study of risk factors for childhood asthma in Costa Rica, sensitisation to house dust mites was a statistically significant key factor for asthma after adjusting for age, gender, area of residence and maternal smoking during pregnancy (Celedon et al. 2001). Altitude appears to have a strong influence on likelihood of sensitisation to house dust mites and to grass pollens, related only partly to frequency of exposure at different altitudes, although climatic factors, such as the lower indoor humidity at high altitudes than at sea level, influence numbers of mites (Charpin et al. 1988). Lower levels of sensitisation to house dust mites (but conversely higher levels of sensitisation to grass pollens), have been reported for children living in the Alps (Charpin et al. 1991). The influence of climatic regions on house dust mite populations and hence on sensitising or on triggering attacks of atopic disease is also indicated by studies of differences between coastal and inland regions in Australia (Peat et al. 1993): the allergens have less effect in dry, rural regions.
Inner city environments include high exposure to cockroaches, which may be at least as important as house dust mites in triggering the onset of atopic disease. A study on children with recurrent wheezing in Chicago, USA, found that cockroach allergen sensitivity starts early in life and may be the only sensitising allergen for many young inner city children (Alp et al. 2001). In this study of 196 children aged 5 months to 16 years, 24% of the youngest children (aged under 4 years) had cockroach allergen sensitivity and the youngest patient with a positive reaction was only six months old. Only 13% of these youngest patients were skin test positive to dust mite allergen. In a study of 1041 children aged 5 to 12 years with mild to moderate asthma in eight North American cities, dust containing both dust mite and cockroach allergens was found to be an important determinant of sensitisation, with no demonstrable relationship for cat, dog or mould allergens. High levels of dust mites may also be associated with high levels of other allergens, making it hard to distinguish independent effects of particular allergens.

With this mix of allergens, it is perhaps unsurprising that some studies have found no significant effect of HDM, or in studies examining a local range of allergens. Lau et al. (2000), in a prospective birth cohort study in 5 German cities, found no association between early indoor allergen exposure and prevalence of asthma, wheeze or bronchial hyperresponsiveness. Indoor levels of fungi and cat allergens, but not house dust mites, were significantly related to sensitisation (skin prick and lung function tests) in a study of 485 adults in Australia (Dharmage et al. 2001). Fungi and moulds in damp housing can exacerbate asthma – and a home does not have to be damp to have a wide range of these organisms: a Belgian survey identified at least 12 species in 30% of homes surveyed, including new and renovated dwellings (Nolard 2001). In these surveys, the level of allergic complaints, and proportion of positive skin prick tests or IgE antibodies, correlated with the frequency of moulds identified in the home. However, such evidence does not prove a causal effect and studies on the health effects of damp housing have produced conflicting results as well as evidence of the influence of co-factors, including socio-economic status, heating, smoking, ventilation and family mobility (Austin & Russell 1997; Peat et al. 1998).

The consensus appears to be that mites and moulds aggravate established atopy, rather than acting as causes of increased susceptibility in man (Warner 1999), although Sporik and Platts-Mills (2001), in a review of the literature on allergens and asthma, suggest that some of the conflicting results could be explained by different study locations and environments: they argue that a low allergen environment could still be protective against asthma. Sensitisation as early as six months (Alp et al. 2001) raises questions
about other early exposures that have been claimed to be causal, such as hygiene and reduced microbial exposure. While cockroach and dust mite sensitisation studies cannot indicate whether such allergen exposure predisposes to atopy, they are important in the search to identify the timing of predisposing and sensitising factors. They provide possible further evidence that the ‘window of opportunity’ for immune system priming and predisposition to atopy occurs during pregnancy or the first few months of life.

3.6.4 Industrial and traffic air pollution

Rapid variation in air pollution appears to trigger asthma attacks (Charpin et al. 1988), but interest in rising levels of air pollution as a cause of the rise in atopy waned because of inconsistent evidence regarding a dose response effect. Some recent studies have prompted re-examination of air pollution as a cause or trigger of asthma.

Traffic emissions were reported to increase atopic sensitisation for nine-year-old children living near major roads and in suburban areas in West Germany (Krämer et al. 2000). Outdoor traffic exposures were related to allergic rhinitis, hay fever and wheezing in children living in the most congested areas. Ozone in urban air has been implicated as associated with an increased risk of asthma (Miller 2001) and heavy exercise in high ozone environments has been found to increase the risk of asthma in children with no history of the disease (McConnell et al. 2002). The relative risk of developing asthma was 3.3 for children who played three or more sports in such areas, compared to those playing no sports, although sport activity had no effect in low ozone areas. The authors suggest that heavy breathing, associated with vigorous exercise, would allow ozone to penetrate more deeply into the lung, including sites associated with the heaviest morphological damage in animal experiments. This finding accords with the view that many cases of asthma derive from a combination of tissue abnormalities and atopy. Indoor exposures have also been implicated as possibly causative of atopic disease, rather than only triggering attacks: examples include gas appliances (Ponsonby et al. 2001) and formaldehyde vapour (e.g. from synthetic materials in the home) (Rumchev et al. 2002).

Occupational asthma is well described, including an increased risk for workers in detergent factories (Cullinan et al. 2001), although this provides little insight on the predisposing factors involved. Bjorksten 1999, reviewing results from environmental studies, commented that deteriorating air quality can only marginally explain the observed regional differences in allergic disease prevalence and studies of cities with different levels of air pollution have shown no consistent correlation with levels of clinical atopy (Warner 1999). Apart from the recent reports regarding ozone, there is no
evidence that environmental pollutants induce asthma, although there is evidence that they can incite it.

3.6.5 Smoking

Smoking has not been implicated as causing atopy/asthma, although it is well established as an exacerbating factor. Higher levels of adult asthma and hay fever are associated with ever having smoked (Upton et al. 2000). The strong association of maternal smoking with subsequent asthma (Weitzman et al. 1990) suggests possible environmental interaction with the growing foetus or during early infancy. While noting that maternal smoking may be an important factor in inducing transient wheezing in early life, Warner (1999) commented that “the extent to which parental smoking influences the development of atopy has been extensively discussed and it is doubtful that it has much, if any, impact.” Furthermore, cigarette smoking decreased from 42% to 25% between 1965 and 1993 in the USA and therefore is an unlikely explanation for the rise in asthma reported for Americans in the last 30 years (Grant et al. 1999).

3.6.6 Stress

‘Stress’ has been increasingly reported to be a cause or aggravator of disease, including asthma, although the term is poorly defined and the results of studies on stress and atopic disease vary according to the risk proxy or exposure categories chosen. High levels of ‘stress’ as measured, for example, by negative life events and a poor home environment, have been reported to predict the onset of asthma in children genetically at risk (Mrazek et al. 1999, Sandberg et al. 2000). Stress factors have also been implicated in causing increased susceptibility to infectious disease (Grey 1993, Haavet and Grünfeld 1997, Stanwell-Smith et al. 1994). While multiple chronic stressors and severe negative life events appear to increase the risk of asthma attacks (Sandberg et al. 2000), such adverse influences appear to act on pre-existing disease or pre-existing genetic susceptibility. Thus they are not generally viewed as having a causal role.

3.6.7 Socio-economic factors: social class, affluence and poverty

Several socio-economic factors may influence opportunities for microbial exposure. For example poor standards of water supply and other household amenities provide less opportunity to adhere to hygiene practices in the home, or low educational achievement may be associated with poor understanding of how to apply hygienic practice. Social class or socio-economic group has been studied in relation to levels of atopy: other studies
have focused on economic status as the main factor, including risks posed by increasing affluence as well as by poverty.

**Social class/socio-economic group**

In his review of the first decade since his hypothesis, Strachan cited the evidence of an inverse relationship between socio-economic status and allergic disease, with hay fever and eczema more prevalent in more affluent families (Strachan 2000). The variation with parental socio-economic status appears to be independent of the offspring’s later socio-economic status in adulthood, emphasising the importance of early influences. As a proxy measure of exposures, socio-economic group/social class has been valuable in interpreting different epidemiological patterns of disease, but an evidence-based approach requires such proxies to be supported by documented evidence of increased microbial exposures. As Nielsen *et al.* (1999) observed in a study of socio-economic factors possibly contributing to juvenile chronic arthritis (JCA), high and low income groups are “very heterogeneous with regard to social variables other than income”. In their study, children who lived on farms had a lower incidence of JCA than those in urban flats, and they concluded that the hygiene standards on farms were good in the sense that “exposure to human infections is low”.

Studies in the inner cities of the USA (Salvi and Holgate 2001) suggest that the association of asthma and atopy with higher social class/socio-economic group may be changing, particularly for asthma, with increasing incidence in lower income groups. Thus the previously observed socio-economic gradient may be levelling out, although it is too early to determine whether the risk is now equal, or changing, in specific income/social class categories. Socio-economic factors also have a strong influence on diet and thus diet may be a confounder in studies of atopic dysfunction and socio-economic group/social class: this is further considered in relation to the dietary hypothesis as an explanation for the rise in atopic disorders (**Section 3.6.8**).

**Affluence and poverty**

Changing levels of affluence in developed countries have been suggested to contribute to the rise in atopic disease. Levels of affluence, or indirect assumptions of such levels through measures of social class/socio-economic group, have also been used as proxies for changes in other exposures, such as hygiene amenities, hygiene practices and diet. Given the wide range of assumptions of exposure related to economic status, it is unsurprising that the relationship between atopic disorders and socio-economic group is unclear. Poverty has been identified as a risk factor for
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asthma in the United States (Platts Mills et al. 2000), with levels of asthma increasing over the last 40 years in the poorest sections of the population. Poverty is also known to be a major factor involved in exacerbating or initiating asthma and other atopic disease in susceptible individuals (Rona 2000). The association between income and asthma also applies to developing countries: in a cohort study in Ghana, an increased risk of asthma was associated with low household income, low school leaving age and residence in high poverty areas (Lintonjua et al. 1999).

3.6.8 Nutrition and diet

"...if the dietary hypothesis is correct [regarding the cause of the asthma epidemic], the favourable trend in eating habits between 1985 and 1991 may already be having a beneficial effect."

(Seaton et al. 1994)

Increasing affluence allows a more varied, but not necessarily healthier diet; while on the other hand it allows better nutrition in pregnancy and infancy. Low birth weight is more common in low socio-economic groups and has been identified as a risk factor for asthma (Schwartz et al. 1990) as well as less resilience to poor living conditions and a higher risk of coronary heart disease (Barker et al. 2001). Improved nutrition has led to increases in birth weight and birth head circumference: an association with birth head circumference has been reported for subsequent raised IgE levels (Godfrey et al. 1994) and for asthma (Fergusson et al. 1997). The mechanism may be that increased fetal growth trajectory is achieved at the expense of immune system development, since this involves rapidly dividing tissues that may be compromised by the demands for fetal head and body growth. Since the 1960s, the UK diet has changed out of all recognition, including an unprecedented variety of processed foods and exotic ingredients. Studies in the UK and elsewhere, such as the USA, suggest that changes, such as lower consumption of raw food, fresh fruit and vegetables, are more marked in lower socio-economic groups (MAFF 1997, Billson et al. 1999, Pollard et al. 2001, FSA 2002). Government campaigns to encourage more fruit and vegetable consumption, and the increased availability of salads throughout the year, may be reversing this trend, but only in the last decade – and with less impact in lower income groups because of the higher cost of fresh food.

Diet has been implicated as a predisposing factor for atopy by the studies of Butland et al. in the UK (1999) and in Saudi Arabia (Hijazi et al. 2000). In the former study low fresh fruit intake was associated with frequent wheeze and speech limiting attacks of asthma in adults, after accounting for smoking: as with the affluence/poverty effects, the issue is whether causation
or exacerbation of existing disease is involved. The Saudi Arabian study is of interest because of the rapid changes in that society due to increased affluence. As in other changing societies, asthma has increased in urban areas of Saudi Arabia. Hijazi et al. showed that diet may be the most important change in such societies, and therefore most likely to explain the recent trends in atopic disease. The dietary factors involved include eating at fast food outlets, as well as lower intake of milk, vegetables, fibre and foods rich in Vitamin E. Dietary vitamin E was reported to inhibit IgE responses to allergic stimuli in a random sample of 2633 adults (Fogarty et al. 2000). In a study of adult-onset wheeze in Aberdeen, intakes of vitamin E and plasma levels of ascorbate (measure of Vitamin C) were inversely related to adult wheeze in the manual social class and among current smokers (Bodner et al. 1999). Other dietary changes, such as increased sodium relative to potassium, increased fat intake or decreased omega-3 fatty acids and solid food such as eggs from an early age, may influence subsequent sensitisation to inhalant allergens (Platts Mills et al. 2000). A questionnaire based study of Australian preschool children identified a high dietary intake of polyunsaturated fats as a risk factor for a diagnosis of asthma within the previous 12 months (Haby et al. 2001).

The dietary hypothesis, as an explanation of trends in atopic disorders, has biological plausibility in terms of the nutrients needed for healthy immune system development, such as polyunsaturated fatty acids and antioxidants (Hijazi et al. 2000). Thus, as an indirect effect of affluence, dietary changes may be responsible for the increased predisposition to atopic disease. The dietary hypothesis has also been linked to the notion that there has been a shift in the population susceptibility to atopic disease (Seaton et al. 1994), possibly also linked to increasing population mixing and greater genetic diversity (Warner 1999). In an investigation of whether reduction in childhood infections or change in diet could explain increases in asthma and atopic disease, Seaton and Devereux (2000) found that low intake of vitamin C was associated with a seven-fold risk of bronchial hyper-reactivity; by contrast, the lowest intake of saturated fats gave 10-fold protection. In a separate study, the lowest intake of vitamin E was associated with a five-fold increase in adult-onset wheezy illness, while the lowest intake of vitamin C doubled the risk. Dietary intakes were confirmed by measurement of vitamins and triglycerides in plasma. Seaton and Devereux conclude that changes in the diet of pregnant women may have resulted in the birth of cohorts of children predisposed to atopy and asthma.

Another indicator of the influence of a more varied diet may be the reported rise in food allergies: the incidence of adverse reactions to foods is 2-8% in young children and 1-2% in adults (Helm and Burks 2000). Helm and
Burks favour the hygiene hypothesis as an explanation for the rise in food allergy, although arguing mainly on the basis of the immunological model of the Th 1/Th 2 balance and animal studies of induced allergic reactions, for example, after administration of peanut allergen or cow’s milk protein. They also raise the issue of accumulation of new crops and metabolites in crop plants that enter the food chain. Food allergies vary significantly with age: for example, crab, milk, and egg white allergy was highest in the 2-6 year old group in a study of 2008 patients with allergic disease (Lee et al. 2000), suggesting that the age of exposure to food allergens may be particularly important, providing clues about development and dysfunction of the immune system. The role of defective gut-specific IgA responses to food allergies in children (Kalliomäki et al. 2001) is a further indication of the inter-relationship between diet, immunity and allergy.

The dietary hypothesis merits examination in terms of trends in types of food consumed and trends in atopic disease, as well as intervention studies. One prediction rising from the dietary hypothesis is that more favoured socio-economic classes in countries such as the UK should show a reduced prevalence of atopic disease compared with less favoured ones, since fresh fruit and vegetable consumption is greater in higher socio-economic groups. If this prediction is correct, future trends in atopic disease will be inversely related to socio-economic group. This contains an assumption about continuing social class trends regarding diet, but Seaton (2001) has also observed that the decline in new episodes of asthma reported by Fleming et al. (2000) supports the dietary hypothesis, as the decrease in incidence could be attributed to higher consumption of foods containing these nutrients since the mid-1980s. The observed reduction in the average intake of fresh fruit, vegetables and fish occurred just before and during the decades of the rapid rise in asthma. The importance of such foods in providing fatty acids or antioxidant vitamins has been confirmed by several recent studies showing an association between increased risk of wheezy illness and low intake of these nutrients (Soutar et al. 1997, Bodner et al. 1999, Hijazi et al. 2000, Haby et al. 2001, Devereux et al. 2002). The argument against the dietary hypothesis includes the point that it seems unlikely for either dietary deterioration or dietary improvement to simultaneously affect all age groups and all regions (Fleming 2001, response to Seaton 2001). It is relevant to this review to observe that just such a phenomenon has been suggested for hygiene practice and hygiene amenities, in support of the hygiene hypothesis. While diet alone may not be responsible for the apparent mass population effect regarding atopy and asthma, the evidence for a dietary effect is much more persuasive than that provided, mostly on the basis of proxy exposures, for hygiene.
3.6.9 Obesity and lack of exercise

Obesity is defined as a condition in which fat stores are enlarged to an extent that impairs health: it is the effect of chronic energy imbalance. While the tendency to obesity may be partly genetic, it can be predicted from early patterns of weight gain, including birth weight and possibly also from whether the child was breastfed (Armstrong et al. 2002). Obesity is increasing in developed countries and the timescale often cited for the recent rapid increase is the last 30 years (e.g. in USA, Kucmarski et al. 1994), the same period over which atopic disorders have shown a dramatic rise. In European countries rising trends have been shown at least since the 1980s in Netherlands, Switzerland and France (Livingstone 2001) with marked increases over a slightly longer period in the UK, as in the USA. A recent survey estimated 19% of 5-year old children in the UK as overweight and 7% as obese (Reilly et al. 1999). Between 1972 and 1994, triceps skin fold thickness increased by 8% and 7% in English boys and girls, respectively, with larger increases in Scottish children (Hughes et al. 1997). A large population survey of children in the UK showed that the greatest increase has occurred since the mid 1980s (Chinn and Rona 2001); while there was little change in prevalence of obesity between 1974 and 1984, there was an approximately two-fold increase in the next decade. Contributing factors include a comparable decline in outdoor exercise and much higher recorded television viewing. The quality of the indoor air environment may also help to explain asthma morbidity in an increasingly sedentary generation (Grant et al. 1999). Obesity is a global problem: the population of obese adults worldwide was estimated to be 300 million in the year 2000, 100 million more than in 1995 (WHO 2001b).

The body mass index (BMI: weight in kilogrammes divided by square of height in metres) is positively associated with the risk of adult-onset asthma (Camargo et al. 1999). The risk of asthma has been related to pre-existing obesity (e.g. in childhood), although there have been inconsistent results regarding whether both men and women are affected: the risk appears to be higher in women (Tantisira and Weiss 2001). A randomised controlled trial of the effect of weight loss on asthma showed improvement in lung physiology (such as forced expiratory volume) and reduced need for medication (Stenius-Aarnialia et al. 2000). Increasing BMI correlated with an increased risk of childhood asthma and atopy in a prevalence study by von Mutius et al. (2001) and BMI remained significant after adjustment for other factors.

The consistency of the relationship with obesity, the temporal association and the dose response curve has prompted the theory that obesity caus-
es asthma (Gibson 2000). This is not yet a widely held view, since the biological mechanisms remain unclear (Tantisira and Weiss 2001). Obesity and associated hormonal changes could influence inflammatory mechanisms in the lung, or act mechanically on the tissues, preventing full extension of bronchial muscles (Platts Mills et al. 2000). If obesity acts rather as the trigger for asthma, it would not affect hypotheses and theories about the underlying aetiology of atopic disorders, such as the hygiene hypothesis.

Platts Mills et al. (2000) claim that the key changed factor is less exercise. It has been known since the 1960s that full expansion of the lungs decreases lung resistance and it has been demonstrated that bronchial hyperresponsiveness (BHR) develops in normal individuals who do not take a deep breath for 40 minutes (Skoot et al. 1995, Parham et al. 1983). Prolonged physical activity involving lung expansion could have an anti-inflammatory effect: such effects have been described and Fredburg et al. (1999) suggest, on the basis of in vitro studies of bronchial smooth muscle, that extension of this muscle is a more potent bronchodilator than the drug isoprenaline. Physical activity is notoriously difficult to measure and there are epidemiological limitations in the interpretation of studies, such as small sample sizes, definitions of activity and differences in analytical procedures (Livingstone 2000). Trends of decreased activity are nevertheless evident, plus the circumstantial evidence of trends such as video and computer games, children being driven to school and safety issues that deter outside exercise. This would appear to be an area requiring urgent research and meanwhile Platts Mills et al. observe:

“Any discussion of diet should recognise that for diseases such as arteriosclerosis, hypertension and diabetes, it is the relationship between diet and activity that determines the outcome. It would not be surprising if the same were true for asthma.”

(Platts Mills et al. 2000)

3.6.10 Maternal age

One of the possible confounding factors in studies of family size is that maternal age tends to be higher in small families and a greater risk of atopic disease has been reported in the children of older mothers (Bråbäck and Hedberg 1997) in addition to the established gender bias towards boys (Strachan 2000). The immune system development may be biased towards a Th 2 response in the children of older mothers, possibly because of an evolutionary bias towards younger mothers. The risk of childhood asthma is greater for infants with a maternal history of asthma than those whose fathers have this history (Litonjua et al. 1998), indicating the possible importance of
maternal imprinting (preferential expression of maternal genes in the fetus) (Kurz et al. 2000, Daniels et al. 1996), as well as the influence of the maternal-fetal interface (Tanisira and Weiss 2001).

3.6.1 Genetic predisposition to atopy and genetic diversity

“The sequencing of the human genome offers the greatest opportunity for epidemiology since John Snow discovered the Broad Street pump.”

(Shpilberg et al. cited by Clayton & McKeigue 2001)

As mentioned in Section 2, genetic predisposition is a major factor in susceptibility to atopy. While the rise in atopy has occurred within too short a time frame to be explained by a genetic shift in the population, genetic predisposition to asthma and other atopic disorders applies to a large proportion of the population. For example, estimates of genetic effects on asthma in Finland vary between 35%-87% for asthma (Laitinen et al. 1998, Nieminen et al. 1991) and 74-82% for hay fever (Räsänen et al. 1998). Identification of chromosome markers for predisposition appears to be raising previous estimates based, necessarily, on phenotypic expression of atopy in association with family history. Frequency of hypersensitivity to allergens has been shown to vary from 2-4% in China, India and Africa to 20-30% in Britain, USA and Australia (Salvi and Holgate 2001).

The rapid progress in sequencing of the human genome provides hope that immune system dysfunction will soon be better understood; and linkages between chromosomes may help to unravel some of the associations between susceptibility to both atopy and infection. Markers in 19 chromosomal regions have shown evidence of linkage to asthma, atopy or related phenotypes that indicate promising areas of research on the molecular pathways involved in both susceptibility and likelihood of phenotypic expression (Ober and Moffatt 2000). The observation that endotoxins bind to CD14 has raised the possibility that there may be environmental interaction with the CD14 gene during the in utero and postnatal developmental stages, although genetic influence on serum CD14 has so far been demonstrated for atopy rather than asthma (Baldini et al. 1999, Tantisira and Weiss 2001). Genetic factors may also govern the infiltration of T helper lymphocytes into the airways in human asthma cases: this activity may be linked to an abnormality in the T-bet gene, or loss of the transcription factor expressed by this gene. This is supported both by studies in mice and the finding that lung T cells from asthmatics contain significantly less T-bet than those from people with asthma.

Genomic studies of different ethnic groups may also help to explain differences in atopy. At least three atopy-susceptibility gene loci have been
identified in the Hutterites, a founder population of European ancestry (Ober et al. 1999). The remarkably high prevalence of atopic diseases in English speaking communities across the world could be related to shared genetic ancestry, as well as the uniquely wide genetic diversity in terms of ethnic backgrounds for these communities. Genetic diversity, such as wide differences in histocompatibility complex (MHC) genes result in frequent genetic disparity between mother and fetus: this may set the stage for promoting the atopic phenotype (Warner 1999). The propensity to develop allergic disease is strongly influenced by genetic composition, but a complex relationship is involved in determining whether the propensity will manifest itself (Marsh 1999). The change in incidence has taken place over too short a time to be explained by genetic changes in populations, hence the increasing interest in environmental interaction. Clayton and McKeigue (2001) observe that ‘interaction’ is often poorly defined in this context:

“Despite current enthusiasm for study of gene-environment interactions, the closely related issue of how to define and interpret interaction between environmental factors remains unresolved after two decades of debate.”

They suggest that epidemiologists should focus rather on using genetic associations to test hypotheses about causal pathways, particularly where these are amenable to intervention.

The migration effect

An important piece of the puzzle is suggested by studies of asthma/other allergic disease in communities after migration to different countries or markedly different environments. An example is the Tokelau Island Migrant Study developed to monitor the effects of migration on health following migration of more than half of the islanders to New Zealand after a hurricane in 1966. In a study of children aged from infancy to 14 years, the prevalence of asthma in children on Tokelau was 11%, compared with 23% of children in New Zealand, including those born in Tokelau as well as those born in New Zealand (Waite et al. 1980). Thus, children from Tokelau had acquired a higher risk of developing asthma after migration, conforming to the risk of children native to New Zealand, with differences confirmed in later studies (Crane et al. 1989). Migrant Tokelauans also had higher rates of reported rhinitis and/or eczema. While the children experienced safer water supplies and sanitation in New Zealand, Crane (2001) suggests that other the key environmental differences included the very low allergen exposure on Tokelau: no grasses, very little time spent indoors and no cats or dogs. So although experiencing rural/possibly ‘unhygienic’ early environments, migrant Tokelauans
had no protection against this onslaught of allergens in their new country.

Studies of another isolated community on the island of Tristan da Cunha are helping to identify the genes for asthma, although in this rapidly evolving field the genetic basis is still only partially understood. The 300 inhabitants on this remote island were inbred, with little exposure to the outside world: a study found that 57% had partial evidence of asthma while 23% had a definitive diagnosis (Zamel 1995). Nearly half (47%) of the entire population was atopic, with higher levels of atopy in asthmatics (74%) than non-asthmatics (32%). Such studies suggest that the influence of any exposures cannot be interpreted without taking the genetic susceptibility into account.

3.7 Epidemiology of autoimmune disease and other diseases of immune system dysregulation

The importance of timing is pertinent to the extension of the hygiene hypothesis to autoimmune disease, since such diseases may present in middle age or in the elderly. Most of the 40 or so known autoimmune diseases are relatively uncommon, in comparison with atopic disorders: thyroid disease, such as Hashimoto’s thyroiditis and Graves disease affects around 3% of women; rheumatoid arthritis affects 1%, also with a female excess; systemic lupus erythematosus is found in 0.12% of the general population, also more commonly in females; and 0.1% of children have types I diabetes (Mackay 2000).

It has been suggested that an excessively hygienic environment during the critical windows for immune system development may increase the predisposition to some of these diseases. Juvenile diabetes, arthritis and chronic bowel disease have been particularly studied from this perspective, particularly because of a reported rise in incidence over a similar period to that of the rise in atopic disorders.

3.7.1 Diabetes

Childhood diabetes is rising and is more common in first-born children and affluent families, similar to the epidemiological findings of some studies on atopy (Anderson and Watson 2001). Reduced exposure to common infections in infancy was reported to increase the risk of diabetes in a UK study (Gibbon et al. 1997) and a protective effect of chicken pox was reported in an Australian study (Glatthaar et al. 1988), but these results may relate to smaller family size and less opportunity for case to case spread, rather than to hygiene factors. Gale (2002) has proposed that the decline in pinworm infestation (Enterobius vermicularis) since the mid 20th century may
be the ‘missing factor’ that formerly protected against childhood diabetes. The advantages of early social mixing, and hence greater exposure to common infections, were suggested in an interview based case control study of diabetes in Yorkshire (McKinney et al. 2000). The study was designed to test the hygiene hypothesis statement regarding reduced exposure to common microbial infections early in life. An ‘infection exposure index’ was derived a priori for the study including reported infections under 1 year, attendance at day care under 1 year and presence of other children in the household. Frequency of attendance at day care facilities below the age of 1 year was associated with an apparent protective effect against childhood Type 1 diabetes. The authors concluded that the study supported the hygiene hypothesis, although no protective effect was found for overcrowding, presence of older children in the household and deprivation indices. Conversely, Verge et al. 1994 reported an association with early attendance at day care in a case control study of childhood insulin dependent diabetes in Australia: they also found a separate raised risk associated with recent infection within three months of first diagnosis (children under 5 were excluded from analysis of this exposure). No specific type of infection was implicated.

A study of the variation in incidence of Type 1 diabetes in Italy showed a lower risk in rural areas (Cherubini et al. 1999): the lower risk did not appear to be explained by differences in water supply, but the authors postulated more homogeneous genetic background (less migration into rural than urban areas) might be an important factor. Another puzzling feature of the epidemiology of diabetes is that children with Type 1 diabetes appear to have a lower risk of atopic disease (Bingley et al. 2000). This may relate to the mediation of this type of diabetes, as well as some other autoimmune diseases, by the Th 1 pathway, in contrast to the Th 2 lymphocyte response implicated in atopic disease. Insulin-dependent diabetes mellitus (IDDM) has also been linked to early dietary exposure to cow’s milk infant formula (Verge et al. 1994) and to older maternal age at delivery (Bingley et al. 2000).

3.7.2 Juvenile arthritis

A Danish study investigated the socio-economic background of cases of juvenile chronic arthritis (JVA) to explore the possibility of a hygiene link as well as the family size factors (Nielsen et al. 1999). The matched case control study cohort studied included all 220 confirmed cases of JVA, measuring factors such as family size, urban or rural residence, overcrowding, type of housing and parental income. While only children with high-income parents had a statistically significant greater risk of JVA, the investigators found no evidence to support either hygienic or unhygienic conditions in the aetiology of JVA. Children living in an urban flat had nearly a three-fold greater risk compared
with those living on a farm. Pugh et al. (1993) reported an association between JVA and previous exposure to parvovirus B19, Influenza A, Coxsackie B4 virus infection and enteric pathogens, such as *Salmonella* spp, *Campylobacter* spp and *Chlamydia*.

### 3.7.3 Chronic bowel disease

The aetiology of chronic inflammatory bowel diseases, such as ulcerative colitis and Crohn’s disease, is mostly unknown, but shows wide variation between countries, with particularly high levels in the developed world (Langman et al. 1983). Environmental, as well as genetic influences, have been postulated. Crohn’s disease has become more common in developed countries during the past 50 years (Gent et al. 1994). Trends for ulcerative colitis are less consistent, for example, reported as falling slightly in a Scottish study comparing rates between 1968 and 1983 (Barton et al. 1989). The three-fold rise in Crohn’s disease over this period prompted a study to examine whether delayed exposure to enteric infections could be responsible (Box 3.7a).

**Box 3.7a: Domestic hygiene and Crohn’s disease**

Gent et al. (1994) tested the hygiene hypothesis in a matched case control study of 133 patients with Crohn’s disease and 231 patients with ulcerative colitis patients, comparing their early housing conditions in three English districts. Controls were age and sex matched and from the same general practice lists as the cases. Crohn’s disease patients were significantly more likely to have had a hot water tap in their first house and a separate bathroom, compared with controls: adjustment in the statistical analysis for social class in infancy increased the odds ratios. There was no clear association with household amenities for the ulcerative colitis patients, although they were much less likely to have had an appendicectomy, a finding also observed in other studies. The authors concluded that “good domestic hygiene may protect from exposure to a full range of agents that programme the immune system of the gut during infancy.” They also predicted that improvement of domestic hygiene in developing countries could potentially lead to a steep increase in the incidence of Crohn’s disease.

McCormick and Manning (2001) reported a study designed to test whether the hygiene hypothesis applied to the Irish itinerant ‘travelling’ community: a questionnaire survey of gastroenterologists and bowel surgeons revealed that none could recall ever having seen a ‘traveller’ with idiopathic inflammatory bowel disease. They postulated that a less hygienic environ-
ment might protect the travelling community from later bowel disease. The study on Irish itinerants was uncontrolled and therefore both recall and ascertainment bias might partly explain the results, as well as no information on other factors that would explain a low prevalence in itinerant/low socio-economic groups, such as diet, or indeed any information on their hygiene practices. The study by Gent et al. (1994) was designed to reduce bias by using matched controls and they also investigated other household amenities, such as flush toilets, mains drainage and any water tap (perhaps surprisingly for this era, several of the cases and more of the controls recalled no water tap in their early homes). Only hot water and a separate bathroom were more significant early exposures for the cases. Both studies used the proxy exposure of household amenities to infer hygiene conditions. The conclusion by both sets of investigators, that an over-clean environment may cause such bowel disease, would need verification from hygiene studies in itinerant and low income groups; poor hygiene practice cannot be assumed to be an established factor, as discussed in regard to farming and rural families. The largest confounder in the proxy measures of bathrooms and hot water supply is income, which could affect a wide range of factors relevant to bowel disease. Inconsistent results have been reported for risk of appendicitis in studies on bathroom facilities Barker et al. (1988) and Coggon et al. (1991) (see 3.4.3.IV). Further research is needed on whether hygiene or other factors are involved in the findings regarding Crohn’s disease.

3.7.4 Other diseases involving the immune system

In addition to the atopic and autoimmune diseases that directly involve immune responses, other disease groups have been added to the list of diseases related to inappropriate immune responses and thus potentially diseases to which the hygiene hypothesis would apply. **Childhood leukaemia** [particularly acute lymphoblastic leukaemia (ALL)] is of particular interest because both infectious and reduced microbial exposure aetiologies have been suggested, including the ‘Greaves hypothesis’ (Greaves 1993) that delayed exposure to infection is involved (Box 3.7b).
Box 3.7b: Microbial exposure and childhood leukaemia

- In a population based case control study in Quebec, Canada, a raised risk of childhood ALL was associated with older siblings, antibiotic treatment of the mother during pregnancy and being born second or later, whereas early day-care attendance was protective (Infante-Rivard et al. 2000): there was no association with a marker for social mixing, a proxy for microbial exposure. In a German case control study (Kaatsch et al. 1996), a significant association was reported between childhood leukaemia and vaccination, fewer virus related infections, breastfeeding and less social mixing. Smith et al. (1998) reported an inverse relationship between hepatitis A virus and ALL, which they attributed to improved hygiene during the 20th century.

- Reduced microbial exposure was suggested to be involved in a marked (70%) increased risk of leukaemia in children aged 1-4, compared with cohorts born 20 years previously (McNally et al. 2000, McNally et al. 2001). Over-estimation of trends has also been suggested due to ascertainment bias, such as introduction of new diagnostic techniques (Feltbower et al. 2001).

- Three recent case control studies found no overall evidence to support a reduced microbial exposure aetiology (Rosenbaum et al. 2000, Neglia et al. 2000, Dockerty et al. 1999; Dockerty et al. also reported an increased risk from early Influenza A infection and poorer social circumstances, supporting other evidence of an infectious aetiology (Kinlen 2001, Kinlen and Balkwill 2001, Alexander et al. 1997, Birch et al. 2000).

- Greaves postulated that delayed exposure to infection in developed countries could explain the reported rise in ALL (Greaves 1993); Michalek and Horvath (2002) reported lower seropositivity to human herpesvirus 6 in children with any paediatric cancer prior to treatment, compared with healthy controls. The Greaves hypothesis does not explain the excess of leukaemia in children younger than 2 years (Kinlen and Balkwill 2001).

Overall the evidence is stronger for infection causing leukaemia than in protecting children against it, and this also applies to other cancers such as cutaneous lymphomas (Goodlad et al. 2000). Genetic susceptibility appears to be involved with an excess of cases of leukaemia and lymphomas in males, which may be linked to increased susceptibility to infection (Birch et al. 2000). The association with either infection exposure or the lack of it needs further investigation to clarify the causation path and the role of timing of exposures in leukaemia or other cancers.
Atherosclerosis, the major cause of coronary heart disease, has been suggested to be an inflammatory process with an infectious aetiology (Mozar et al. 1990, Auer et al. 2002): poor hygiene/sanitation is implicated as causative, with no reports of protection from early microbial exposure (Box 3.7c). While detailed examination of the large field of atherosclerosis research is outside the scope of this review, it is relevant to the growing evidence of chronic diseases attributed both to infection and to dysfunctional inflammatory processes.

Box 3.7c: Atherosclerosis, infection and hygiene

- Mozar et al. (1990) proposed that improved sanitation and food hygiene could be responsible for the decreasing mortality from heart disease in the last quarter of the 20th century, although also acknowledging the effects of dietary control (e.g. of cholesterol) and treatment of associated hypertension.

- The possibility that arteriosclerosis might be inflammatory was first proposed by the German pathologist Virchow in 1856; and the agreement since then that it represents a response to injury to the endothelium of arteries gives credence to the ‘virus hypothesis’, supported by studies showing adverse effect on the heart and arteries by Coxsackie B4, herpes simplex virus and cytomegalovirus (Mozar et al. 1990).

- In a study of 218 patients referred for coronary angiography, Auer et al. (2002) reported that patients seropositive for more than four of six pathogens (hepatitis A, Chlamydia pneumoniae, Helicobacter pylori, herpes simplex virus and influenza types A and B) had a significantly higher prevalence of coronary artery disease. Auer et al. commented that this supported ‘studies showing an association of poverty and cardiovascular disease, in that infection would be more common in people living in disadvantaged circumstances.

3.7.5 Multi-level causation and the autoimmune diseases

The issue of hunting for risk factors in the autoimmune diseases was aptly summarised by McKinlay and Marceau (2000) in a recent review concerning diabetes, noting that there are illnesses for which dozens, even hundreds, of ‘independent’ risk factors have been reported. They suggest that risk factor epidemiology generally focuses on the isolated contribution from one factor, while overlooking competing influences from other levels:

“Although the ‘discovery’ of a new risk factors creates the illusion of progress, we do not know how much any one factor contributes to the total explana-
tion, and whether modification of that risk factor would appreciably alter the prevalence of the condition.”

Thus, although the proposed risk factors for diabetes include sex, ethnicity, family history, age, obesity, body-fat distribution, socio-economic status and the ‘new’ factor of the hygiene hypothesis, the contribution of these factors to diabetes remains uncertain. In general, the evidence for the hygiene hypothesis in auto-immune diseases is not convincing, although the studies have revealed other interesting associations and links with infection that merit further investigation. Platts-Mills and colleagues (2000) observed that the change in asthma in recent decades has much in common with the epidemic increase in diseases such as Type II diabetes or obesity. This lends support to the value of the multi-factorial approach: Platts-Mills et al. acknowledge that none of the current theories for the rise in asthma can yet explain the progressive increase in such diseases, but suggest that the decline in physical activity is one factor well worth pursuing.

3.8 Summary of the epidemiological evidence

This section has reviewed the epidemiological evidence for a link between susceptibility to atopy or autoimmune disease and infection or microbial exposure. This evidence is summarised below and will be further discussed in the Section 5. The particular focus of this review is whether the evidence indicates that any postulated reduction in microbial exposure could be linked to ‘hygiene’ in terms of amenities or practices. These issues are further discussed in Sections 4 and 5.

3.8.1 Evidence relating to microbial factors

3.8.1.1 Family size

The most consistent finding in support of a link between the postulated reduced microbial exposure and atopy (particularly hay fever and eczema) is related to family size. The inverse relationship between family size and risk of subsequent atopy has been reported by several studies and the role of larger household size in spread of infection during outbreaks has also been established in the field of infectious disease surveillance. However, there are puzzling discrepancies such as a stronger effect on brothers than sisters, lack of an overall association with family size in some studies. There are also differences depending on the type of atopic disorder or marker used to define atopy, such as skin prick tests, clinical case definition or inhalation tests. The protective effect associated with bed sharing (Svanes et al. 1999) and that observed in relation to day nursery attendance...
(a possible proxy for large families) support the results on family size, although the day nursery exposure results are inconsistent, with some studies not showing a protective association. To date, no generally accepted explanation has been found for the family size effect other than the proposed increase in opportunities for cross-infection. Alternative possibilities for the effect include immune system differences relating to sibship order, in other words, an effect depending on maternal influences on development in utero that alter with subsequent pregnancies.

3.8.1.1. Childhood infection

If the extent of cross infection within large families is an important factor, one might reasonably expect to find an inverse correlation between prevalence of atopy and confirmed history or evidence of specific or non-specific infections in early childhood. The epidemiological evidence for this is inconsistent and no individual infection or combination of specific infections has emerged as protective for subsequent atopy. Strachan, in a recent review, comments that the evidence of common specific and non-specific infectious diseases in infancy and childhood offers “no support for the ‘hygiene hypothesis’” (Strachan 2000). Wold (1998) similarly observed that “there is no strong evidence, at present, that the lacking factor is infections”. Matricardi also suggests that the attempts to link evidence of infection to atopy have raised as many questions as answers (Matricardi 1997). These questions relate to the type, number and duration of infection and the tissue, organ or system involved. As Matricardi observes, a great diversity of infections have been reported as inversely related to atopy; and therefore one must be very cautious before attributing a cause-effect link. Overall, there has been a greater emphasis on studying infections spread by the orofaecal route and their ability to stimulate the immune system (Holt 1996, Cookson and Moffatt 1997, Matricardi 1997, Matricardi and Bonini 2000a). In studies of the frequency of childhood illness, the evidence for ‘microbial load’ is stronger for viral respiratory infections than gastrointestinal infections (Holt et al. 1997, Martinez 1997, Berstad and Brandtzaeg 2000, Illi et al. 2001.) However, the protective role of the load of early childhood infections is contradicted by two large recent studies (Bodner et al. 1998, Bager et al. 2002).
Exposure to endotoxins in early childhood, particularly on farms, has attracted recent attention as possible protective factors in the microbial load (Braun-Fahrlander et al. 2002) as well as cautious concern that these bacterial products may not be the answer to healthy immune development (Douwes et al. 2002). The current evidence regarding microbial load does not determine whether the key exposure is via a more ‘natural’ (microbe-rich) diet or via cross contamination/airborne exposure in the farming environment. Studies on endotoxins, including those related to high-risk occupations such as wastewater workers, suggest that these may spread by both the oral-faecal and respiratory routes, as well as via food. In order to determine whether hygiene practices could or have influenced this type of exposure, it will be necessary to obtain more precise data on the most likely form of transmission to humans. If endotoxins are predominantly spread by the oro-faecal route in farming environments, then hand and surface hygiene could have a significant effect on transmission. If airborne transmission predominates, then hygiene practices are unlikely to have a major impact. It will also be important to observe and document hygiene practices: what is the evidence that people on farms wash their hands or surfaces less than in other environments?

3.8.1.III Unhygienic contact and improved household amenities

The epidemiological evidence for links between reduced microbial exposure and hygiene rests entirely on assumed proxy measures of good or poor hygiene, such as family size and rural or urban exposures in childhood, or poverty. This involves two assumptions: first, that such factors are inevitably associated with unhygienic surroundings and poor hygiene behaviours. This may apply in some circumstances, but cannot be assumed. The second assumption is that unhygienic surroundings are evidence of increased microbial exposure: for example, it could be the case that people who are aware of the risk of infection transmission are more careful when the surroundings are obviously unclean. The lack of any direct evidence, for example, quantified differences in hygiene standards/facilities in association with differences in atopic disorders, presents a severe limitation for application of the hygiene hypothesis to domestic hygiene. The trials with mycobacterial vaccines have demonstrated possible beneficial effects on diseases of immune dysregulation, but these findings do not prove that improved hygiene is responsible for immune system disorders.
3. IMMUNE SYSTEM DISORDERS: TRENDS AND EPIDEMIOLOGICAL LINKS TO MICROBIAL EXPOSURE

3.8.1.IV Breastfeeding

Breastfeeding for at least 3 months, and possibly for longer than 9 months, is associated with decreased risk of atopy and asthma in the majority of studies examining this factor, but it also protects against some infections. This could indicate either, that non-invasive microbial exposure is the more important factor in regulating the immune system, or, that breastfeeding offers a protective effect independent of any infection exposure. In regard to the hygiene hypothesis, the evidence on breastfeeding does not support an inverse relationship between infections and subsequent atopy, since breastfeeding protects against both. Alternatively it would support a link between susceptibility to both immune dysfunction and to invasive infection. Firm conclusions are not possible because of the many confounders involved in assessing studies on breastfeeding: thus the effects of breastfeeding may be linked with other factors. Only one study investigating possible associations with breastfeeding, hygiene practice and atopy was identified for this review (Sherriff et al. 2002a&b): in the recent analysis of data from the ALSPAC cohort, children who had never been breastfed during the first six months were more likely to have high ‘hygiene scores’ than breastfed children. However, this finding was not statistically significant after adjustment for other factors such as maternal smoking, education and housing.

The evidence on microbial factors is summarised in Table 3.8a below.
### Proposed risk factor | Strength of evidence | Comment
--- | --- | ---
**Factors directly linked to microbial exposure**

**Childhood infections**
- Inconsistent results for specific infections: some studies report a protective effect from non-specific mild infections, but others do not, with no consensus on the type of infection that may be protective, e.g. respiratory vs gastrointestinal infection.
- The inconsistent evidence has led to the suggestion that the total infection burden, or total microbial turnover, is the protective factor. There is no evidence that it is necessary to become ill to acquire protection.

**Endotoxin exposure**
- Evidence relates mainly to assumption of exposure on farms (see proxy measures below) although lower levels of atopy have been reported in those exposed to confirmed higher levels of endotoxins in farmhouse dust, compared with non-farm households (Gereda et al. 2000; Braun-Farhlander et al. 2002).
- Involves an assumption that it is not necessary to become ill to acquire protection, since postulated effect appears to be independent of level of clinical infections. High endotoxin exposure (e.g. in wastewater workers) associated with minor health effects but not an increased level of infectious disease.

**Factors modifying the response to infection**

**Vaccination/antibiotics**
- No consistent evidence implicating an aetiological role for any vaccine or for courses of antibiotic therapy in early childhood.
- Research possibly hampered by difficulty of removing or controlling for confounding factors.

**Breastfeeding**
- Appears to protect both against infection and against subsequent (atopic) asthma and other forms of atopy; a few contradictory studies re: atopy protection.
- Effects re: atopy appear to be depend on relatively long duration of breastfeeding: confounding factors include the influence of the body mass index and family history; effect seems to be independent of an association with cross infection/hygiene.

**Probiotics**
- May help to prevent both diarrhoeal infections and the development of atopic disorders, particularly eczema.
- More research is needed on their effects.

**Proxy measures of microbial exposure**

**Family size/sibship/birth order**
- Family size effect is among the most consistent evidence, although some studies have not found an association with family size and analysis of sibship, birth order and gender have produced further inconsistencies.
- Even if large family size protects against atopy, its effect has been estimated to account for only a small fraction of the rise in atopy. High maternal parity (2 or more live births) was associated with high hygiene scores in the ALSPAC cohort (Sherriff et al. 2002a).

**Farming vs urban environment**
- Significant evidence that a farming environment is protective.
- Not clear whether the protective effect derives from microbial or non-microbial factors.

**Close contact with other children**
- Inconsistent: some studies suggest protection from close contact, others have reported no effect.
- Possible association with social or other factors, e.g. the studies comparing East and West German children.

**Socio-economic**
- Poverty reported as a risk factor for asthma.
- Little evidence of consistent association between socio-economic status and microbial exposure in industrialised countries.

Table 3.8a: Summary of evidence re: atopy for microbial factors
3. IMMUNE SYSTEM DISORDERS: TRENDS AND EPIDEMIOLOGICAL LINKS TO MICROBIAL EXPOSURE

3.8.2 Evidence relating to non-microbial factors

As with the microbial factors, the evidence for the contribution of non-microbial factors includes supporting as well as conflicting results. The evidence, summarised in Table 3.8b, appears to be strongest for changing elements in the diet and obesity, possibly associated with decreased physical activity, although there is as yet no epidemiological evidence for the latter, related to the difficulty in measuring activity accurately. The diet/obesity ‘hypothesis’ is supported by a combination of biological plausibility and the evidence suggesting that trends in diet and obesity show a chronological correlation with the recent rise in atopy. Recent studies linking asthma incidence to exercise in high-ozone environments may reflect the role of tissue architecture and development, rather than atopy, in the aetiology of asthma.

<table>
<thead>
<tr>
<th>Proposed risk factor</th>
<th>Strength of evidence</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental pollution</td>
<td>No causal effect established, including smoking.</td>
<td>Many confounding factors: generally viewed as inciting/exacerbating factors, although recent studies propose a causal effect for asthma from frequent vigorous exercise in high ozone environments.</td>
</tr>
<tr>
<td>Stress</td>
<td>Adverse events/deprived early conditions may predispose both to infection and to atopic disorder.</td>
<td>Both stress and the degree of adversity/deprivation poorly defined in most studies.</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>Low birth weight possible risk factor for asthma; poverty exacerbates asthma.</td>
<td>Immune system development thought to be particularly at risk in malnutrition.</td>
</tr>
<tr>
<td>Diet factors</td>
<td>Asthma/atopy associated with low intake of fresh fruit/vegetables/vitamin E/increased fat intake.</td>
<td>Temporal association of rise in atopic disorders with documented changes in diet.</td>
</tr>
<tr>
<td>Obesity</td>
<td>BMI positively associated with risk of adult onset &amp; childhood asthma; increasing obesity in children.</td>
<td>Temporal association of rise in atopic disorders and obesity trends.</td>
</tr>
<tr>
<td>Exercise</td>
<td>Prolonged physical activity has anti-inflammatory effect; circumstantial evidence of increasing sedentary lifestyle in childhood.</td>
<td>No epidemiological studies – difficult to measure physical activity. Possible interaction between diet and exercise.</td>
</tr>
<tr>
<td>Genetic predisposition</td>
<td>Genetic predisposition may be higher than previously thought; wide ethnic variation in susceptibility &amp; migration effects suggesting environmental interaction.</td>
<td>Importance of distinguishing between atopic and non-atopic asthma; research at early stage for markers for infection susceptibility and immune dysfunction susceptibility.</td>
</tr>
</tbody>
</table>

Table 3.8b: Summary of evidence re: atopy for non microbial factors i.e factors not implicated in the hygiene hypothesis
3.8.3 Evidence relating to autoimmune disease and other diseases linked to immune system dysregulation

There is conflicting evidence for the role of reduced exposure to infection in the aetiology of juvenile arthritis and leukaemia. The evidence is more supportive in the case of type 1 diabetes and Crohn’s disease. However it is difficult to argue the case on the grounds of biological plausibility, since there is also evidence that infection has a causative role in arthritis, diabetes, leukaemia and atherosclerosis. The immunological mechanisms involved in the effect of either absence or presence of infection in early life is also unclear. For example, type 1 diabetes is predominantly an interferon \( \gamma \) based disease, involving the Th 1 pathway, in contrast to the Th 2 pathway predominantly involved in atopy. While epidemiological evidence suggests a role of changing exposure to infection for diabetes, it is difficult to explain in the immunological terms used to support the hygiene hypothesis. It would imply that one cause (reduced microbial exposure) could affect opposite pathways, or alternatively that infection is associated with the balance and regulation of both the Th 1/Th 2 pathways. The evidence is also inconsistent, for with a reported protective effect associated with day care facilities in the diabetes study by McKinney and colleagues (2000) or the reverse effect of day care attendance reported by Verge et al. (1994). More investigation is needed on the interactions and specific exposures involved.

The studies implicating hygiene amenities in the aetiology of Crohn’s disease are intriguing and as yet unexplained by other factors, although confounders such as social class and diet may be involved. A well conducted case control study showed a possible protective effect from not having hot water and a separate bathroom in early childhood (Gent et al. 1994). The evidence for the influence of such amenities on subsequent appendicitis is conflicting. Hygiene practice or observed hygiene behaviours have not been studied in relation to any of the autoimmune diseases.

3.8.4 The overall epidemiological evidence

There is some individually striking evidence linking atopy to changed microbial exposure, particularly in regard to a shift from farming to less ‘animal rich’ and urban environments. However, the evidence is inconsistent and in some studies, directly conflicting. There is thus little indication that specific pathogen exposure, or acute, intense infectious disease, is required to prime the immune system as part of normal development. The concept of total microbial burden or turnover has been proposed to take account of the inconsistent results. Studies on probiotics and breastfeeding,
factors that would be expected to modify the body’s response to infection, appear to indicate protection both against infection and atopy. This suggests that it may be too simplistic to propose a direct relationship between total microbial turnover and tendency to atopy. The microbial factors, thus, require considerable further investigation.

In contrast, the evidence linking atopy to hygiene practice in the home, as a proxy measure of reduced microbial exposure, is relatively weak and circumstantial. It is based almost entirely on unsubstantiated assumptions about the way in which living conditions and socio-economic factors influence our hygiene habits and how these, in turn, affect exposure to microbes. With the exception of a very few studies, hygiene amenities have not been quantified or examined; and no studies have been conducted involving direct observation and measurement of hygiene practices and associated microbial exposures in relation to this group of diseases. Higher standards of household amenity, such as hot water supply, appear to be more closely associated with autoimmune diseases than with atopic disorders: the evidence for this effect is strongest for Crohn’s disease but conflicting for diabetes and juvenile arthritis.

The epidemiological data also provide evidence of dramatic alteration in other lifestyle factors during the time span for the rapid rise in atopy. Perhaps the most convincing of this is the effect of dietary trends, associated also with the trend towards more sedentary childhood activity (computers, TV) and an alarming rise in obesity. The current research focus on farms has not yet taken account of findings such as the greater strength of evidence in young farmers compared with others of their generation with different environments and lifestyles. Maziak comments on the influence of immunological theory in epidemiological research, particularly relating to the ‘farm effect’:

“We have been overwhelmed in the past decade or so by studies speculating something that could have led to the up-regulation of the T-helper-1 response or down-regulation of the T-helper-2-response, or both, whenever a decrease in asthma or its risk was noted in certain populations, and vice versa. As a result, we became less prone to considering other environmental factors that can contribute to asthma, but would not easily fit within the T-helper-1 and T-helper-2 education paradigm, such as social or lifestyle differentials of the disease.”

(Maziak 2002)
4.1 Introduction

This section describes international trends in infection, trends within England and Wales and other parts of Europe and studies on infection transmission based on the follow up of outbreaks as well as on laboratory simulations. In terms of overall microbial exposure, the data on infection recorded in surveillance systems represent only a small proportion of the exposure to pathogenic and non-pathogenic microbes. Nevertheless, changing trends in the type and frequency of recorded infectious disease are important to an evaluation of the hygiene hypothesis, particularly the idea that declining levels of childhood infections or of overall microbial load has occurred. If this assumption were correct, we would expect to find evidence of greatly reduced infection transmission, or marked changes in microbial exposure, over the last 30 years in the home and in other settings. Within the same period, we would also expect to find evidence of marked alteration in hygiene practices; and evidence of a decline in measured frequency occurrence of micro-organisms in the home and of opportunities for exposure to such organisms.

Definitions of what is meant by microbial exposure, infection and infectious disease were covered in Section 2.

4.2 International infection trends

Infection is still a major worldwide cause of mortality, with, for example, rising levels of malaria, tuberculosis and diarrhoeal disease. At the start of 2000, the United Nations (UN) reported that at least 1.1 billion people still lacked access to water supply and almost 2.5 billion had no access to proper sanitation (United Nations 2000). These billions of people include some of the poorest and unhealthiest in the world (WHO 1995, 2000b). Every year, between 12 and 13 million children die from the combined effects of malnutrition and infection (WHO 1999). While no longer experiencing this level of premature death, industrialised countries also have their share of gastrointestinal infection, with up to 30% suffering from foodborne diseases each year; and about 2–3% of cases of foodborne diseases lead to long-term ill health (WHO 2000a) (Box 4.2).
Box 4.2: International morbidity and mortality related to foodborne and other intestinal infectious disease (Sources: WHO 1999, WHO 2000a/b, Murray and Lopez 1996)

It was estimated by the WHO in 1999 than an estimated 4 billion people, including 1.5 billion children suffer diarrhoea due to water or foodborne infections every year.

2.2 million children under the age of 5 die from diarrhoeal disease annually (WHO 2000b).

12-13 million children die from the combined effects of malnutrition and infection (WHO 2000b).

2-3% of cases of foodborne disease cause chronic ill health (WHO 2000b).

Malnutrition affects nearly 30% of people in the developing world (WHO 2000a) and 800 million people face food deficit. These play a major role in their ill health, particularly infectious disease carried by unsafe food and water (Murray and Lopez 1996).

In industrialised countries, up to 30% of people suffer from foodborne diseases each year (WHO 2000a).

In developed countries this type of infection causes much lower morbidity and mortality than in the circumstances of poor sanitation and malnutrition found in developing countries, but it still makes a major contribution to morbidity. Increased travel between the developed and developing world and rapid means of transport have also ‘globalised’ the risk of acquiring infection (Kaul et al. 1999), so that the general trend has been an increase in infectious disease, internationally, over the last half century and one that is likely to continue, particularly regarding resistant infections, newly identified infections, such as HIV, and resurgence of old threats such as cholera and TB. Deaths attributable to infectious disease in the USA rose by 22% between 1980 and 1992, jumping from 5th to 3rd rank in the most important causes of death (Anon 1996).

4.3 Infection trends in England and Wales and other parts of Europe

“It is widely believed that the vast improvements in the living conditions of people in general and the immense strides that medical science has made in this century have reduced infectious diseases to a problem of minor importance. This belief is helped by the method of classifying infectious diseases in a way that obscures their importance. In fact…infectious diseases are as prevalent and as important as ever.”

(Kaprio L, 1984, preface to Velimirovic 1984)
Leo Kaprio, then Regional Director for the World Health Organization in Europe, made the above statement at a time of rapid rise in atopic disease. Velimirovic (1984) demonstrated, in an analysis of infectious disease morbidity and statistics for Europe, that the International Classification of Diseases (ICD) failed to reflect the frequency and importance of infections. The plea for better and more standardised surveillance systems in Europe continues today, but the list of notifiable diseases in most countries still reflects the concerns, and often also the outdated terminology, of earlier eras. Despite the emergence of infections with high mortality such as Human Immunodeficiency Virus (HIV), surveillance systems are still insufficient to accurately document the true level of infectious disease in Europe, and many other parts of the world. Regarding the issue as to whether infection was declining in the mid-1980s, Velimirovic concluded that there was evidence of a decline in the major infections, such as cholera and typhoid fever; and that the main change was in reduced morbidity and mortality rather than frequency. The reduction in morbidity and mortality attributed to treatment or prophylaxis (such as immunisation) and to improved socio-economic and working conditions.

4.3.1 Gastrointestinal infection in England and Wales

“The three major organisms implicated in gastrointestinal diseases, Campylobacter, E. coli O157 and Salmonella, share the same characteristics, namely that they do not produce preformed toxins in food, they do not develop spores and they are all heat sensitive. Given these features, and with the possible exception of Campylobacter infection, they should theoretically be preventable by good general and food hygiene measures alone. It is somewhat chastening, therefore, to note that the incidence of each of these infections was the highest ever recorded.”

(PHLS 1999)

During 1997 the reported incidence of food poisoning reached its highest ever level (PHLS 1999). This was also one of the warmest years of the last century (Department of Health 2002). Bentham and Langford (1995) have reported a significant correlation between the monthly incidence of food poisoning notifications and average ambient temperature in England and Wales. It therefore seems likely that longer hot spells and the now confirmed upwards trend in global temperature will lead to further increased levels of food poisoning in the future.

The general trend has been an increase in food poisoning notifications since the early 1970s and current trends in reported cases and outbreaks of gastrointestinal disease do not indicate a marked decline in outbreaks asso-
4. TRENDS IN INFECTIOUS DISEASE, MICROBIAL EXPOSURE AND HYGIENE PRACTICE IN THE HOME

ciated with the home or elsewhere. The proportion of such outbreaks associated with home-related factors has remained fairly constant. Although overall food poisoning figures for *Salmonella* have declined since 1998 (PHLS 2000a), the commonest cause of bacterial gastrointestinal infection, *Campylobacter*, shows a continuing rise: almost 54,000 cases were identified during 2000 (PHLS 2001a), associated with 16,443 days of illness and 236 hospital admissions (PHLS 2001b). The increase in *Campylobacter* reports occurred throughout the period of rising atopic disease: while initially this may have been related to the wider introduction of laboratory techniques for *Campylobacter* identification in the 1980s, the subsequent continued rise appears to reflect a real increase in incidence. By contrast, dysentery (e.g. related to *Shigella sonnei* infection) reached a peak in the mid-1990s and has since declined: this infection traditionally has a long-term cyclical pattern related to waxing and waning herd immunity. The concept of herd immunity refers to the proportion of subjects with immunity in a given population (John and Samuel 2000).

Infection morbidity due to viruses appears to be rising and, as with *Campylobacter*, has shown no decline. There is certainly no evidence of a fall in campylobacteriosis since the majority of laboratories introduced routine screening for the organism in the 1990s. The recent reduction in food poisoning related to *Salmonella* organisms, after a steady rising trend over several decades, appears to be due mainly to control of the epidemic serotype *Salmonella typhimurium PT104* in poultry and eggs.

It has been long accepted that the figures recorded from surveillance significantly underestimate the true incidence and prevalence of infectious disease. In the UK, reported cases of intestinal infectious disease (IID) reached a peak of 100,000 cases in 1998 (PHLS 2002), while the true figure was estimated at nearer two million cases (PHLS website for 1998 data). The extent of the under-estimation was revealed following the publication of a community based IID survey in England and Wales conducted in 1995/6 and reported early in 1999 (Wheeler *et al.* 1999). This showed that many minor cases are not reported to general practitioners, and that the true figure is nearer to 9.5 million cases, suggesting that one fifth of this population suffer from IID every year. According to the food standards agency about 50% of these infections are foodborne, the remainder being due to person to person transmission. Since many of these cases are ‘sporadic’ (not part of a recognised outbreak), and are not picked up by surveillance, it is reasonable to assume that a significant proportion of these infections occur in the community and in the home. The results of the study by Wheeler *et al.* suggest that around half of the unreported 9 million cases are viral in origin, particularly
Norovirus (formerly Norwalk-like virus), but also rotavirus. Studies from UK, Netherlands, Spain and Italy suggest that as much as 50-80% of food-borne Salmonella and Campylobacter infections arise in the home (Sheard 1986, Hoogenboom-Vergdegal and Postema 1990, Scuderi et al. 1996, Hernandez et al. 1998). Schmidt (1998), in a WHO report, also concluded that most food-borne IID occurs in the home.

Because of cyclic and seasonal variation, it is necessary to examine the surveillance data over 20 or so years in order to establish an overall increase or decrease. The trend in gastrointestinal infections has been generally upwards since CDSC commenced surveillance in 1977 (Figure 4.3) and an upward trend was also evident in earlier surveillance figures from the Public Health Laboratory Service. These data show no evidence of a decline in gastrointestinal infections during the key period for the rise in atopic disease; in fact, the reverse. Examining long-term trends also avoids the distortions by single cause outbreaks or temporary declines in particular years that are possibly related to herd immunity factors. For example, a rise in multi-resistant Salmonella typhimurium, DT104 in 2000 was epidemiologically related to consumption of salad vegetables in food eaten away from home or in takeaways (PHLS 2000b).

More recent trends can be examined by comparing the cumulative reports of infections with a similar time period in consecutive years. Recent comparison data reported from CDSC confirmed the inexorable rise in Campylobacter infections, but also a temporary decline in rotavirus. The general trend for rotavirus is an increase over the past 20 years, representing a major cause of diarrhoeal disease in infants (PHLS 2000a). Similarly, there has been an overall upward trend in norovirus over the last 20 years: a very modest decline occurred in the first few weeks of 2001. Salmonella infections in England and Wales reached a peak in 1997 and thereafter declined, related to the control of the epidemic organism S. enteritidis PT4 in poultry flocks. This phage type of S. enteritidis became the commonest identified type of Salmonella in the 1980s and 1990s, but now represents only about 33% of all salmonellosis in England and Wales (PHLS 2001b).
4.3.2 Non-gastrointestinal infections in England and Wales

Reported infections spreading by the respiratory route have also shown no overall decline, with the exception of those controllable by immunisation programmes, such as measles. The true incidence of respiratory tract infections is likely to be much higher than those few infections that are ascertained in laboratory reporting systems or by statutory notification. This particularly applies to the common, milder types of upper respiratory tract infection, such as the common cold and influenza-like illness attributed to a range of viruses. Consultation rates for influenza and influenza-like illness are well documented in the UK through the Royal College of General Practitioners surveillance scheme: a peak was noted in the winter of 1998-1999 of 200-400 per 100,000 population (PHLS 2002): although influenza epidemics show a cyclic pattern and vary in severity, and thus also in the level detected by surveillance, there was no overall change preceding or during the rapid rise of atopic disorders. Similar patterns and peaks of influenza activity have been reported from other European countries. Respiratory syncytial virus (RSV) peaks annually between November and February in temperate countries such as the UK: while it has declined slightly since 1997 (PHLS 2002), there was no recorded decline during the rapid rise of atopic disorders. As for the common cold, there is no evidence of any change in
incidence in recent decades or indeed over the last century, including the age groups affected by such infections. The evidence suggesting differences in frequency of colds and other minor infections related to day nursery attendance and other exposures, reviewed in Section 3, may indicate differences in susceptibility between population subgroups, but it cannot be used to infer any overall increase or decrease in these infections in the population.

**Tuberculosis** re-emerged in England and Wales in the late 1980s after years of gradual decline (Box 4.3). Most people exposed to invasive mycobacteria do not develop progressive disease, therefore it must be assumed that levels of subclinical or asymptomatic infection have also increased since 1987.

**Box 4.3: The rise in TB**

TB has increased by 34% since 1987 (PHLS 2001c). Tuberculosis is caused by *Mycobacterium tuberculosis*, and some other species of mycobacteria. The provisional total of tuberculosis notifications in England and Wales in 2000 was 10.6% higher than in 1999. Two thirds of the increase has occurred in London region, with smaller increases in South East and Trent health regions. A large proportion of these infections have been identified in men, particularly those in the 25-64 years age group (PHLS 2001c). People recently arriving in the UK from countries with high prevalence of tuberculosis are at particular risk.

**Measles**, one of the viruses implicated as possibly protective against atopic disease, has greatly declined in the UK following the introduction of the combined measles, mumps and rubella vaccine in 1988. A national immunisation campaign in 1994 averted an expected epidemic of measles and reduced population susceptibility by around 85% (Department of Health, Welsh Office, Scottish Office and DHSS Northern Ireland, 1996). Thus, the dramatic decline in measles post-dated the onset of the rapid rise in atopic disease. A single measles vaccine was introduced in 1968, but poor uptake and high prevalence of the infection meant that vaccine coverage was never sufficiently high to have an effect on virus transmission (Department of Health et al. 1996).
4.4 Infection transmission in the home

The search for a plausible explanation for the rise in atopy through reduced microbial exposure or infection implicitly involves the home environment, since this is the most likely setting for the postulated essential exposures for immune system development, although these may also occur in other community locations, such as play groups, schools, farms and outdoor environments. There have been few studies of the home environment in the context of degree of microbial exposure: rather, studies have focused on assumed proxy measures such as decreasing family size, which is held to present reduced opportunities for cross-infection. This subsection considers evidence for microbial exposure and infection transmission within the home and whether there is evidence of a decline. This includes data from microbiological and surveillance studies within the home and other community settings.

4.4.1 Sources of evidence for infection transmission in the home

Data showing how and to what extent infection transmission occurs in the home comes from a range of sources (Table 4.4).

| 1. | Laboratory simulation of infection transmission via contaminated food/articles used to wipe surfaces, etc. |
| 2. | Cross sectional audit surveys of the distribution of micro-organisms in the home. |
| 3. | Microbiological, epidemiological and environmental investigations following outbreaks or where a known source of contamination has been in the environment. |
| 4. | Studies of the persistence and dissemination of micro-organisms, for example, the duration for which an organism can be identified or isolated after known contamination, or the rate of spread to other items or surfaces after introduction of the organism. |
| 5. | Studies of the infective dose required to cause infectious disease. |
| 6. | Studies of the pathogenicity of the organism: its ability to invade and cause disease. |
| 7. | Studies of the host response to infection: the implications of impaired immunity or genetic susceptibility to particular infections. |

Table 4.4: Sources of evidence on infection transmission in the home

In terms of infection transmission, the home environment has been under-researched. The difficulty of conducting detailed studies within the home contributes to this lack of quantifiable evidence, in comparison with data available from other environments such as hospitals. While cross contamination has been shown to contribute to infection outbreaks in community settings, there are difficulties in conducting epidemiological trials of the impact of hygiene procedures on infection rates in the home, including the
size of the required study population, cost, ethical constraints and the problem of controlling or measuring the differing effects of exposure to pathogenic micro-organisms.

Audit studies have demonstrated a wide distribution of microbes, including potentially harmful microbes, in the home. The sample sizes in these studies are relatively small and most of the potentially harmful species identified in such studies are likely to be harmful only to particularly vulnerable individuals, such as those with immune suppression. Such evidence is, however, supplemented by studies on the persistence and dissemination of pathogens (harmful microbes) in the home, investigations following outbreaks, evidence of the importance of the infective dose, and the risk of microbial contamination causing illness being related to the number of micro-organisms present on hands and other surfaces in high-risk areas or situations. The type of organism is clearly also relevant: some are highly invasive pathogens; others need to be present in enormous numbers to have a health effect; others are merely commensal organisms that do not cause disease. Some of the latter may provide protection against disease, such as the lactobacilli and coliforms within the intestinal microflora. Finally, there is evidence about the host role in infection: some individuals have greater susceptibility, such as those with immune deficiency or impaired immunity due to age or chronic disease.

4.5 Microbial contamination in the domestic environment

Pathogenic, potentially pathogenic and commensal species of microorganisms are all introduced continually into the home on people, food, pets, and sometimes via the air. Contaminated water is an occasional problem even in European countries with rigorous controls. Additionally, sites where stagnant water accumulates can become a primary reservoir of microbes: examples include sinks, U-bends and toilets. Cleaning cloths and facecloths also provide a moist environment that readily supports microbial growth and transfer to other surfaces (Scott and Bloomfield 1990a, 1990b, 1993). In addition, the home contains fungi, moulds, insects and occasionally rodents, many of which have the potential to cause disease.

Audit studies have found that micro-organisms, including some potentially pathogenic species, are commonly found in all areas of the home environment (Finch et al. 1978, Scott et al. 1982, Speirs et al. 1995, Josephson et al. 1997, Rusin et al. 1998). There is no evidence to suggest that the pattern or level of microbial contamination in the home has significantly altered in the 20 years between the earliest and most recent of these studies. While raw food is probably the main source of contamination in kitchen areas, there is
evidence of the contributing role played by surfaces such as draining boards, sinks, U tubes, nappy buckets, dishcloths and cleaning utensils: wet sites, in particular, may act as permanent sources or reservoirs allowing the establishment of free-living bacterial populations.

In the bathroom or toilet, enteric bacteria probably originate from the toilet or directly from people, although permanent reservoirs of bacteria readily survive in baths, basins, cleaning cloths and face cloths, as demonstrated by laboratory studies of the survival of Gram-negative species (e.g. *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp. and *pseudomonas*) in samples of sink U-tube, toilet water and in contaminated wet cloths (Scott and Bloomfield 1990a, 1990b, Scott 1990). Most of the bacterial species isolated in these studies are not normally pathogenic to the healthy adult, but this type of evidence suggests an abundant population of micro-organisms in the home, including both pathogenic organisms (*Box 4.5*) as well as non-invasive species. Pathogenic organisms can also be shed, or transferred by contact, into the environment by infected family members or animals, or by carriers of pathogenic organisms, who may show no symptoms of disease.

**Box 4.5: Listeria, Salmonella and Campylobacter contamination in the home environment**

- *Listeria* spp. were found in 47% of 213 homes (kitchen sinks, dishcloths and washing up brushes, the refrigerator and the toothbrush) (Beumer *et al.* 1996) and from fridge surfaces in 2.2% of homes (Spiers *et al.* 1995).


- Barker and Bloomfield (2000) demonstrated that in 4 out of 6 homes containing a Salmonella case, the causative strain was isolated from faecal soiling under the flushing rim of the toilet and scale material in the toilet bowl, despite the fact that samples were taken 3 or more weeks following notification of the infection.

- Wilson *et al.* (1998) showed that, where there was a confirmed case of *Salmonella*, the same strain could be isolated from dishcloths in 6% of the homes.

- McDermid and Lever (1996) showed that *S. enteritidis* PT4 could remain viable in aerosols for up to 2 hours, providing the opportunity for spread during food processing and preparation.
4.5.1 Food and transmission of infection in the home

Pathogens introduced into the home on meat, poultry and other foods are readily dispersed by contact with hands and other surfaces. Surveys of chickens in the UK between 1979 and 1998 indicated contamination rates between 25 and 79% for Salmonella (PHLS 1989, Anon 1995) and 80-90% for Campylobacter (Anon 1999b, Bolton et al. 1999, Kramer et al. 2000). Recent data suggest that contamination rates have now fallen: the UK Food Standards Agency (FSA 2001) has quoted an average contamination rate in chickens of 50% for Campylobacter and 6% for Salmonella. However, regional figures vary: for example, Salmonella contamination in 17.4% of frozen chickens in Scotland and Campylobacter contamination in 89% of fresh chicken. A recent UK study (Jorgensen et al. 2002) showed that Salmonella and Campylobacter were present in 25% and 83% of raw chickens, respectively. Based on this data it can be estimated that in the UK at least 1 in 25 homes prepares a meal with contaminated chicken every day of the year. Similar or higher rates of chicken contamination have been reported from other European countries, such as the Netherlands, France, Italy and Germany (Humphrey 2001) and from a study conducted in Wales (Harrison et al. 2001). A UK study by Hardy et al. (2000) reported that Campylobacter was isolated from 5.8% of 243 samples of poultry outer packaging obtained from retail outlets. None of the samples were positive for Salmonella. Harrison et al. (2001) reported that 34% of poultry packaging samples were contaminated with Campylobacter and in 3% of cases the contamination was found on the outer surface. Salmonella was isolated from 11% of packaging, but was not detected on the outer surface.

A recently published study in the UK (Chapman et al. 2001) confirmed that both cattle and sheep are important reservoirs of E. coli O157, especially during the summer months. These investigators showed that 0.4-0.8% of meat products purchased from butchers were positive for E. coli O157.

4.5.2 Pets and transmission of infection in the home

Pets may bring potentially infectious organisms into the home. Domestic cats and dogs can act as reservoirs of Salmonella, Campylobacter and other enteric pathogens (Bruner and Gillespie 1966, Moreno et al. 1993) and resistant staphylococcal infections, such as Methicillin Resistant Staphylococcus aureus (MRSA) (Cefai et al. 1994). The paws of dogs and cats have been found to have high levels of bacterial contamination, leading to the conclusion that children in particular are at risk of salmonellosis from their pets (Morse et al. 1976). In a recent study (Harrison 2000), 19 species of...
**4. TRENDS IN INFECTIOUS DISEASE, MICROBIAL EXPOSURE AND HYGIENE PRACTICE IN THE HOME**

*Campylobacter*, including *C. upsaliensis* and *C. jejuni* were isolated from 100 faecal specimens obtained from a London cattery.

**4.5.3 The influence of poor housing on infection: fungi and moulds**

Studies have indicated a link between airborne bacteria and fungi, associated with poor or damp housing or damp environments such as shared bathrooms, and the incidence of respiratory allergies such as asthma (Flannigan 1991, Huguenin-Dumittan 1977, Nolard 2001). There have been a number of studies demonstrating a high prevalence and variety of fungal species in the home and studies on home and hospital outbreaks show their ability to cause outbreaks or increased prevalence of infections.

**4.5.4 Cross contamination and cross-infection from an infected source in the home**

There are several ways, apart from direct person to person, or animal to person transmission, in which contamination can spread in the home with resulting human exposure:

- cross contamination by hands;
- cross contamination via surfaces;
- cross contamination by cleaning materials such as cloths;
- aerosolisation of pathogens (sneezing, coughing, vomit);
- inadequate storage or preparation of food, leaving viable micro-organisms or allowing multiplication to a level that can cause disease.

Cross infection from an infected source in the home to a new host depends on a number of factors, including the number of microbial particles shed from the source into the environment. Although vomiting or diarrhoea eliminates organisms from the gut, it increases the potential for contamination of the environment and spread of the infection. In the following sections laboratory studies of the dispersal, survival and transfer of bacteria and viruses via hands and other surfaces, together with examples of outbreak investigations which implicated cross contamination as the likely cause are reviewed. These data have also been reviewed by Beumer *et al.* (2002).

**4.5.6.1 Cross contamination via hands and other surfaces**

The importance of hands as a means of transfer of micro-organisms from an infected source, from one contaminated surface to another, from hands to food, or directly from hand to mouth, is well accepted (Todd *et al.* 1983, Bauer 1990). There is a consensus in the medical and scientific commu...
nities that hand washing is one of the most fundamental ways of preventing cross infection, by removing transient organisms acquired from patients or the environment (Larson 1988, Rammelkamp et al. 1964; Salzman et al. 1967, Casewell and Phillips 1977, Maki 1978, Rotter 1984, Maki 1989, Bauer et al. 1990, Jarvis 1991, Aiello and Larson 2002). Poor personal hygiene in food handlers was identified as the second leading cause of illness in a review of foodborne illness between 1983 and 1987 (Bean et al. 1990).

School outbreaks of gastrointestinal infection have been attributed to cross contamination via unwashed or poorly washed hands (Kaltenthaler et al. 1995), including evidence that schools with higher hand coliform counts were more likely to have had a reported outbreak of gastro-enteritis in the past. Hand to mouth transfer has been implicated in E. coli O157 infections (Adak et al. 1996, Mead et al. 1997) including the finding that people preparing food in the homes of cases are significantly less likely to report washing their hands or work surfaces than those in control homes.

A number of laboratory based studies (Scott and Bloomfield 1990a, Humphrey et al. 1994, Cogan et al. 1999, Neely and Maley 2000) indicate that bacterial contamination on environmental surfaces can survive in significant numbers for periods of at least four hours, with some species surviving for at least 24 hours. Viral species including rotavirus, rhinovirus, adenovirus, poliovirus, herpes simplex virus and hepatitis A virus can also survive for significant periods on dry surfaces (Mahl and Sadler 1975, Nerurkar et al. 1983, Sattar et al. 1986, Ansari et al. 1988, Ward et al. 1991, Mbithi et al. 1991)

Other studies show that, when surfaces become contaminated the organisms are readily transferred via hands, cloths and other means to other surfaces, in numbers sufficient to represent an infection hazard (Scott and Bloomfield 1990a, 1990b, 1993, Hilton and Austin 2000). Recent studies demonstrate that significant transfer can occur between hands, foods and kitchen surfaces (Chen et al. 2001) and between household objects, hands and the mouth (Rusin et al. 2000). The recognised importance of hand washing has possibly led to an under-emphasis on the role played by inanimate surfaces in the spread of infection in the home.

The risk of infection transmission in the home is supported by evidence of spread within families from a child infected at a day-care centre (Morrow et al. 1991; Fornasini et al. 1992; Osterholm et al. 1992), although direct case to case transmission as well as via surfaces is likely to be involved. Clostridium difficile has been shown to occur in day-care centres and to be transferred to families (Ahmed et al. 1993, Smith 1984). Contamination with this organism was common in the environment of patients with this disease.
It was often found on floors, toilets, bedding, mops, scales, and furniture in areas where the patient had been present and was isolated from the hands and stools of asymptomatic hospital personnel. It was also found on surfaces in a patient’s home.

4.6 Cross contamination and food poisoning

The data show that food preparation in the kitchen carries a particularly high risk of cross contamination via cloths, carving knives, mincing machines and other equipment. Chopping boards and cleaning cloths are amongst the most frequently implicated vehicles (Box 4.6a).

*E. coli* is a normal inhabitant of the gut, but invasive and resistant strains, such as *E. coli* O157, cause serious illness and outbreaks, which are almost invariably attributed to cross contamination from raw meat and poultry during preparation for sale or in the kitchen (Box 4.6b). *Campylobacter jejuni* emerged as a human pathogen in the 1980s and is now the commonest reported cause of bacterial gastrointestinal infection in the UK. Cross contamination related to the handling and consumption of poultry has been implicated as a cause of outbreaks of *C. jejuni* (Pebody *et al.* 1997) (Box 4.6c).

**Box 4.6a: Infection spread via chopping boards and cleaning cloths**

Chopping boards have been implicated in several outbreaks (Sanborn 1963), for example, a *Salmonella typhimurium* outbreak at a naval hospital associated with turkey: the organism was isolated from the chopping board, even though it had been cleaned after use. Another outbreak aboard a ship, due to *Salmonella chester*, occurred in two phases. Roast pork was the suspect vehicle in phase one, while turkey sandwiches were the suspect vehicle in phase two. Both foods had been carved on a cutting board placed on a table used to thaw frozen turkeys. The juices from the thawing turkeys apparently contaminated the cutting board.

*Salmonella enteritidis* was isolated from several environmental sites in the kitchen, including a cleaning cloth, after an outbreak arising in a public house (Holby *et al.* 1997). The suspected vehicles were pre-cooked food, presumed to be contaminated within the kitchen. Several sites were contaminated with *Salmonella* 9 days after the outbreak. Repeat sampling was negative only after thorough cleaning and the organism could still be sampled from a join between two work surfaces.

Unpasteurised cheese produced on a farm caused an outbreak of *Salmonella berta* through cross contamination (Ellis *et al.* 1998).
Zhao et al. (1998) showed that bacteria can be readily transferred to chopping boards after cutting and handling contaminated raw poultry; also that large numbers of bacteria can survive on the chopping boards for at least 4 hours – thus able to cross-contaminate fresh vegetables if the boards are not cleaned or disinfected.

Cogan et al. (1999) demonstrated that, following preparation of chickens contaminated with *Salmonella* and *Campylobacter* in 20 domestic kitchens, these species could be isolated from 16.8% of the hands and from hand and food contact surfaces sampled. In a further study (Cogan et al. 2002) involving a limited number of sites (hands, cloths, chopping boards, utensils, tap handles) the numbers of bacteria disseminated was evaluated. For *Salmonella*-contaminated surfaces, although counts of <10 were recorded on 18.3% of occasions, on 22 (18.3%) and 2 (1.7%) occasions counts were >10 and >1000 respectively. For *Campylobacter*-contaminated surfaces, high counts were isolated more frequently with 20% of sites recording counts of >1000. High counts of both species occurred on chopping boards despite the fact that participants were asked to clean the boards between preparing the chicken and the vegetables.

**Box 4.6b: E. coli O157 and cross contamination**

A widespread outbreak of *E. coli* O157 was associated with cross contamination in a butcher’s shop in Scotland in 1998 (Pennington 1998).

Hamburgers prepared in the home were implicated as a major cause of sporadic outbreaks of *E. coli* O157:H7 infection (Mead et al. 1997). Case households were more likely to report not washing their hands or work surfaces after contact with raw beef: they were also more likely to report placing cooked hamburgers on an unwashed plate that had previously been in contact with raw beef.

**Box 4.6c: Campylobacter and cross contamination**

A restaurant outbreak in 1998 was traced to cross contamination of food with *C. jejuni* from raw chicken (Anon 1998). Inspection of the kitchen showed the food preparation area to be too small to separate raw poultry and other foods adequately during preparation. The cook had cut up raw chicken before preparing salads and lasagne, which were statistically associated with illness. The lettuce or lasagne was probably contaminated with *C. jejuni* from raw chicken through unwashed or inadequately washed hands, cooking utensils or the countertop.
In a review of the mechanisms of transmission of foodborne infection in the UK, Roberts (1990) concluded that although most outbreaks are the result of poor temperature control of raw and cooked foods, many are directly or indirectly associated with cross contamination. Cross contamination was implicated in 6% of outbreaks and poor hand hygiene in about 4%. The data in Box 4.6d suggest that the proportion of food poisoning outbreaks attributed to cross contamination has increased rather than declined.

**Box 4.6d: Continuing evidence of cross contamination in the home**

Cross contamination was identified as a contributory factor in up to 14% of *Salmonella* outbreaks (Roberts 1986).

A UK survey of food poisoning in the domestic environment between 1992 and 1995 suggested that cross contamination contributed to 28% (28 of 101) of reported outbreaks (Ryan *et al.*, 1996).

Evans *et al.* (1998) reported that cross contamination was a contributing factor in 39% of general outbreaks of foodborne disease between 1995 and 1996.

Gillespie *et al.* (2002) reported that the most common faults in food hygiene in foodborne outbreaks linked with private homes were inappropriate storage, inadequate cooking and cross contamination (39%, 31% and 20% respectively).

### 4.7 Person to person spread of contamination and cross infection

The assumption is usually made that the unhygienic contact which produces person to person spread of infection involves either direct or airborne contact, or transfer via the hands. This ignores the possibility that the hands may have become contaminated by contact with contaminated surfaces. Several studies have attributed transfer via both hands and environmental surfaces as causing outbreaks (Box 4.7).
Box 4.7: Secondary spread of infection due to environmental contamination

A *Salmonella typhimurium* outbreak in a university hall of residence spread via shared toilets. Transmission was also enhanced by the shortage of toilets in the residential blocks (Palmer *et al.* 1981). The last case in this outbreak was a cleaner who emptied a commode used by a severely affected student and disposed of lightly soiled sheets, indicating that infection was via an environmental source.

*S. typhimurium* was isolated from ward dust and from sputum of patients after a hospital outbreak in which laundry workers and domestic staff were infected through contact with contaminated bed linen (Datta and Pridie 1960).

Secondary spread from a hospital outbreak of *S. typhimurium* outbreak in the USA, occurred in staff whose contact with infected patients involved handling only sheets and specimen bottles (Steere *et al.* 1975).

Linton *et al.* (1977) demonstrated that *E. coli* strains from chicken carcasses became part of the majority coliform flora of one volunteer who had handled, cooked and eaten the chickens at home. None of the strains had been detected in the faecal flora prior to handling the chicken. The chicken *E. coli* strains remained in the faecal flora of the food handler for about 10 days.

Parry and Salmon (1998) calculated that a member of the household caused between 4 and 14% of secondary cases, or asymptomatic infections, of *E. coli* O157.

Cross contamination, for example by sick relatives or pets, was identified as the main cause of infection in a study of 50 homes in which children under 4 years were known to be infected with *Salmonella* spp. (Schutze *et al.* 1999). Other family members were found to have had illness at the time of the illness of the index patients in 34% of homes.

From the results of recent investigations, it is increasingly accepted that IID in the community is by no means all food-borne, and that secondary person-to-person spread within families, particularly of viral infections, is often the cause. Person-to-person spread can occur via hands or surfaces, via food prepared in the home by an infected person, or by airborne spread (due to aerosolised particles resulting from vomiting or fluid diarrhoea). Evans *et al.* (1998) reported that, whereas 174 out of 233 outbreaks of infection in the UK attributed to *Salmonella* were “mainly food borne” and 15 regarded as “mainly person to person”, 607 of 680 reported outbreaks of Norovirus (formerly called SRSV) infection were attributed to person to person transfer.
and only 21 were reported as foodborne. In a more recent study of data from 4083 outbreaks, Le Baigue et al. (2000) suggested that 19% of *Salmonella* outbreaks were transmitted by other means and that under half of *E. coli* O157 outbreaks were foodborne. From studies demonstrating the spread of *C. jejuni* in commercial kitchens, Dawkins et al. (1984) concluded that, since cross contamination via work surfaces is equally likely to occur in domestic kitchens, it is not unreasonable to assume that cross contamination or careless personal hygiene in the home may account for many of the reported sporadic *Campylobacter* infections.

*Shigella sonnei*, the organism responsible for epidemic dysentery, spreads readily in the environment, especially during epidemic periods, such as 1992 in England and Wales, when 17,000 cases of dysentery were reported (Evans and Maguire 1996). Once a case has occurred within the home or within a school, it is difficult to eradicate the organism without careful attention to hand hygiene and cleaning of washbasins, toilets and surrounding areas (Khan 1982). Outbreaks are often centred on nursery schools and other community locations, but there is also substantial evidence for spread within the home. The infectious dose is very small and young children are implicated in the spread of shigellosis to their families.

Other evidence of persisting cross contamination includes the continuing frequency of staphylococcal (including Methicillin Resistant *Staphylococcus aureus*, MRSA) and streptococcal infections. MRSA is now notorious as a cause of infection within hospitals, but cross contamination in the home is one of the reasons why it is so hard to eradicate MRSA from communities (Cefai et al. 1994). *Group A streptococcal infections* spread rapidly in family, hospital and nursing home settings (Schwartz et al. 1992). Investigation of a nursing home outbreak of streptococcal skin lesions revealed the presence of the organism on carpets and soft furnishings as well as in throat swabs (Sarangi and Rowsell 1995).

The pathogen *Listeria monocytogenes* can survive at low temperatures and cross contamination is therefore a hazard within refrigerators as well as in food preparation areas. In an outbreak following a supper at a private home, the food vehicle was identified as a rice salad. It had been prepared 24 hours in advance and stored at room temperature until eaten. The role of cross contamination in this outbreak was demonstrated by isolation of *L. monocytogenes* from other foods left over from the supper, and from the blender and the freezer in the home of the cook (Salamina et al. 1996).

The opportunistic pathogen *Legionella pneumophila* spreads via the respiratory route, causing a range of illness including severe pneumonia. It sur-
vives in even relatively hot water supplies and can be isolated from showerheads, particularly if water has been stagnant in the shower piping for a few days. There is potential for spread in the home via aerosols during routine use (Bollin et al. 1985). *L. pneumophila* was also isolated from 30% of hot water distribution systems in apartment buildings in Finland (Zacheus and Martikainen 1994), the highest concentration being in the shower water. Like *Legionella*, the emerging pathogen *Cryptosporidium parvum* is widespread in the environment but has only been linked to water associated outbreaks since the early 1980s. It is a protozoan organism that may be transmitted via food or water. A major outbreak in Milwaukee, United States due to inadequate water treatment resulted in more than 400,000 people being ill and 100 deaths (Fox and Lyttle 1994).

4.8 Viral infections and contamination of the home environment

Because of the difficulty of isolating viruses in the environment, investigations have focused mainly on bacteria. Yet viruses, particularly Norovirus (formerly Norwalk-like virus or small round structured virus) are a significant cause of outbreaks of gastrointestinal infection, topping the figures for gastrointestinal outbreaks (Evans et al. 1998). Between 1992 and 1994, norovirus accounted for 27% of gastroenteritis outbreaks (Djuretic et al. 1996), rising to 43% for outbreaks during 1995-1996 (Evans et al. 1998). They are a major cause of outbreaks in institutions, such as nursing or residential homes for the elderly, schools and nurseries. Other important viral infections of the gastrointestinal tract include rotavirus, astrovirus and calicivirus.

During and after viral infections, virus particles may be shed in large numbers in many body fluids including blood, faeces, saliva, urine and nasal secretions. Evans et al. (1998) reported that rotavirus infections accounted for 3% of all reported food borne outbreaks. Outside of the hospital environment, rotavirus outbreaks are most usually reported for infants and young children in day care centres (Keswick et al. 1983, Pickering et al. 1988). Low humidity and crowding indoors is an important factor in the spread of this virus (Crowley et al. 1997) but surface to finger/surface to mouth transmission also readily occurs (Ward et al. 1991, Butz et al. 1993, Akhter et al. 1995). Ward et al. (1991) demonstrated that 13 out of 14 adult subjects became infected after consuming rotavirus (10^3 focus forming units) in a controlled laboratory experiment. Astroviruses and enteric adenoviruses have been associated with gastroenteritis outbreaks in schools, paediatric hospital wards and nursing homes (LeBaron et al. 1990). Rapid spread of virus outbreaks is both via the faecal-oral route and by infectious aerosols of projectile vomit, particularly in winter vomiting disease associated with norovirus.
While no studies have been carried out to investigate the prevalence of viral species at environmental sites and surfaces in the home environment, the evidence from investigations of environments, such as cruise ships, hotels and restaurant kitchens suggests that it is highly likely that viruses abound in the home following a viral illness in one of the family members. Norovirus has proved to be a difficult organism to eradicate in cruise ships and hotels, where recurrent outbreaks occur due to persistence of the virus on various surfaces such as carpets, curtains and toilet areas (Ho et al. 1989, Cheesbrough 1998). Pathogen transmission in child care facilities (a private home and a child care centre) has been studied by using modified cauliflower virus DNA as an environmental marker (Jiang et al. 1998). The DNA markers were stable for up to a month in the environment. The markers were introduced into the environment through DNA treated toy balls and spread in the environment was traced by detection of the DNA with PCR. The DNA markers spread within a few hours in both facilities. The marker-treated objects were removed after 1 day, but the markers continued circulating for up to 2 weeks. Hand contact with contaminated surfaces played an important part in the transmission of the markers. After introduction into the child care centre, the markers were detected in the children’s homes, on toys and environmental surfaces and from the hands of family members. The sudden and projectile vomiting associated with norovirus is a potent means of contaminating the environment. Outbreaks have been traced to vomiting in a sink (Patterson et al. 1997) or on carpets (Cheesbrough et al. 1997) (Box 4.8).

**Box 4.8: Norovirus and infection via a kitchen sink and carpets**

An outbreak of gastroenteritis followed a wedding reception, with 50% of guests affected. A kitchen assistant had vomited in the sink and subsequently cleaned it with a chlorine-based disinfectant. The sink was used to prepare potatoes on the following morning. The source of the outbreak was found to be the sink, demonstrating that SRSV is relatively resistant to environmental disinfection and can remain infectious on surfaces for several hours (Patterson et al. 1997).

In another outbreak, two carpet fitters became ill after removing a carpet from a hospital ward side room. An outbreak of norovirus had occurred earlier on the ward, with the final case symptoms ceasing 13 days before the removal of the carpet. Routine vacuuming every day since the outbreak had not removed the virus. (Cheesbrough et al. 1997)

Studies of the transmission of rhinovirus have shown high levels of self-inoculation via nose picking and eye rubbing; and also that perpetrators tend to repeat such behaviours (Hendley et al. 1973). However, interventions, such as disinfection of surfaces or by iodine application to the fingers could interrupt the transmission (Hendley and Gwaltney 1988).
In a review of respiratory virus transmission in the home, Goldmann (2000) concluded that these viruses exploit multiple modes of transmission. Family outbreaks of rhinovirus colds may be linked to survival of the virus on environmental surfaces at ambient temperatures. Sattar et al. (1993) and Hendley et al. (1973) showed that rhinoviruses could survive for several hours on the hands, and that self inoculation by rubbing of the nasal mucosa or conjunctivae with virus-contaminated hands infects susceptible hosts. Hygiene interventions reduced the level of respiratory and gastrointestinal infections in a pre-school day care centre, indicating that cross contamination had been an important factor in their spread (Krilov et al. 1996). The measures implemented included environmental surface cleaning and disinfection, including toys. Hall & Douglas (1981) demonstrated that direct and indirect contact transmission of respiratory syncytial virus (RSV) were more important than droplet (aerosol) transmission and also demonstrated, with Hall et al. (1978, 1980), that infants with RSV infection secrete large quantities of the virus, with the potential to survive for more than 5 hours on impervious surfaces, such as counter tops.

4.9 The importance of the dose of infection

The likelihood of cross infection (as opposed to cross contamination) depends on the number of microbes/particles that reach the new host: the greater the number of particles, the greater the risk of infectious disease. The evidence demonstrates the possibility of infection transmission from relatively low levels of contamination of surfaces, by person to person contact and via the faecal-oral route (Guthrie 1992). Estimates of the number of microorganisms required to cause infection have been derived from investigations with healthy adult volunteers (Box 4.9a).
Box 4.9a: Effective doses of pathogens based on studies with volunteers or outbreaks

The effective dose of *Salmonella* and *E. coli* varies with the particular strain from $10^2$ to $10^3$ cells up to $10^6$ to $10^7$ organisms (McCullough and Eisele 1951, Ferguson and June 1952, Lipson 1976). Waterman and Small (1998) demonstrated that the oral infectious dose of *Salmonella* is relatively low (50-100 organisms) when consumed as part of a contaminated food source.

Lower doses have been reported for favourable vehicles e.g. contaminated chocolate: 1-6 cells of *Salmonella nima* (Hockin et al. 1989), 60-65 cells of *Salmonella eastbourne* (Craven et al. 1975), 50-100 organisms of *Salmonella napoli* (Gill et al. 1983) and <10 organisms of *S. napoli* (Greenwood and Hooper 1983), and cheese (between 1 and 6 cells of *Salmonella typhimurium* from cheddar cheese were calculated as the infective dose for six patients who became ill after consuming the cheese (D’Aoust 1988).

The infectious dose for *E. coli* O157 is reported to be as small as 10 (Willshaw et al. 1994) and a median of less than 100 organisms per uncooked hamburger, in an outbreak in which the hamburgers were consumed after cooking (Griffin et al. 1994).

Ingestion of only 500 organisms of *Campylobacter* can cause human illness (Tauxe 1992).

An inoculum of up to $10^6$ cells of *Staph. aureus* may be required to produce pus in healthy skin but a lower dose of $10^2$ may be sufficient where the skin is occluded or traumatised (Marples 1976).

Intraocular injection of as few as 60 cells of *Pseudomonas aeruginosa* was sufficient to cause eye infections in rabbits (Crompton et al. 1962).

The infective dose for norovirus is of the order of 10-100 particles; over 30 million particles may be shed during vomiting (Caul 1994).

The minimal infective dose for rotavirus is as little as one cell culture-infective unit (Ward et al. 1986). The minimal infective dose for rhinoviruses via the nasal route has been found to be <1 TCID$_{50}$ (Couch 1990), whereby TCID$_{50}$ is the tissue culture infective dose giving 50% infection of cells. Parainfluenza viruses have an infective dose via the intranasal route of 80 TCID$_{50}$ (Smith et al. 1966). A minimal infective dose of less than 10 pfu (plaque forming units) has been demonstrated for poliovirus (Minor et al. 1981).
For some pathogens in ‘favourable’ vehicles that enhance survival and multiplication, the dose can be as small as 1-6 cells. This may explain why food borne transmission of organisms such as *Salmonella* occurs more readily than faecal-oral transmission. One of the first *Salmonella* strains for which a small infective dose was demonstrated was *Salmonella typhi*, the cause of typhoid fever (Box 4.9b): small infective doses have now been demonstrated for many other *Salmonella* serotypes.

**Box 4.9b: Typhoid fever, Typhoid Mary and domestic hygiene**

Typhoid fever is spread only by humans, and its success via the faecal-oral route is partly related to the relatively low infectious dose of *S. typhi* (*S. enterica* serovar *Typhi*) (<1000 cells, Bell and Kyridades 2002), as well as its persistence in asymptomatic carriers of the infection (Shanson 1999, Chin 2000). *S. typhi* also readily survives in water: before improvements in water treatment and sanitation in developed countries, typhoid fever was a major cause of morbidity and mortality and is still estimated to cause 17 million cases worldwide (Chin 2000). Its high prevalence in the 19th and early 20th centuries made the enteric fevers (typhoid and paratyphoid fever) familiar domestic hazards: in the classic famous case of ‘Typhoid Mary’ in the early 1900s, a cook caused several cases and outbreaks of enteric fever through hand contamination of foods – her case demonstrated the existence of an asymptomatic carrier state, as well as the role of hygiene in preventing spread from carriers, contaminated water and food.

The evidence demonstrates that infection can arise from relatively low levels of contamination on surfaces, by person to person contact and via the faecal-oral route (Guthrie 1992). The evidence reviewed in 4.5 to 4.8 suggests that, regardless of whether or not they have declined, levels of microbial exposure are still sufficient to cause infectious disease. The data show that high counts of a wide variety of micro-organisms can be isolated from both the internal and external environment. Logically, these levels must also be capable of causing subclinical or asymptomatic infection, all of which would be expected to stimulate an immune response.

### 4.10 Endotoxins in the home environment

Airborne endotoxins have been cited as important beneficial stimulants of the immune system, where the benefit translates to reduced subsequent atopic disease. Park *et al.* (2000) measured endotoxin dust in 20 homes in Boston, USA, over a 15-month period. High levels were identified, for example, on kitchen and bedroom floors, but variation in levels related to humidity and season. Measures of dust in two outdoor locations showed a strong
seasonal variation, with higher endotoxin levels in the summer. This study was designed to investigate the impact of seasonal variation on endotoxin levels, rather than hygiene, but if cleaning had a major impact, it would be expected to reduce or mask other causes of variation.

4.11 Trends in hygiene and household amenities

The hygiene hypothesis, as originally stated, proposes that the rapid rise of atopic disorders in the last 30 years of atopic disorders is linked to improved household amenities and to less opportunity for ‘unhygienic contact’. Temporal trends in hygiene and amenities are therefore important in assessing the hypothesis, in addition to studies investigating the impact of household amenities.

The rise of ‘hygiene’ in the late 19th and early 20th century

The modern practices of hygiene, water treatment and sanitation are relatively recent developments in terms of human evolution. Widespread access to clean water, soap and chemicals to aid cleaning dates back, with only a few exceptions, to the last hundred or so years. It was common to re-use dirty bandages in the 19th century and, as we know from the work of Semmelweiss on the postnatal wards in Vienna (Loudon 1992, Miller 1982), hand-washing after examining a corpse was regarded as eccentric in the mid 1850s. In the absence of confirmed knowledge of pathogens, and the prevalence of vague theories of disease transmission linked to miasma (Box 4.11) the application of hygienic practice was often erratic or even altogether absent.

Box 4.11: The miasma theory

Until the latter part of the nineteenth century, the transmission of infectious disease was predominantly explained by miasma theory. Miasmas were held to be poisons in the air, particularly emanating from rotting animal and vegetable material, the soil and stagnant water. Hence malaria, literally ‘bad air’, which seemed all the more aptly named to those who observed that people living by estuaries and in wetlands were particularly susceptible to ‘ague’ and ‘marsh fever’, common synonyms for malaria when it was prevalent in Europe. Similarly ‘spotted fever’, now known as typhus, was more prevalent in slums and crowded, squalid conditions: the derivation of typhus is the Greek tephos, meaning bad or unpleasant smoke.
4.11.1 Water supplies, soap and frequency of washing

In previous eras, the application of hygiene was partly dictated by the scarcity of domestic water supplies. A constant supply of water, much less of hot water, was still rare in mid-nineteenth century England (Hartley 1978). In earlier times there was a widely held belief that one could overdo the benefits of washing and that it could be detrimental to resistance to disease, possibly particularly in northern cultures where a bath could be a chilly experience: 

"Wash your hands often, your feet seldom and your head never."

English proverb (Strauss 1968)

This traditional suspicion of over-frequent washing may partly explain the populist interpretation and debate about modern 'clean' lifestyles, including the much higher availability of showers and baths. The trend towards the modern pattern of more frequent bathing and laundering in the USA and UK dates from 1890 to 1915. The emerging emphasis on cleanliness has been claimed to play an essential but generally ignored role in the sanitary revolution and the associated control of infectious disease (Greene 1984). Late 19th century and early 20th century legislation, such as the Public Baths and Wash Houses Act requiring local authorities to provide baths and laundry facilities for people without domestic water supplies, encouraged higher standards of cleanliness; and abolition of tax on soap was followed by a doubling of soap consumption between 1861 and 1891 (UKCPI 2002).

The supply of filtered water, municipal chlorination of water supplies and marked increase in soap use and bathing predates the rapid rise in atopic disorders by nearly a century. Soap manufacture in the USA more than doubled between 1904 (700,000 tons, 8.4 kg per capita) and 1919 (1,700,000 tons, 16 kg per capita) (Greene 1984). The increasing popularity of showers in the USA occurred between the 1940s and 1960s, with the proportion of American homes with bathtubs and/or showers increasing from 61% in 1940 to 87% in 1960 (Greene 1984).

4.11.2 Water treatment and sanitation

Chlorination of water supplies dates from the end of the 19th and early 20th centuries. In the UK, chloride of lime was used to control a waterborne outbreak of typhoid in 1897, followed by increasing chlorination of municipal supplies during the early 20th century (Galbraith 1994). Chlorine as a water disinfectant for a mains supply was first used in 1908 in the USA (Cantor 1994) and in 1910 in the UK (Galbraith 1994). Understanding of particular types of waterborne pathogens led to other water treatments such as sand fil-
tration and flocculation – and on the importance of separating drinking water supplies from sewerage systems. Availability of water for personal and domestic hygiene, and safe disposal of excreta, has been claimed to have a greater impact on health than improved drinking water quality alone (Esrey et al. 1991).

Progress in sanitation followed the first connection of house drains and cesspools to sewers in 1848 in England: the first public lavatory was opened in 1852 in Fleet Street, London (GLIAS 2002). It was nearly fifty years before such systems were common – and dumping of untreated sewage into the sea was not prohibited until 1998 (Halliday 1999). While the ‘sanitation revolution’ continued throughout the first part of the 20th century in developed countries, there appears to be no association with chronological trends in water and sanitation and the rapid rise in atopy over the last 30 years.

4.11.3 Home hygiene trends

The discovery of the transmission of infection by micro-organisms also led to much greater emphasis on hygiene within the home. Prior to the 20th century, home hygiene was most obvious in the approach to food preparation and storage. While the transmission of infection was not understood, avoiding the contamination or putrefaction of food, such as protecting it from rodents and insects, was a commonsense precaution. During the 20th century there was increasing emphasis on cleanliness in the home, with advice on regularly cleaning walls, ceilings and other areas partly prompted by the fear of infection before the antibiotic era. An increasing number of products and equipment were developed for home cleaning during the last century, but other social changes during the latter part of the century changed the approach to housework and its extent:

- Domestic help became less available and more expensive;
- Women increasingly worked outside the home and had less time for housework;
- Hygiene was perceived as less important with the arrival of vaccination and antibiotic therapy for many of the old infectious enemies, such as diphtheria and typhoid fever.

These changes led to a more superficial approach to home cleaning, with speed and aesthetic factors more important than the traditional emphasis on hygiene and disease prevention.
Most of the cleaning agents in use since the 1960s/70s are variants and mixtures of chemicals that have been available for a considerable time, such as bleach (hypochlorite solution – available as chloride of lime since the late 18th century) or creosol based disinfectants, introduced in 1877. The first detergent powders, introduced at the beginning of the 20th century, were developed by adding bleach to soap.

Usage of soap, detergents and cleaning products has continued to rise over the last 50 years, though at a lower rate than observed in the first quarter of the 20th century.

<table>
<thead>
<tr>
<th>Per capita consumption (kg) of soaps and detergents</th>
<th>1969</th>
<th>1977</th>
<th>1988</th>
<th>1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toilet soap</td>
<td>0.806</td>
<td>0.859</td>
<td>0.702</td>
<td>0.630</td>
</tr>
<tr>
<td>Hard surface cleaners</td>
<td>0.730</td>
<td>1.794</td>
<td>2.977</td>
<td>2.060</td>
</tr>
<tr>
<td>Scourers</td>
<td>1.029</td>
<td>0.942</td>
<td>0.668</td>
<td>0.490</td>
</tr>
<tr>
<td>Hard soap</td>
<td>1.188</td>
<td>0.746</td>
<td>0.445</td>
<td>0.370</td>
</tr>
<tr>
<td>Dishwashing detergents</td>
<td>1.183</td>
<td>2.272</td>
<td>3.794</td>
<td>3.120</td>
</tr>
<tr>
<td>Fabric washing detergents</td>
<td>6.957</td>
<td>7.951</td>
<td>8.376</td>
<td>8.890</td>
</tr>
<tr>
<td>Others (by difference)</td>
<td>1.807</td>
<td>3.496</td>
<td>5.756</td>
<td>3.040</td>
</tr>
<tr>
<td>Total soaps &amp; detergents*</td>
<td>13.700</td>
<td>18.060</td>
<td>22.718</td>
<td>18.600</td>
</tr>
</tbody>
</table>

Table 4.11.3

*Including Fabric Conditioning products which have no cleaning action

Source of data: Extracted from Statistical Tables published by AISE (Association Internationale de la Savonnerie, de la Detergence et des Produits d’Entretien) 49, Square Marie Louise, B-1000 Brussels.
The above figures show an apparent steady rise in per capita consumption of soaps and detergents to a peak in the late 80s, followed by a decline to 1994. However, changes in product classification as well as formulation distort the picture such that only broad assessments are possible. For example, the late 1980s saw rapid growth of liquid fabric washing and rinse conditioning products which would increase the weight of product consumed, but not ‘cleaning power’ deployed. Conversely, the introduction of more concentrated liquid and powder products in the late 80s and early 90s will have had the opposite effect (Pickup J, Personal communication). Assessing these figures in the light of major formulation trends Pickup (2003) estimated that the real increase in cleaning product usage across the 12 European countries covered over the 25 year period would have been of the order of 50% i.e. around 2% per annum. Such changes are considerably smaller than the variation in usage between countries, ranging from 8kg/cap in Finland to 30 kg/cap in Spain, though different national formulation preferences again distort this picture.

With regard to temporal trends in use of particular product types, Table 4.11.3 indicates the greatest increases have been in use of dishwashing products and hard surface cleaners, although the rise in the latter was substantially offset by the decline of hard soap and scouring products previously used for this purpose (Pickup 2003). Detergents based on synthetic surfactants, rather than soap, first came into general use in the 1950s, thus too early to be associated with the rise in atopy, biodegradable surfactants being introduced because of environmental concerns, such as foaming on rivers, in the early 1960s.

The other notable trend in hygiene products is the introduction of ‘anti-bacterial’ agents into cleaning materials, storage containers and other products in the mid 1990s with the aim of reducing microbial exposure. This occurred too recently to be considered as relating to the rise in atopic disorders.

In relation to the central tenet of the hygiene hypothesis, that childhood exposure to microbes has decreased, the key issue is whether the increasing availability of a wide variety of cleaning/disinfection agents and changing trends in hygiene practices have greatly reduced the total microbial exposure, particularly in early life. If there is evidence of continuing microbial exposure at levels sufficient to stimulate an immune response, the link with hygiene practices is much more tenuous. In assessing whether this ‘reduced opportunity for unhygienic contact’ bears a relationship to changes in our cleaning and hygiene habits, we need to evaluate both the bacteriolog-
ical studies of the impact of hygiene procedures on cross contamination and microbial exposure; and also the various case control studies and other investigations assessing the effect of intervention on infection rates. These factors are discussed in the next part of this section.

4.12 The impact of cleaning and hygiene practices on cross contamination and microbial exposure in the home

In applying a ‘hygiene’ procedure to sites and surfaces in the home, the purpose is to prevent further spread (and ultimately limit human exposure) by reducing the micro-organisms to a level which is no longer harmful to health. In practice a hygienically (as opposed to visibly) clean surface can be achieved either by removal of organisms from the surface, by inactivation in situ using a disinfection process, or a combination of both approaches. Since detergent-based cleaning involves mechanical removal of the microbes, to be effective in removing the majority of germs it must be applied in conjunction with a thorough rinsing process. People readily assume that wiping, using a cloth rinsed in soapy water, to produce a visibly clean chopping board achieves a surface that is also hygienically clean. In reality this may remove a large proportion of the bacteria but it also spreads residual bacteria in sufficient numbers around the surface and onto the cloth to be spread to other surfaces (Scott and Bloomfield 1990a). For surfaces which cannot be effectively rinsed, such as kitchen surface areas or other critical contact surfaces such as taps and flush handles, door handles and nappy changing areas, the means to achieve a hygienically clean surface is by cleaning followed by, or accompanied by chemical disinfection.

In recent years a range of laboratory and ‘in homes’ studies have been carried out which demonstrate that where hygiene procedures are used for a specific purpose, at a specific time, they can be effective in interrupting the chain of bacterial and viral transmission under use conditions in the domestic situation. Thus for example studies by Ward et al. (1991) and Sattar et al. (1993) demonstrated that disinfectants at recommended use dilutions were effective in preventing transfer of rotavirus contamination from dried stainless steel discs to finger pads. Oral consumption of the disinfectant-treated virus by licking the fingertips caused no infection in 14 subjects, whereas 13 of 14 subjects who consumed the untreated virus became infected. In a similar set of experiments these workers showed that the disinfectant prevented transfer of rhinovirus contamination from dried surfaces to hands (Sattar et al., 1993). Scott and Bloomfield (1993) showed that significant reduction in contamination of surfaces and cloths could be achieved by ensuring that contaminated cloths are not used in food preparation activities.
Yet while such studies indicate that hygiene can be successfully achieved by these processes, several of the ‘in-homes’ studies have demonstrated the persistence of pathogens and other micro-organisms after cleaning or disinfection procedures, particularly where these are done as part of day to day routine home cleaning. Some examples are given in Box 4.12. Other examples are reviewed by Beumer et al. 2002. Such evidence suggests that hygiene practices, as normally applied in the community, are often inappropriately conducted, for example, detergent based cleaning is carried out without rinsing, or disinfectant products are not properly applied. The ‘in homes’ study of Scott et al. (1984) (Figure 4.12) suggests that detergent-based cleaning can actually increase the spread of microbes in the home. Some recent evidence also shows that in some situations detergent-based cleaning with rinsing is insufficient to deliver hygiene, probably because the contaminating organisms are strongly adhered to the surface. Cogan et al. (2002) showed that detergent-based cleaning was not fully effective in removing Salmonella from contaminated hands and food preparation surfaces and was insufficient to decontaminate cleaning cloths.

**Box 4.12: Persistence of pathogens after cleaning procedures**

De Wit et al. (1979) showed that, following domestic preparation of chickens artificially contaminated with *E. coli* K12, the organisms could be isolated from surfaces such as chopping boards, cloths, the kitchen table and sink surface even after washing up. In 16/26 kitchens hygiene was achieved using detergent-based cleaning, but in 10 kitchens *E. coli* K12 was found after washing-up.

Schutze et al. (1999) carried out an investigation of 50 homes in which there was an infant infected with *Salmonella* species, such as *S. typhimurium* or *S. Newport*. Isolates of *Salmonella* were recovered from 19 (38%) homes. In all but two homes the same serotype was isolated from the environment, from another family member, or from pets in the home.

Cogan et al. (2002) evaluated the numbers of bacteria disseminated on hands, cloths, chopping boards, utensils and tap handles: samples taken immediately after meal preparation, on 8.3% and 50% of occasions respectively, had counts of *Salmonella* and *Campylobacter* exceeding 100 cfus per sample area with 4.8% and 35% of sites showing counts of >1000. Where surfaces were cleaned using the typical bowl washing routine, but then thoroughly rinsed under running water, a more significant reduction in the risk of contamination could be achieved, although 23% of 60 sites sampled still showed contamination with *Salmonella*, with 3.3% of sites showing counts of greater than 100 cfu.
Humphrey et al. (1994) recovered *Salmonella enteritidis* PT4 from fingers and utensils, sometimes after washing with soap and hot water, following preparation of egg dishes using artificially contaminated eggs – including recovery from dry films of either batter or eggs on work surfaces up to 24 hours after contamination.

An ‘in homes’ study (Scott et al. 1984) showed that, prior to cleaning, only 1 in 5 (20%) of 10 selected hand and food contact and other sites in the kitchen, bathroom and toilet could be considered as hygienic (<10 cfu per 25 cm²). After detergent-based cleaning, the proportion of contaminated sites actually increased to 68%.

‘Washing’ is insufficient for decontamination of cloths where they have become heavily soiled and the organisms strongly attached (Scott and Bloomfield 1990b).

Preventing infection transfer in the home depends not only on the inherent effectiveness of cleaning and disinfection procedures but also on the surface to which it is applied and, most particularly, on the time of application. The critical influence of these factors on both the extent and consequences of microbial exposure is rarely appreciated. The 1984 ‘in homes’ study of Scott et al. (Figure 4.12) showed that, although disinfectant products were effective in reducing microbial contamination levels, the effects were relatively short lived. After a relatively limited period (90 mins to 3 hours), most sites and surfaces become substantially recontaminated. This is probably due to reuse or, for wet sites or surfaces (e.g. for damp cloths), the regrowth of residual survivors not destroyed by the hygiene process. In a further study of the effects of daily application of disinfectant over a period of 3 days, there was no evidence of any sustained or cumulative effect in terms of the frequency occurrence of sites rated as ‘hygienic’.

Similar data was obtained by Josephson et al. (Table 4.12), 1997, who concluded that casual or irregular use of disinfectant cleaners is unlikely to reduce the risk of pathogens on kitchen surfaces. In this study, the effects of disinfectant usage, on a range of hand and food contact sites in the home, was determined over a period of 6-9 months. In phase 1 of the study sites were cleaned without disinfection. In phase 2 of the study homes were supplied with a disinfectant and encouraged, but not instructed, how to use it. In phase 3, the target surfaces were cleaned and disinfected immediately (5-10 min) before sampling. Although unfocussed disinfectant usage had little impact, in almost every case the incidence of contamination significantly decreased with targeted use of a disinfectant.
4. TRENDS IN INFECTIOUS DISEASE, MICROBIAL EXPOSURE AND HYGIENE PRACTICE IN THE HOME

Figure 4.12: Effectiveness of disinfection of soap and water cleaning at environmental sites in the domestic environment (From Scott et al. 1984)

Table 4.12: Number of samples with contamination in kitchen areas with and without use of a disinfectant cleaner (Josephson et al. 1997)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Percentage of samples with contamination in 10 households</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heterotrophic Bacteria</td>
</tr>
<tr>
<td>No disinfectant</td>
<td>98.8</td>
</tr>
<tr>
<td>Irregular use</td>
<td>95.2</td>
</tr>
<tr>
<td>Targeted use</td>
<td>58.4</td>
</tr>
</tbody>
</table>

4.13 Intervention studies

Evidence of the ability of impact of hygiene practice to reduce pathogen exposure has come from case control studies in which the effects of hygiene intervention on infection rates were monitored. In a recent review, Aiello and Larson (2002) evaluated the results of 64 intervention and observational studies between 1980 and 2001 although most of the studies relate to day care centres, schools or residential homes, or to communities in rural areas of developing countries where the concentration of people and activity provides the most cost effective setting for evaluating the impact of hygiene.
Box 4.13 contains examples of some such studies. In virtually all of the studies, handwashing was one of the factors studied. In some studies, this was combined with other hygiene interventions. Since the studies involved the implementation of infection control programs involving several steps, it is difficult to attribute the effectiveness of the hygiene procedures to one specific aspect because they were inherently multifaceted.

Box 4.13: Handwashing and other intervention studies

A recent study in which US Navy recruits were ordered to wash their hands five times a day, Ryan et al. (2001) showed that respiratory infections such as coughs were 45% lower than for a control group.

Surveys of the day-care centre environment have showed contamination of the surfaces, toys, food areas, nappy (diaper) changing areas, and the hands of children and adults (Ekanem et al. 1983, Holaday et al. 1990, Van et al. 1991a,b, Osterholm et al. 1992, Laborde et al. 1993). These studies also indicate a dose-response effect, for example, in the high coliform bacterial contamination revealed on toys and the association of high faecal contamination levels with increased risk of diarrhoea (Laborde et al. 1993).

A hand washing programme over a 35-week period was shown to be effective in halving the level of diarrhoea in day-care centres (Black et al. 1981). Staff training programmes also reduced diarrhoea rates in day-care in comparison with day units not offered a programme (Bartlett et al., 1988). One of the difficulties in evaluating such programmes is that multiple protective measures are involved, such as hand washing, use of vinyl gloves, alcohol-based hand rinses and other factors (Butz et al. 1990).

Guidance about hygiene re: nappy changing and adherence to hand washing successfully controlled a widespread outbreak in day care centres in Kentucky (Mohle-Boetani et al. 1995, CDC 1992).

Hand washing was identified as an important control measure in an outbreak of enteroinvasive E. coli at a school for mentally retarded adults and children in Missouri (Harris et al. 1985).

The ineffectiveness of general, as opposed to specific targeted cleaning activities, in reducing exposure to infection is suggested by the results of a recent study reported by Larsen and Duarte (2001). These workers carried out a retrospective study of home hygiene practices and prevalence of infection amongst household members in 398 households of an inner city popula-
tion. Results showed that only two specific practices, using a communal laundry and not using bleach in communal laundering, were predictive of increased risk of infection transmission. Other general cleaning practices such as daily personal bathing or showering, daily cleaning of bathrooms and toilets, frequent changing of dish-sponges (1-14 days), or use/non use of antimicrobial cleaning products for these activities showed no significant correlation with infection rates.

4.14 Observational studies of hygiene practices in the home

In the preceding section we observed how targeted hygiene interventions in community settings can reduce infection rates. The hygiene hypothesis proposes that changes in domestic cleaning and hygiene practices in recent years have reduced exposure to microbes. This begs an assumption that patterns of hygiene behaviour in the home are of a type and quality that will reduce exposure to pathogens. A number of observational studies have been carried out to assess the true standards of hygiene practice in the home.

Griffith et al. (1998) carried out an observational study of 108 UK participants to estimate the risk of food poisoning following domestic food preparation. Only a small proportion of consumers (4.6%) fully implemented appropriate food safety measures while 3.7% prepared food in a way that seriously violated recommended practice and exposed them to a high level of risk. The vast majority (95.4%) failed to implement one or more basic hygiene procedures, due to lack of knowledge or failure to implement known food safety procedures. Fifty eight percent of volunteers did not wash their hands after handling raw meat and poultry whilst 28% cut up food on a dirty chopping board. These results complement the studies of Cogan et al. (1999, 2002) described previously, which showed the extent to which pathogens are spread from a contaminated chicken during food preparation in a domestic kitchen. Some follow-up studies (Griffith and Humphrey unpublished) have now shown that following preparation of a chicken salad meal in a domestic kitchen using a contaminated chicken, the target organisms could be isolated from the salad on a number of occasions.

In a video study of domestic food handling practices in Australia (Jay et al. 1999), the most common unhygienic practices observed were: lack of handwashing before handling of food; poor handwashing technique; inadequate cleaning of kitchen surfaces; involvement of pets in the kitchen; and lack of separate hand and dish towels. These observed practices were inconsistent with the responses by participants to a food safety questionnaire.

Using polio vaccine virus as an indicator of viral contamination from
faeces, Curtis et al. (2003) carried out a study of the hygiene practices of mothers with young children in England. Of a total of 234 domestic surface samples from 10 households, 13% were positive for poliovirus. Hand contact sites were most frequently contaminated, such as bathroom taps, door handles, toilet flushes, liquid soap dispensers, nappy changing equipment and potties. Observations suggested that only 43% of childcarers washed their hands after changing a dirty nappy compared with 76% who washed them after toilet visits. 15% of bathroom sites were positive for poliovirus. Nappy changing took place mainly in the living rooms. Contact with living room surfaces and objects during nappy changing was frequent and evidence of faecal contamination was found on 12% of living room surfaces and 10% of kitchen surfaces. Such studies suggest that in relation to standards of hygiene practice specifically aimed at preventing transmission of pathogens in the home, public adherence to simple rules of hygiene remains poor.

4.15 Summary of infection trends

This section has reviewed trends in infectious disease, together with the evidence on the occurrence and transmission of microbes in the home environment, the impact of hygiene procedures and trends in hygiene amenities and behaviours. A number of microbiological problems and shortcomings must be taken into account in interpreting these data. These include difficulties in quantifying microbial or pathogen exposure, including selective or under-reporting/under-ascertainment of infection. There is also scanty evidence on the presence of asymptomatic infection, and colonisation and prevalence of (and exposure to) non-invasive micro-organisms in the environment. There are problems in determining the real incidence of infectious disease arising in the home and in ascertaining the underlying causes of sporadic episodes of infection or outbreaks occurring in this environment. Under reporting and under-ascertainment applies particularly to minor gastrointestinal infection (Wheeler et al. 1999).

The underlying causes for most cases of sporadic infections are rarely established. Even in outbreaks, it is usually much easier to identify the source, for example, contaminated chicken, than the contributing factors, such as food handling faults. Thus, figures on the proportion due to food handlers are less reliable. Also, while it is relatively straightforward to examine the process involved in cooking and storing food, it is socially difficult to interrogate food handlers about their personal hygiene, especially when an outbreak has occurred in someone’s home. Given these reporting and ascertainment problems, it is likely that the level of home acquired infection is far higher than that recorded in surveillance data.
4. TRENDS IN INFECTIOUS DISEASE, MICROBIAL EXPOSURE AND HYGIENE PRACTICE IN THE HOME

While there is only limited quantitative epidemiological data on the extent to which infection outbreaks are the result of poor hygiene, and even less data on exposure to non-pathogenic organisms or microbial by-products, the evidence presented in this section gives no support to the postulated significant decline in either during recent decades. The implication of ‘reduced infection burden’ or ‘reduced microbial exposure’ and the associated evidence is summarised in Table 4.15. This examines seven premises related to reduced microbial exposure. The conclusions from this are summarised under the headings of different types of exposure below.

4.15.1 Trends in infectious disease

Infections with a high mortality have generally declined, particularly in developed countries, but some have either appeared or re-surfacd (for example: HIV, TB). There is no evidence of a general decline across the broad range of infectious diseases in developed countries. For example, there is no overall evidence of a decline in gastrointestinal infection, with rising campylobacter enteritis and viral causes of gastroenteritis. The community based study of Wheeler et al. (1999) demonstrated the vast underestimation of IID, particularly sporadic cases occurring in the home. Salmonellosis has fallen but only since the late 1990s and there is reliable evidence of a previous continuous rise over several decades. The decline in hepatitis A (since 1994 in the UK), associated with vaccination, post-dates the start of the rise in cases of atopic disease, although it had already been in decline since the 1940s. This longer term decline in HAV may be related to generally improved housing and hygiene standards, but does not show any convincing temporal relationship with trends in atopic disorders. There is a mixed pattern for respiratory infections, with decline in some bacterial causes of respiratoy infection and those that spread by the airborne route. In the UK, the decline in measles since 1988 is not temporally associated with the start of the rise in asthma and other atopic disorders and TB has been rising through the 1980s and 1990s.
### Table 4.15: Reduction in microbial exposure or infection

<table>
<thead>
<tr>
<th>Type of postulated reduction</th>
<th>Evidence for and against the proposition that microbial exposure has declined</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Infections with a high mortality have declined worldwide/or have declined within industrialised countries.</td>
<td>Some infections, such as typhoid fever and typhus fever have declined, but mainly in industrialised countries. Now Cholera is not an indigenous risk in most developed countries, but has re-emerged as a major problem in some parts of the world. Survival from infection has undoubtedly improved due to availability of effective treatment and greater host resistance due to better nutrition. Treatment and removing the source of infection (e.g. separating sewage from drinking water) has had a major impact but infection worldwide is still a major killer and some serious infections have emerged or have re-emerged in industrialised countries such as the UK (e.g. HIV/AIDS and TB).</td>
</tr>
<tr>
<td>2 General decline across the broad range of infectious disease.</td>
<td>One infection, smallpox, has been eradicated by immunisation and other diseases for which a vaccine exists have also declined, e.g. measles, <em>Haemophilus influenzae</em> infection, hepatitis A. Some types of food poisoning have declined in the very recent past (late 1990s), e.g. salmonellosis, related to better control of infection in poultry. Others have emerged as a major problem, such as <em>Escherichia coli</em> O157 infection in meat or untreated water supplies. Animal infections are still a major problem, as evidenced by the recent UK outbreaks of Bovine Spongiform Encephalitis and Foot and Mouth disease.</td>
</tr>
<tr>
<td>3 Gastrointestinal infections have declined.</td>
<td>Decline in typhoid fever and cholera occurred too early to be linked with changes in atopy trends; other causes of gastrointestinal infection were on the increase during and before the rapid atopy rise e.g. <em>Campylobacter</em>, <em>Salmonella</em>, viral gastroenteritis. Poultry control of epidemics of <em>S. enteritidis</em> and <em>S. typhimurium</em> (late 1990s) post-dates rise in atopy.</td>
</tr>
<tr>
<td>4 Respiratory infections have declined.</td>
<td>No obvious decline, particularly of viral infections, but difficult to assess as such infections tend to be cyclical or seasonal. Diphtheria has declined since the 1940s in England and Wales: TB is now rising in the UK, particularly in cities with large numbers of high risk communities, such as London.</td>
</tr>
<tr>
<td>5 Subclinical infection/colonisation by environmental micro-organisms with low invasiveness has declined.</td>
<td>Not routinely measured so difficult to assess: there is no convincing evidence of lower levels of environmental organisms within the home or in institutions, except where hygiene practices have targeted high-risk areas such as operating theatres. The postulated decline in exposure to ‘beneficial’ micro-organisms is based on assumptions about less exposure in the modern lifestyle (less ‘rural’ exposure or outdoor activities in natural environments), or relate to trends that predate the rise in atopy (pigs no longer kept in the kitchen, pasteurised milk, treated water).</td>
</tr>
<tr>
<td>6 Reduced exposure to innocuous organisms.</td>
<td>As above [5] plus increasing interest in changes in gut microflora, suggesting different patterns of exposure are affecting the early colonisation of the gut, e.g. less likely to be colonised by lactobacilli in developed countries.</td>
</tr>
<tr>
<td>7 Exposure to microbial by-products.</td>
<td>E.g. endotoxins. Reduced exposure to animals and aerosolised animal faeces in rural environments is particularly implicated.</td>
</tr>
</tbody>
</table>

The Hygiene Hypothesis and Implications for Home Hygiene
4.15.2 Trends in exposure to environmental organisms

The postulated decline in ambient levels of non-invasive microorganisms, or those with low pathogenicity, is not supported by microbiological studies in homes and institutions. This does not exclude the possibility of a different pattern of early microbial exposure, related to food intake as well as to the move away from rural environments, with consequent effects on gut colonisation during development of the immune system.

The consumption of household cleaning products has increased during the critical period of the rise in atopy, although this has been of the relatively modest order of 50% over the last 35 years, and there is no evidence that we spend more time or energy on routine home cleaning. Microbial audit surveys of the home also suggest that routine daily or weekly cleaning has only a limited influence on the day to day levels of non-pathogens and potential pathogens in the general home environment, and one which by no means eliminates microbial exposure. Re-colonisation of surfaces rapidly occurs and many microbial species are adapted to survival for long periods, particularly on damp surfaces but also with the small amount of moisture available in apparently dry areas and soft furnishings. It has been suggested that cleaning and disinfection could gradually alter the microbial population of the indoor environment, in a similar manner to the documented changes in gut colonisation over time and in industrialised regions, but the available evidence does not support this. Reduced exposure to microbes found in outdoor rural environments may also be a factor, although this has more to do with structures and lifestyle than to cleaning and disinfection.

4.15.3 Trends in exposure to pathogens

The perception that more cleaning and cleanliness will directly result in reduced exposure to microbes has lead to a general assumption that ‘cleaning’ is synonymous with ‘hygiene’. Hygiene is concerned with controlling exposure to pathogenic microbes: the loose general usage of ‘cleaning’ in this sense is misleading and has confused the hygiene hypothesis debate. The evidence presented in this section suggests that routine daily cleaning habits, with the possible exception of showering and bathing, have little effect in reducing exposure to the human, animal or foodborne pathogens that are continuously brought into the home. The evidence shows that the primary routes for transmission of pathogens in the home are via hands, hand contact surfaces, food preparation surfaces and cloths and that normal day to day activities provide ample opportunities for exposure to foodborne pathogens or pathogens from infected people or pets. The prevention of infection from exposure to these pathogens depends on timely, targeted and effective
hygiene procedures. This is strongly supported by the evidence base, but results from observational studies suggest that consumer adherence to this basic rule of hygiene remain poor. Such data correlates with evidence suggesting that the significant proportion of IID transmitted via food or from person to person occurs in the home.

Although it is possible that the pattern of microbial exposure in the home has changed, the evidence argues strongly against a decline in overall microbial exposure. It would appear that the ability of the microbial flora in the environment to persist, and re-colonise, has not been affected by hygiene practices. The indoor environment remains a rich mixture of dust mites, other insects, bacteria, viruses, fungi and moulds – and our opportunities for exposure to these are increased, since a rising proportion of time is spent indoors (Platts-Mills 1994). The fact that this all pervading microbial exposure only occasionally results in infectious disease must relate to the ability of the immune system to deal with these threats. Since the infectious dose for many pathogens is very small, a dramatic and persistent reduction in environmental microbial exposure would be required to make an impact on the occurrence of infectious disease, unless this were efficiently targeted at the critical times and places. There is no evidence that any such reduction has occurred.

Regarding the postulated small family size effect in reducing microbial burden, there are several issues to be considered. Overcrowding and large household sizes are accepted factors in increasing the possibility of infection transmission: early investigations of diseases such as meningococcal meningitis and septicaemia established the importance of such factors as distance between beds in shared accommodation. Aerosol/airborne transmission is more likely in overcrowded environments; but transmission by most infection routes depends on behavioural factors. There is no evidence that there has been a revolutionary behavioural change in recent decades in the form of more frequent hand washing during food preparation or after toilet visits, or of other hygiene-related activities. Cross infection is still a major factor in infection transmission and the evidence suggests that this is more strongly related to education and understanding of infection risk, than to family size.
5 DISCUSSION OF THE EVIDENCE ON TRENDS IN IMMUNE SYSTEM DISORDERS, INFECTION AND HYGIENE

5.1 Introduction

The central tenet of the hygiene hypothesis is that declining microbial exposure is a major causative factor in the increasing incidence of atopy in recent years. The timing of such exposure appears to be important for protection against atopic disease, the possible ‘window’ for the effect being pregnancy and early infancy. The hypothesis further states that the reason why this key exposure no longer occurs, or occurs to an insufficient extent, is the trend towards smaller family sizes, improved household amenities and higher standards of personal cleanliness – in effect, cleaner homes.

In Section 3, the trends in atopic disease and epidemiological studies were reviewed, followed in Section 4 by trends in infectious disease, microbial transmission and microbial exposure. In this section, the evidence from these two fields is assessed in relation to two separate questions:

• How strong is the postulated link between the rise in atopy and a reduction in microbial exposure?

• Could such reduced microbial exposure be related to improved hygiene practice, household amenities and/or personal cleanliness?

There are several sources of evidence in relation to the first question, for or against an inverse relationship between atopy and reduced microbial exposure:

i. Epidemiological studies of the relationship between prevalence of atopy and measures of infection and infection exposure (Section 3);

ii. The temporal relationship between the rise in atopy and trends in infectious disease (Sections 3 and 4);

iii. The temporal relationship between the rise in atopic disease and trends in microbial exposure (Sections 3 and 4);

iv. The biological plausibility of the immunological link between microbial exposure and atopy (Sections 2 and 3).

Evidence in relation to the second question, for or against a relationship between reduced microbial exposure and hygiene practice, derives from the following sources:
5. DISCUSSION OF THE EVIDENCE ON TRENDS IN IMMUNE SYSTEM DISORDERS, INFECTION AND HYGIENE

i. Epidemiological studies of the relationship between the rise in atopy and proxy measures of cleanliness and hygiene (Section 3);

ii. The temporal relationship between the rise in atopy and trends in hygiene practice (Section 4);

iii. The influence of hygiene practice on microbial contamination and microbial exposure in the home (Section 4);

iv. The relationship between the route of exposure to microbes which reduce immune dysfunction and the potential influence of hygiene practice on microbial exposure via this route (Section 4).

5.2 Evidence for or against a relationship between atopy and reduced microbial exposure

5.2.1 Epidemiological studies of the relationship between prevalence of atopy and measures of infection and infection exposure

The review of the evidence suggests a multifactorial aetiology for atopic disorders, auto-immune disease and other types of immune dysfunction, with no single factor, for example pollution, diet, genetics or microbial exposure, accounting for all the different types of atopic disease, or for the rapid rise observed in the latter part of the 20th century. There is also evidence of a possible recent decline in the incidence of asthma in the UK, although it is too early to say whether the trend in this, or other atopic disorders, is changing. Nevertheless, various studies cited in subsections 3.4 and 3.5 suggest that reduced or changed exposure to micro-organisms could be associated with the rise in atopy. Several different types and routes of infection have been reported to be inversely related to atopy, that is, protective against expression of atopic disorders. The routes include both airborne and faecal-oral routes, and the implicated micro-organisms, or their components, include mycobacteria (both the species causing human tuberculosis and saprophytic species), Measles virus, Hepatitis A virus [HAV] and the endotoxins of gram-negative bacteria. Exposure to HAV early in life has been used as a marker of exposure to unhygienic conditions, for example by Matricardi and colleagues (1997, 2000), because of the established association of HAV with large family size and low socio-economic status. That it is only a marker is suggested by the conflicting results regarding the influence of HAV on later atopy. Inconsistent evidence from studies has prevented identification of the protective role of any specific micro-organisms, or definition of the contribution made by the location of the study, socio-economic levels, genetic variation and differences in diet. Matricardi (1997) has argued for caution...
in attributing a cause and effect link to this diversity until questions can be answered concerning the type of infection, number of infections, the tissue, system or organ involved and the duration of exposure. If the infection load has not markedly declined, except in the severity of its clinical effects, then the recent rise in atopic disease must be due to other factors. Infection, either in exposure or in absence, may of course play a part, since the immune system exists, partly, to respond to micro-organisms. The evidence of a continuing infection/microbial exposure burden does not in itself refute the infection exposure tenet in the hygiene hypothesis. Further more detailed examination of trends may indicate a role for particular infection or species of micro-organisms. Meanwhile the absence of evidence for a single microbial cause has led to the suggestion that the key factor may be the total microbial burden:

“It now appears that environmental ‘danger’ signals regulate the pattern of immune responses in early life. Microbial burden in general, and not any single acute infectious illness, is the main source of these signals.”

(Martinez 2001)

The possibility that endotoxins – a component of all gram-negative bacteria – have an important role in this mix of microbial exposure, is the subject of current research in this field (Weiss 2002, Braun-Fahrländer et al. 2002), particularly in regard to the lower prevalence of atopy in the children of farming families. Such evidence implicates differences in the composition of the microbial load, rather than its quantity. In particular, it suggests that there is something very special about the farming environment, as compared with other types of rural exposure or the urban environment. Whether the overall ‘microbial burden’ has decreased in recent years takes us to the next question, that of the temporal relationship of trends of microbial exposure and atopic disorders.

5.2.2 The temporal relationship between the rise in atopy and trends in infectious disease

“A common perception of serious communicable disease seems to be that they are predominantly problems of the developing world. This is wrong. First, the burden of disease from ordinary indigenous infections is considerable... Second, England and Wales are facing new infections.”

(PHLS 2002, p7)

If the trends in infection and atopy are related, then the data should show that the reduction in infectious disease (and microbial exposure, where measurable) should have occurred just prior to the start of rapid rise in the
incidence of atopy in the 1960s/1970s. Infections should also have continued to decline during the first couple of decades, at least, of the observed rise in atopy. Also, if the window of opportunity for immune system ‘priming’ is pregnancy and early infancy, one would expect to find evidence of a dramatic decline in reported infectious disease 3-6 years before the steep rise in atopy, particularly of specific infections identified as having a priming role.

As discussed in Section 4, the assumption that the rise in atopy is related to a decline in infectious disease is understandable since the majority of data on infectious disease trends has been derived from national surveillance data. Such surveillance focuses on serious, systemic infection and on outbreaks: the sporadic cases of infection that are common in the home environment are under-estimated or missed altogether in these systems, as demonstrated in the community based study of infectious intestinal disease in England (Wheeler et al. 1999, FSA 2000). As a result, an overall decline in infectious disease has been inferred from the decline in infection mortality, in hospital admissions for infection, and the success of vaccination programmes and antibiotics. The continuing burden of infectious disease, particularly that of sporadic or non-notifiable cases, tends to be overlooked. Even more neglected is the proportion of infection likely to be acquired in the home environment: in addition to the evidence from the UK, this has been estimated to account, for example, for 74% of salmonella infections in Italy (Scuderi et al. 1996) and for a high proportion of foodborne infections in Europe as a whole (Schmidt 1998). Thus it would appear that infection arising in the home is still common and that microbial exposure in the home to a wide variety of organisms is a universal experience, with no evidence of a decline in general microbial exposure.

Regarding temporal trends for specific infections, there is firm evidence from national surveillance data that specific infections have declined, particularly since the introduction of antibiotics and a wider range of vaccines from the 1940s onwards: examples include measles, mumps, rheumatic fever, tuberculosis and hepatitis A (HAV). These particular infections were cited recently as showing an inverse relationship with the incidence of immune disorders (Bach 2002). Yet there is no convincing temporal relationship of trends in these infections with those of atopy from the national surveillance of infections in the UK, a country with high incidence and prevalence of atopic disorders and one of those showing the rapid post-1970 rise. The decline in TB started much earlier, and has shown a recent rise in London and other metropolitan areas over the last decade. The decline in measles in the UK dates from the introduction of the combined measles, mumps and rubella vaccine in 1988. Similarly, the decline in HAV since 1994 in the UK is associated with the introduction of an effective vaccine and
postdates the rise in cases of atopic disease, although it had already been in decline since the 1940s. The longer term decline in HAV may be related to generally improved housing and hygiene standards, but shows no convincing temporal relationship with trends in atopic disorders.

As discussed in subsection 4.3, there has also been a continuing rise in many gastrointestinal infections documented by national surveillance during this time period and no evidence of any decline in common respiratory infections such as colds and influenza. Some proponents of the hygiene hypothesis hold that the earlier decline of other serious infections, such as cholera and typhoid, is relevant to the argument of reduced microbial burden. The decline in these diseases, associated with improvement in water, sanitation and personal hygiene, occurred in the late 19th and early 20th century in industrialised countries: if responsible for the greater phenotypic expression of atopic disorders, the rapid rise observed in the later 20th century should have occurred much earlier. An alternative possibility is that there are genetic links between greater susceptibility to infection and also to immune dysfunction. Enhanced cytokine responses have been reported in virally infected patients with asthma, apparently leading to prolonged lymphocyte and eosinophil accumulation in the lungs (Salvi and Holgate 2001). If the rise in immune dysfunction relates to the decline of exposure to lethal infections over the last hundred years, it could be due to survival of a higher proportion of children with genetic susceptibility to immune disorders. In the studies suggesting a possible protective relationship from early childhood infection, it is notable that the infections recorded are mild and certainly not life threatening: Varner (2002) has proposed that a genetic predisposition to atopy protects against infection in early life, and that human evolution has favoured individuals with this predisposition. If correct, this could explain the lower frequency of early, mild childhood infections in atopic children, compared with those who do not develop atopy. The unravelling of the human genome is still at a very early stage and further research may clarify such aspects of disease susceptibility.

5.2.3 The temporal relationship between the rise in atopic disease and trends in microbial exposure

Microbial exposure includes not just the reported or ascertained infections, but also the general burden of mostly mild infectious disease that eludes the surveillance systems. It also includes the extensive amounts of exposure which must occur, but are contained by the immune system without any observable clinical response. Thus there is no precise measure of the overall burden of microbial exposure. The most consistent proxy evidence is
the decline of family size over the last century, and the inverse relationship between family size and hayfever or eczema reported by Strachan and others (Section 3). In addition to the differences, as yet unexplained, related also to gender and birth order, there appears to be no temporal relationship between family size and the recent rise in atopy: the major decline in family size dates from the early 20th century in the UK and in most other industrialised countries where atopic disorders have increased. To date, no explanation has been found for the smaller family size effect other than the proposed decrease in opportunities for cross infection through direct person to person exposure to human borne pathogens and commensal micro-organisms. Wickens et al. (1999) estimated that declining family size could account for only a small proportion of the rise in atopy between 1961 and 1991. Nevertheless, the protective effect of a large family is supported by the lower level of atopy/asthma associated with bed sharing and day nursery attendance in early childhood, although again the results are inconsistent, with an absence of a protective effect in some studies.

Although there are no consistent data showing how types and levels of environmental strains have altered in the home over the past 30 years, the microbiological studies suggest that the home environment still abounds with microbes, with inevitable exposure to a wide range of pathogens, commensals and environmental species as part of normal day-to-day living. The evidence cited in subsections 4.4 to 4.10 shows that pathogens are introduced continuously into the home on contaminated food, or by infected people or pets. The data also show the extent to which these organisms are shed or otherwise dispersed from these sources, and their ability to survive and be transferred in significant numbers via hands, cloths, surfaces and aerosol transfer. If the protective association of large families and day nurseries is related to microbial exposure, it must therefore relate to transfer of human commensal organisms, or case to case spread of minor childhood infections, in addition to the mix of micro-organisms present in all home environments.

The fact that environmental exposure appears unchanged does not exclude the possibility of a different pattern of early microbial exposure. Altered patterns of food intake as well as to the move away from rural environments have the potential to affect the pattern of gut colonisation during development of the immune system.

5.2.4 The biological plausibility of the immunological link between microbial exposure and atopy

Biological plausibility refers to whether a theory is based in currently estab-
lished scientific knowledge. Immunological research has produced evidence of a complicated relationship between microbial exposure and immune responses, including the possibility of either enhancing or suppressing such responses. Specific infections have been shown to act on different components of the immune pathways, for example, measles, respiratory syncytial virus and helminth invasion (Section 3). The link with any specific infection and subsequent atopy has proved more elusive: although it is theoretically plausible, it is not supported by epidemiological research except in the possibility of a general microbial effect, such as by endotoxins (produced by a wide range of bacteria) or ‘microbial load’. Advancing knowledge of particular genes associated with atopy, for example the recently identified ADAM-33 gene associated with asthma (Shapiro and Owen 2002), may explain how genetic determinants of immune dysfunction could also affect responses to microbial stimulation. At the current state of knowledge, the suggested association between atopy and types or levels of microbial exposure remains theoretical. It is relevant to observe that a previous, but comparatively recent, theory of phenotypic expression of asthma rested partly on early infection as a cause, also on the grounds of biological plausibility. Specific types, and timing, of infections have been implicated with regard to the aetiology of some forms of auto-immune disease and cardiac disease (Section 3). The increasing influence of biological plausibility in research, and its perceived overall importance in establishing relationships in evidence-based medicine, is discussed later in this section.

5.3 Evidence for and against the proposition that reduced microbial exposure is related to improved hygiene practice, household amenities and personal cleanliness

Contrary to what one would expect if the hygiene hypothesis were correct, in the sense of our modern environment being ‘too clean’, there is no evidence that the microbial burden in homes or the proportion of cases or outbreaks of infection have been significantly reduced as the result of “higher standards of cleanliness and hygiene practice”.

The evidence for the effect of improved household amenities, such as water supply, baths, showers and sewerage, is complicated, as demonstrated in the studies on appendicitis and chronic bowel disease. Obviously it has improved hygiene in the broadest sense in terms of lowered exposure to faecal and other waterborne pathogens, and improved personal hygiene in terms of increased bathing and showering. Where these amenities have been implicated as protecting against immune dysfunction, for example Crohn’s disease, it is difficult to separate this assumed effect from other associated factors such
as low income and diet. The fact that household amenities do not appear to have affected the general microbial environment of the home is reassuring in terms of the increased emphasis on the postulated role microbial exposure in priming the immune system. As for hygiene practice, it is easy to see how this could be assumed to have greatly improved in line with the increased amenities: yet the evidence reviewed in this document suggests that both cleaning and hygiene practice is often inefficient or insufficient to reduce or prevent microbial exposure, whether at the level of personal hygiene (the uphill struggle to persuade people to wash their hands after activities with a high risk of microbial contamination) or in food preparation (the demonstration of persisting pathogenic contamination after general cleaning).

Behavioural, educational and other socio-economic factors are determinants in the application of hygiene practice or use of cleaning agents: in the recent studies of hygiene and allergy from the ALSPAC cohort (Sherriff et al. 2002a & b), there was a strong association between measures of low socio-economic status and low educational attainment with high frequency of washing children or using cleaning agents in the home. The type of household environment, particularly the evidence of apparent protection in a farm with high exposure to animals, has been used to support the argument of reduced microbial exposure in other rural or urban settings. Microbial exposure obviously varies between different environments and this is supported by the studies on differences in the gut microbial flora in developing and developed countries, but variation does not imply reduction, rather that the microbial mix may be different. Socioeconomic and behavioural factors also apply to the farming setting, such as differences in diet and increased opportunity for outdoor exercise: poor hygiene practice cannot be assumed in such environments, nor has increased microbial exposure been demonstrated.

5.3.1 Epidemiological studies of the relationship between the rise in atopy and proxy measures of cleanliness and hygiene

An assumption of reduced opportunity for unhygienic contact underpins the hygiene hypothesis. This proposition is based partly on evidence (declining family size, improved household amenities) which makes assumptions for which the inter-relationships are unclear or where temporal associations with the rise in atopy have not been convincingly demonstrated (reduced unhygienic contact, higher standards of personal cleanliness). In the absence of quantifiable data on how much hygiene and cleanliness occurs within homes and community facilities such as day nurseries, proxy exposures have been adopted in epidemiological studies, such as family size, farms and day nurseries. The tension between the evidence (microbial,
immunological) and assumptions (proxy exposures for poor hygiene) in the hypothesis is shown diagrammatically in Figure 5.3.

Figure 5.3: Infection versus proxy exposures in the hygiene hypothesis

Proxy exposures, unless linked to established and documented evidence of degrees of hygiene, cannot be evaluated. The gradual acceptance of the germ theory, as opposed to the miasma theory, as the cause of disease during the latter part of the 19th and early 20th centuries was driven by the discovery that particular pathogens caused particular types of infectious disease, by identifiable routes, and amenable to intervention studies. At present, the evidence in support of the role of hygiene in the aetiology of immune dysfunction is at a comparable level to the evidence for miasmas as a cause of disease in the 19th century: that is, it is based on proxy exposures that may turn out to have no direct role or link to measurable hygiene practice. In the case of the miasma theory, the proxy exposures cited included poor housing, poverty, malodorous environments and presence of sewage. These proxy factors proved to be indirect and sometimes inaccurate markers of the infection risk for example, there is no evidence of risk from untreated sewage unless there is hand to mouth contact or the sewage is aerosolised in a closed environment. Similarly, poverty increases the risk of a range of diseases, but not necessarily, and often not at all, because of poor hygiene.
5. DISCUSSION OF THE EVIDENCE ON TRENDS IN IMMUNE SYSTEM DISORDERS, INFECTION AND HYGIENE

5.3.2 Temporal relationship between the rise in atopy and trends in hygiene practice

During the last half century availability of household amenities and appliances and the range and effectiveness of household cleaning products have increased. These trends were reviewed in subsection 4.11. Water use has increased in all industrialised countries, including use both for personal cleanliness and for kitchen appliances such as dishwashers and washing machines. Yet the trend towards the modern pattern of more frequent bathing and laundering dates back to the beginning of the 20th century, with the most marked increase in soap use, for example, predating the rapid increase in atopy by at least 75 years. The proportion of American homes with showers increased by about 25% between 1940 and 1960 (Greene 1984), but had already reached 61% by 1940: similar rises in showers, although slightly later, have occurred in European countries but the rise in atopy occurred at much the same time throughout the industrialised world. The general and increased use of showers and cleaning products may have had an impact on skin flora and skin irritation associated with some forms of eczema, but with no apparent correlation with other forms of atopy. Although the evidence suggests that we spend more time on bathing, showering and handwashing for aesthetic purposes, recent behavioural studies in the UK show that compliance with handwashing at critical times to prevent transmission of pathogens is poor (e.g. after toilet visits or changing a nappy (Curtis et al. 2003) or during food handling (Griffith et al. 1998).

The evidence gives no support for a temporal relationship between provision of treated water supplies and sanitation and the rise in atopy over the last 30 years. Filtration and chlorination of municipal water supplies dates from the late 19th and early 20th century in most developed countries: only a small proportion of consumers depended on untreated private water supplies after this era, particularly in the UK. The main ‘watershed’ for water treatment is considered to be the Croydon typhoid outbreak of 1937, which demonstrated the importance of well monitored water treatment and chlorination during repairs as well as in day to day supply (Galbraith et al. 1987). Removal of pathogens in modern water treatment also inevitably removes most benign microbial bacterial contamination, such as the saprophytic mycobacteria proposed to have a role in immune system priming (Rook and Stanford 1998), but the temporal relationship does not fit with the rapid rise in atopy. Again, it is important to emphasise that water treatment and sanitation are not appropriate proxies for hygiene practice or behaviours.
While not specifically related to the hygiene hypothesis, as proposed by Strachan, it is important to consider the increasing use of ‘new’ cleaning agents which has recently been brought into the debate, for example by Sheriff et al. 2002 (b). As discussed in Section 3, statistics for use of soaps, detergents and cleaning agents show no correlation between consumption levels in different countries and the prevalence of asthma, even allowing for the fact that interpretation is made difficult by changes in the grouping and the concentration of products (Pickup 2003). Most of the agents in common use were available long before the rise in atopy, and while the introduction of enzymes in detergents in the 1960s has been associated with occupational asthma, prevalence of atopic disorders shows no correlation with recorded use of such agents in different countries, and systematic studies have also failed to find a link with the development of eczema (White et al. 1985, Andersen et al. 1998).

5.3.3 The influence of hygiene practice on microbial contamination and microbial exposure in the home

“…continuing rise in the incidence of the most common causes of serious bacterial gastrointestinal infection… the majority of cases of which should be preventable through good hygiene practices.”

(PHLS 1999, p5)

The important words in the extract above from an annual report from the Communicable Disease Surveillance Centre for 1997, incidentally reporting the highest ever increase in food poisoning in England and Wales, are “should be preventable”: later in the same report, “theoretically” is added to this potential prospect of prevention.

5.3.3.1 Exposure to environmental organisms

Microbial audit surveys of the home as reviewed in subsection 4.11 suggest that routine daily or weekly cleaning has only a limited influence on day-to-day levels of non-pathogens and potential pathogens in the general home environment. Such ‘hygiene’ practices in no way eliminate microbial exposure. Re-colonisation of surfaces rapidly occurs and many species are adapted to survival for long periods, particularly on damp surfaces, but also in apparently dry areas and soft furnishings. Also, there is evidence that detergent-based cleaning can increase the distribution of microbes in the home, for example via cleaning cloths. An analogy has been drawn by some proponents of the hygiene hypothesis, between the influence of antibiotics on the gut flora and disinfection and environmental colonisation in the suggestion that cleaning and disinfection could gradually alter the microbial popula-
tion of the indoor environment: there is no evidence to support this. While reduced exposure to microbes in an outdoor urban environment may occur, such a reduction (or variation) in microbial exposure relates more to structures and lifestyle than to cleaning and disinfection.

5.3.3.11 Exposure to pathogens

The use of proxy markers for cleaning and cleanliness in epidemiological studies appears to contain an assumption that ‘cleaning’ is synonymous with ‘hygiene’ as well as the inference that this significantly reduces exposure to pathogenic microbes. The evidence presented in subsection 4.11 suggests that in reality routine daily cleaning habits have little effect on reducing exposure to pathogens that are continuously brought into the home. The evidence shows that the main sources of these pathogens are food, infected people and pets, together with items such as cloths which can support the growth of a resident population of microbes. Thus, normal day-to-day activities provide ample opportunities for some exposure to foodborne, human or animal pathogens. The evidence shows that the primary routes for transmission of these and other microbes in the home is via hands, hand contact surfaces, food preparation surfaces and cloths. The evidence in subsection 4.13 demonstrates that the optimum means to reduce exposure to these pathogens to a level which is insufficient to prevent infection is by targeting hygiene procedures at these sites and surfaces at the right time e.g. after toilet visits, handling raw food, handling pets etc. Observational studies such as those described in subsection 4.14 suggest that consumer adherence to this basic principle of hygiene remains poor: this agrees with community studies which indicate that a significant proportion of IID is transmitted via food prepared at home, or from person to person within the home.

5.3.4 The relationship between the route of exposure to microbes which reduce immune dysfunction and the potential influence of hygiene practice on microbial exposure via this route

The route of infection or of microbial exposure has an essential bearing on whether the hygiene hypothesis can be used to refer to hygiene in terms of water treatment, zealous cleaning in the home or prevention of contamination of food by intestinal pathogens. Despite the continuing reference to hygiene and reduced infection in relation to the aetiology of immune system disorders, there is no clear evidence on the type of microbial exposure required to produce the postulated beneficial effects on the immune system. It has not been established whether this exposure involves pathogens or commensal/opportunist organisms, benign species or microbial by-products; or
whether asymptomatic infection or overt disease are involved, or indeed whether the route of infection is an important issue. Even if further evidence supports the concept of the total ‘microbial burden’ required for development of a healthy immune system, this notion is insufficiently precise to allow any conclusions as to whether modern hygiene practice has an impact in reducing the required level of microbial exposure.

5.4 Evaluation of the current evidence in terms of criteria for establishing environmental cause of disease

“Although consistent with current dogma, the conclusion that we should mimic the effect of childhood infection on the immune system is premature, if not totally flawed, on two counts. First, association, not causation, has been shown, and, secondly, the proposed mechanism of action is inconsistent with available evidence.”

(Anderson and Watson 2001a)

In the extract above, Anderson and Watson (2001a) were referring in particular to claims that type 1 diabetes is related to a more hygienic environment in early life, but their reference to the influential criteria to distinguish causation from association, originally proposed by Bradford Hill (1965), is pertinent to the analysis of the evidence for the hygiene hypothesis. His nine criteria to assess putative environmental causes of disease form a cornerstone of environmental epidemiology. These criteria as they apply to the hygiene hypothesis are listed in Table 5.4 and shown diagrammatically in Figure 5.4. Of the criteria examined in Table 5.4, the most consistent criterion supportive of the hygiene hypothesis is that of biological plausibility, particularly in terms of immunological research. Yet Bradford Hill attached much less importance to this criterion than to others, such as statistical strength of association, consistency of the association or repeatability of results. Referring to the plausibility criterion, he stated that:

“...this is a feature I am convinced we cannot demand. What is biologically plausible depends upon the biological knowledge of the day.”

(Bradford Hill, 1965, p298)

Nevertheless, biological plausibility has, in recent years, gained increasing importance as a major criterion, partly linked to the increasing emphasis on evidence-based medical practice and partly to the discoveries in immunology and genetics. One of the greatest strengths of the hygiene hypothesis is that, if it could be shown that microbial exposure has declined, the link with atopy is plausible in terms of the emerging research on the development and regulation of the immune system. Comparisons between levels of childhood infection, as investigated by Illi (2001) and others, are also
pertinent, since even if microbial exposure has not declined, the fact that
some children experience more frequent episodes of infection, and that these
children appear to have a reduced subsequent risk of atopic disorder, are
supportive of a beneficial role for microbial exposure in early life. On the
basis of the current epidemiological evidence, this beneficial exposure is
restricted to minor unspecified infection and also to children who may be less
susceptible, for various reasons, to progressive or intense infectious disease.
The evidence-based approach must necessarily exclude the unproved link
with hygiene practice, particularly as it is based on proxy or imprecisely
defined exposures (Figure 5.3). Of the four factors cited in support of the
hygiene hypothesis in Figure 5.3, all can be refuted by the existing infection
evidence:

• Decline in morbidity and mortality of infection has not reduced the over-
  all burden of infection from persisting or emerging types of infection.

• Cross infection remains an important cause of outbreaks/sporadic cases of
  infection.

• There is no convincing evidence of less exposure to environmental organ-
  isms: the evidence points rather at differences in urban/rural lifestyle and
  contributing influence of diet/income/sedentary habits/less outdoor exposure.

• There is no evidence of a link with hygiene practices, although there is
  evidence that effective hygiene practices reduce the spread of infection.
  The studies showing persistence of pathogens in homes or in other set-
  tings after outbreaks shows that hygiene practices are often ineffective.
  Despite evidence of the impact of hygiene education in particular settings,
  there is no evidence of marked improvement in hygiene practice on a
  population basis. Trends of declining time/less domestic help for house-
  work, would also support a proposition that household cleaning has
  become less rigorous in most homes over the last half century.
Table 5.4: Bradford Hill’s criteria for the strength of an epidemiological association between an environmental exposure and disease (Bradford Hill, 1965)

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Re: evidence for hygiene hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <strong>Strength of association:</strong></td>
<td>Statistically significant evidence of protective effect from exposure to various types of infectious disease/species of micro-organisms – but not consistent or specific.</td>
</tr>
<tr>
<td>2. <strong>Consistency:</strong> Association repeatedly observed by different persons, in different places, circumstances and times.</td>
<td>Only in non-specific terms, i.e. evidence from several countries but relating to different types of infectious disease/microbial exposure.</td>
</tr>
<tr>
<td>3. <strong>Specificity:</strong> Is the association restricted to specific groups of people, specific sites of exposure or to specific diseases?</td>
<td>Yes – results of studies suggest that the effect may be highly specific and may also depend on other co-factors, such as farming environment. Specificity important in establishing cause and effect but if the evidence shows restriction to particular groups/exposures, the results cannot be generalised to apply to wider groups, as in the hygiene hypothesis.</td>
</tr>
<tr>
<td>4. <strong>Temporality:</strong> Putative cause must precede effect.</td>
<td>No overall decline in reported episodes of infectious disease prior to the rapid rise in atopic disorders in last 30 years of 20th century. Reduction in mortality from infectious disease has occurred during this time period. Reduction in specific diseases either preceded rise by several decades (e.g. hepatitis A) or subsequent to it (e.g. measles). Some infectious diseases appear to have risen independently from trends in atopic disorders (e.g. tuberculosis). Other factors show stronger temporal association e.g. changes in diet.</td>
</tr>
<tr>
<td>5. <strong>Biological gradient</strong> ('Dose-response' effect)</td>
<td>Yes, for number of non-specific mild infections in early childhood – but not a consistent finding.</td>
</tr>
<tr>
<td>6. <strong>Plausibility:</strong> Biologically plausible from currently known science.</td>
<td>Yes – conforms to current knowledge about maturation and disorders of immune system e.g. balance between Th 1 and Th 2 pathways.</td>
</tr>
<tr>
<td>7. <strong>Coherence:</strong> No conflict with generally known facts of natural history/aetiology of disease.</td>
<td>Yes and no: infectious disease known to be a trigger for asthma and some other atopic disorders and a few infections implicated as causal rather than protective, e.g. RSV, measles (including conflicting results of studies, some showing protective effect, others harmful to immune system).</td>
</tr>
<tr>
<td>8. <strong>Experiment:</strong> Association demonstrated in controlled trials/laboratory animals.</td>
<td>Yes re: saprophytic mycobacteria and trials of vaccines; trials of microbe-free vs. dirty environment for humans neither feasible nor ethical but no cohort study evidence of benefit of ‘dirty’ environment – all evidence retrospective.</td>
</tr>
<tr>
<td>9. <strong>Analogy:</strong> evidence from comparative studies of other species/circumstances.</td>
<td>Yes re: animal studies of immunological development, but results not reliably/consistently transferable to humans.</td>
</tr>
</tbody>
</table>
Note: This can also apply to infection trends in adult life. While hygiene practices have been demonstrated to control or reduce cross infection, the hygiene hypothesis is judged as disproved on this count since the proportion of outbreaks related to cross infection has not declined.
Since the evidence shows that general microbial exposure has not declined, the reasons for differences between children in rates of infection, or in greater or lesser susceptibility to infection, require more research into the possible aetiological factors. This review suggests that these include important roles for diet and lifestyle, as well as in different types and expression of genetic predisposition.

5.5 Theory development and the hygiene hypothesis

As discussed earlier in the review, there are important differences between hypotheses and theories, the latter involving explanation of interrelationships between postulated factors. The philosopher Popper (1902-94), suggested ways of developing or refining a theory, that are relevant to the evolving associations and interpretations linked to the hygiene hypothesis:

1) Include additional variables that help to refine relationships (e.g. cofactors such as diet, rural environment, breastfeeding etc.);

2) Narrow the conditions under which it will apply (e.g. particular types of atopy/particular types of microbial exposure);

3) Develop better operational measures of essential constructs (e.g. timing and route of exposure/evidence from intervention studies).

To apply this approach to the hygiene hypothesis, it is first necessary to consider whether the name is appropriate, given the lack of current evidence implicating hygiene practices.

5.6 Re-naming the hypothesis

“The apparent rise [in the prevalence of allergic diseases]…. could be explained if allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally…. Over the past century declining family size, improved household amenities and higher standards of personal cleanliness have reduced opportunities for cross-infection in young families. This may have resulted in more widespread clinical expression of atopic disease”

(Original statement of the hygiene hypothesis, Strachan 1989)

(‘hygiene’or ‘hygiene proxy’ related exposures shown in bold)
A consideration of the evidence summarised in this Section suggests that, whereas there is good evidence to suggest the validity of the link between microbial exposure and atopy, current evidence for a link with domestic hygiene is very weak. There is, in addition, little justification for the reference to “unhygienic contact” in the original statement of the hypothesis. Using the word hygiene or unhygienic in the context of family contact carries connotations of domestic hygiene practices, which have led to an unhelpful dissemination of loosely defined references, unsupported by scientific evidence, as to whether modern householders are too clean or too zealous in the application of such practices. Nevertheless, the almost constant finding of the importance of small family size, in association with the development of some forms of atopy, is suggestive of a relationship between microbial exposure and atopy. Alternative explanations are conceivable, including influences of the birth order on immune system development, but to date the probable correlation between family size and opportunity for higher microbial exposure is the most strongly supported rationale for the observation. Further research is required to investigate whether specific microbes or infections and routes of transmission can be identified, as recently outlined in an editorial on the hygiene hypothesis and the possible importance of endotoxin exposures on farms:

“We will need longitudinal studies of birth cohorts with appropriate timing of environmental sampling in order to address the issues of dose, timing, cofactors, genetics, and their effects on the development of disease.”

(Weiss 2002)

To this could be added the need for behavioural studies and attempts to unravel the educational and socio-economic influences on hygiene behaviours, diet and exercise. All directly or indirectly impact on opportunities for microbial exposure or on susceptibility to invasion after microbial exposure. Since there is no evidence that cross infection has been significantly reduced, the hypothesis must rest on the reduction in reservoirs of microbes, for example in food, water, reduced numbers of human cases of severe infection and less environmental exposure to animals. Such reservoirs are largely independent of hygiene measures in the control of individuals.

Therefore it is suggested that the hygiene hypothesis should be renamed as a ‘microbial exposure’ hypothesis. Renaming it as a ‘declining infection hypothesis’ would be inappropriate, since the burden of infection has changed rather than showing an overall decline. Also, the relationship between infection and immune system dysregulation may involve complex patterns of exposure to particular micro-organisms, as well as the lack of exposure to others.
A rewording of the hypothesis, based on the statement first made by Strachan, may help to focus attention on determining the true impact of microbes on atopic and other immune diseases, while minimising the very real risks of exacerbating infectious disease by discouraging good hygiene (Box 5.6). While lacking the parsimony of Strachan’s influential statement, the rewording takes account of evidence that has emerged since the time the statement was made; and of new questions raised about the involvement of microbial exposure in the aetiology of immune system dysregulation and the evolving understanding of genetic predisposition and interaction with the environment.

Renaming the hypothesis does not however give hygiene a ‘clean slate’. The focus on hygiene in the debate about atopic disorders has helped to expose problems in 20th century attitudes to hygiene practice. These include the need to address increasing concerns about sustainable biodiversity in the environment, as well as the links between early human development and various environmental exposures. The links between genotype, microbial exposure and disease appear to be far subtler than some of the early interpretations of the hygiene hypothesis suggested: this reinforces the benefits of a more subtle approach to hygiene, particularly in the home environment. The practical implications of this are discussed in the next section.

**Box 5.6: A reworded statement for the microbial exposure hypothesis (previously hygiene hypothesis)**

[The apparent rise in the prevalence of allergic diseases]… could be partly explained if allergic diseases were in some way related to inadequate exposure to particular infectious organisms or to exposure to non-invasive microorganisms in the general environment. Some types of immune dysregulation, particularly hay fever and eczema, are epidemiologically related to smaller family size and birth order, suggesting that smaller families give less opportunity for transmission of the particular infections that may be protective, as yet not clearly identified, but possibly involving micro-organisms of low (or no) pathogenicity and not apparently related to current domestic hygiene practices. The more widespread clinical expression of atopic disease observed in recent years is likely to have a multiple aetiology, including the role of genetic susceptibility, commensal microorganisms, diet, exercise and other factors.
IMPLICATIONS OF THE CURRENTLY AVAILABLE EVIDENCE FOR HYGIENE PRACTICE IN THE HOME

“Even in an era of unprecedented cleanliness and improved public health infrastructure, there is a continued, measurable, positive effect of personal and community hygiene.”

(Aiello and Larson 2002)

6.1 Infectious disease in the home and community

As reviewed in Section 4, the total global burden of infectious disease (ID) is still a major concern, accounting for over 18 million deaths annually: while the majority of infection-related deaths occur in the developing world, infection also causes around 4% of deaths in developed countries (Anon 1996). Although ID mortality is declining in the developed world, the trends on ID morbidity suggest a change in the pattern of diseases rather than a decline in rates. This is partly associated with the emergence of new infections, but also with the rising proportion of those vulnerable to infection in the population. The vulnerable population groups include the elderly: it is estimated that, by 2025, more than 800 million people will be aged over 65 years in the world, two thirds of whom will be in developing countries (WHO 1998). Other groups at risk from infection are neonates, people with chronic or degenerative illness; and immuno-compromised patients discharged from hospital. All of these groups, together with family members who are carriers of HIV or MRSA, are increasingly cared for in the home by a carer, who requires a sound knowledge of hygiene practice. Currently, an estimated 1 in 6 persons in the UK belongs to an ‘at risk’ group and it is likely that the same applies in most European countries (Bloomfield 2001).

In addition to the threat posed by acute infections, pathogens have been implicated as causing or acting as co-factors in the aetiology of cancers and some degenerative diseases (Anon 1996). Examples include Hepatitis B virus (Shanson 1999), Campylobacter jejuni (Skirrow 1998) and Helicobacter pylori (Forman 1998). Foodborne illness has been estimated to result in chronic sequelae in 2-3% of cases (Lindsay 1997); a more recent report from the European Commission (EC 2000) cited evidence of chronic disease, such as reactive arthritis, following 5% of salmonella cases, with 5% also of E.coli O157 cases progressing to the serious and often fatal complication of uraemic syndrome.

The globalisation of infection risk has also increased due to global food markets, increased travel, increased refugees and displaced populations: thus pathogens can more readily reach areas where there is little or no innate resistance.
“Controls at borders and airports can rarely protect against [infections]: multi-drug resistant tuberculosis, imported infections in food, etc. For a variety of reasons, including ever increasing travel and movement of food stuffs across borders, risks of new infections being reintroduced are rising.”

(PHLS 2002)

Until recently the importance of hygiene in interrupting the cycle of disease and poverty was given lower priority than measures to improve the economies of the most deprived developing countries, particularly those in the tropics and subtropics of Africa and south Asia. A recently published report from the Commission on Macroeconomics and Health (WHO 2001a) concluded that the evidence strongly supports a higher priority role for infection interventions. Of the health burdens identified as needing urgent intervention, the Commission identified several that are amenable to hygiene interventions, such as diarrhoeal disease and maternal mortality.

These trends have prompted a growing awareness of the strategic importance of the domestic setting in the chain of infection transmission through the community (Sattar et al. 1999). There are indications that the infectious disease risks in the home are increasing rather than decreasing. Antibiotic resistance is now a global problem with resistant strains such as MRSA spreading into communities (Herold et al. 1998, Zylke 1998). Infection threats include the continued emergence of new hazardous strains of pathogens, and infections caused by species with previously little clinical impact on humans. Verocytotoxin-producing *Escherichia coli* O157 (VTEC) is of particular current concern in the domestic setting. Although infrequent, VTEC causes severe illness with a small infectious dose, thus increasing the risk of person to person transmission. Parry et al. (1998) estimate that the household transmission rate for sporadic *E. coli* O157 in the UK is between 4 and 15%. Re-emergence of old pathogens also represents a significant problem. Tuberculosis has increased in Europe, including more invasive and antibiotic resistant strains, while in the former soviet block countries, diphtheria cases rose 50 fold between 1989 and 1995 (Anon 1996). The need for improved hygiene to reduce the spread of antibiotic resistance has been recommended by recent working parties in Europe (Anon 1999a). Reduced rates of infection and antibacterial resistance have been demonstrated where an approach combining good hygiene and reduced prescribing has been evaluated (Anon 1997, Schmitz et al. 1998).

Over the past two centuries, measures aimed at prevention through hygiene in its broadest sense, such as water, sanitation and waste disposal,
have played an essential role in preventing ID. Globally, escalating treatment costs suggest that hygiene may be the most economically sustainable strategy. Provision of good quality water and efficient sanitation are vital factors, but, particularly for the developing world, it has become apparent that health gains commensurate with investment will only be achieved if steps are also taken to improve standards of hygiene practice in the community. To be effective ‘Home Hygiene’ must cover all aspects of prevention, including food hygiene, personal hygiene and hygiene related to the care of vulnerable groups. In developing countries, it must also address drinking water hygiene, peri-domestic sanitation and the disposal of human excreta and other waste.

The overall conclusion is that hygiene remains the most important cornerstone in the control of infectious disease. Further, it must be concluded that a significant increase in morbidity and mortality from infection would result from any attempt to reduce the integrated practices of sanitation, provision of clean water and hygiene practice. The consequences of infection are more serious in the increasing elderly and immuno-compromised population in the community, requiring hospitalisation with attendant costs.

In their review of studies of hygiene impact in community locations such as day nurseries, Aiello and Larson (2002) acknowledge the influence of the hygiene hypothesis in raising questions about “whether there may be a limit about how clean we should be”. Meanwhile, there is a need to produce guidance on how to prevent the spread of infectious disease within this, currently undefined, limit of desirable cleanliness. Despite concerns about methodological limitations, Aiello and Larson concluded from a systematic review of intervention studies that improved standards of personal and environmental hygiene in these situations could reduce the transmission of infection by over 20%. However, the relative contribution made by individual hygiene practices cannot be assessed from currently available data. Further research is required to assess potential interactions between hygiene interventions, definition of confounding factors, ‘dose-response’ relationships and long-range sustainability of infection reduction in high risk settings (Aiello and Larson 2002). Nevertheless, there is sufficient evidence to show the overall benefit of hygiene practices and to consider how they should be applied to minimise infection risks, while also preserving any potentially beneficial effects of general microbial exposure in the environment. The factors to be considered are:

• the type of infection and route of infection in relation to hygiene practice;

• the implications of trying to sustain high microbial exposure without increasing the risk of infectious disease;
6. Implications of the currently available evidence for hygiene practice in the home

- restoring confidence in hygiene procedures by a more evidence-based approach;
- exploring how to focus hygiene practice and to target areas and activities with a known high risk of infection transmission;
- development of hygiene guidelines and training programmes.

6.2 Type and route of infection and its relation to hygiene practice

For pathogens that are transmitted via hands, other body parts, hard surfaces and textiles, hygiene practices can significantly reduce the infection risk. However, many of the routes favoured by micro-organisms, such as airborne transmission, are only indirectly preventable by hygiene procedures, for example cleaning surfaces contaminated by aerosols containing vomit and other body fluids (Table 6.2). Table 6.2 shows that, of the micro-organisms cited as protecting against atopic disease, hygiene is more likely to reduce exposure to those infections acquired via the intestinal route (e.g. Hepatitis A virus, enterococcal infection), than those that are mainly airborne (e.g. measles, tuberculosis). For the same reason, increased infection risks due to overcrowding and poorly ventilated enclosed areas are only partly reduced by good hygiene practice.

It is well established that respiratory viruses, such as RSV, spread readily via contaminated surfaces and fomites. For viruses such as rhinovirus and Norovirus (formerly Norwalk-like virus), although direct spread by the aerosol/droplet route is probably the primary route for transmission, there is increasing evidence to suggest that transfer via hands and other surfaces is also a significant route of transfer (Goldmann 2000). A study of naval recruits in the USA reported the efficacy of handwashing interventions in reducing the number of coughs and colds requiring a visit to a physician (Ryan et al. 2001): respiratory illness was 45% lower in the intervention group. Airborne transmission is likely to be augmented by cross contamination of surfaces and clothing: there is evidence of inoculation by fingers, for example, by nose picking. Hendley et al. (1973) found that one third of attendees picked their nose and one in 2.7 rubbed their eyes during a one-hour medical lecture.
Selecting targeting of hygiene interventions, as a means of maintaining beneficial microbial exposure, would be an option only if the appropriate types of organisms and/or their mode of transmission was significantly different from those responsible for transmission of pathogens. This would not appear to be a biologically plausible option from the currently understood transmission routes and evidence from research studies.

### 6.3 The possible outcomes of relaxing infection control to accommodate the hygiene hypothesis

"...children brought up in very hygienic homes are more likely to develop asthma. A little dirt may help the immune system – but we must retain a balance – severe infections can be devastating."

(Leaflet distributed to parents of children participating in the ALSPAC ‘Children of the 90s’ research cohort, 2001)

There is no guarantee that exposure to pathogenic micro-organisms will result in colonisation or subclinical infection, rather than invasive disease. While a few proponents of the hygiene hypothesis have gone so far as to suggest that the infection exposure necessary for immune priming should be “intense” (von Hertzen 2000) or at least produce clinical disease (Cookson and Moffatt 1997), others are more cautious and emphasise the need to control the spread of serious infection (Holt et al. 1997). If we assume that the

### Table 6.2: Route of infection and association with hygiene practice

<table>
<thead>
<tr>
<th>Route of infection</th>
<th>Influence of hygiene practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airborne</td>
<td>Minor influence via removal of infected droplets/dust from surfaces. While there is evidence that contaminated hands and surfaces contribute to the level of such infections, the main spread is by aerosol/air currents.</td>
</tr>
<tr>
<td>Intestinal</td>
<td>Major influence via water treatment to remove micro-organisms present in excreta or other environmental sources; also by hygienic food preparation and storage to prevent contamination or survival of the micro-organisms; also by hand washing after 'high risk' activities and removal of intestinal pathogens from surfaces in 'high risk' areas, such as the kitchen, bathroom and nappy changing areas.</td>
</tr>
<tr>
<td>Direct contact</td>
<td>Little influence on case to case spread via direct skin contact, although personal hygiene (frequency of washing etc.) reduces the risk. Many of the direct contact diseases, such as scabies and ringworm, also occur in conditions of good personal hygiene.</td>
</tr>
<tr>
<td>Venereal (sexual transmission)</td>
<td>Personal hygiene makes only a small contribution to the sexual transmission of infection, because the main route is via infected body fluids and direct contact.</td>
</tr>
<tr>
<td>Insect or animal bites</td>
<td>Personal hygiene has no effect but clean clothing reduces spread of some vector-borne infections, e.g. typhus fever, although the main preventive measure is removal of the insect vectors by insecticides.</td>
</tr>
</tbody>
</table>
exposure has to be sufficient to cause disease (for example: measles, tuberculosis, enteritis), encouraging such exposures would cause significant morbidity and mortality. If, however, there is a link between susceptibility to immune dysfunction and greater vulnerability to infectious disease, there might not even be a ‘trade off’ in reduced levels of immune dysfunction.

The outcome of clinical infectious disease may be analysed in terms of their impact on the immune system: (A) survival with a well balanced immune system; (B) survival with chronic effects of infection but protection against atopy; (C) survival with increased susceptibility to atopy; and (D) overwhelming of immune system capacity, causing death (Figure 6.3). The proportion of those exposed that would experience outcomes A, B, C or D in Figure 6.3 would vary with several factors, such as host susceptibility, genetic predisposition and availability of effective treatment. Since only outcome A is acceptable, a policy of encouraging more infection is clearly out of the question.

There is no indication as to how much atopic disease could be prevented by encouraging infectious diseases, or whether more clinically severe immune dysregulation would result, or only ‘minor’ forms of atopy, such as mild hay fever or eczema, would be affected. If, for example, the main effect was a reduction in hay fever, with little or no impact on asthma, the ‘trade off’ would represent a very poor bargain.

Figure 6.3: Immune system regulation outcomes following clinical infectious disease
6.4 The implications of trying to increase non-invasive microbial exposure

“Eating dirt or moving to a farm are at best theoretical rather than practical clinical recommendations for the prevention of asthma.”

(Weiss 2002)

It seems highly unlikely that normal development of the human immune system requires exposure to intense, invasive infection, with all its attendant risks. Such a requirement would be inefficient in evolutionary terms. Nor is it practical, as Weiss suggested, to eat dirt or return to a farm based society to ensure higher microbial exposure. A more widely supported possibility is that we need more general environmental exposure to microorganisms, particularly those with low invasiveness or ability to cause clinical disease, such as the rapid growing saprophytic strains of Mycobacteria. Since untreated water may contain as much as 10^9 mycobacteria per litre, but contains much else besides, including pathogenic organisms, the difficulty is how to preserve the ‘friendly’ species while removing those likely to cause invasive disease, particularly as non-invasive species are more susceptible to disinfection and other treatments. Reduction or elimination of pathogens in water and milk has resulted in the control of many serious infectious diseases, such as typhoid and paratyphoid fever: providing safer water on a worldwide basis is a WHO goal. Claims that we were in some way immunologically better off in the days of untreated water are disconcerting and difficult to interpret from a public health perspective. While water may be less contaminated nowadays, at least in developed countries, there is still ample evidence of contaminated food and transmission of pathogenic micro-organisms by food handling. The idea that there are ‘right’ and ‘wrong’ types of microbial stimulation is academic, unless we could engineer the ‘right’ type of exposure, without introducing more dangerous organisms. An attenuated vaccine containing the ‘right’ type of microbes (such as saprophytic mycobacteria) is one option, and there is evidence of efficacy of a vaccine strategy in animal studies (Wang and Rook 1998) and in some of the trials on humans. Interest in the gut flora (Wold 1998) and the possible benefits of probiotics is another aspect of a ‘putting the right microbes back’ approach. With vaccine or probiotic strategies, there is no conflict with hygiene. Nevertheless, Wold (1998) cited “overly hygienic lifestyle in modern Western countries” as the cause of impaired oral tolerance to innocuous antigens in food and in the air.
6.5 The options for hygiene practice

If it were proven that a high microbial load in early life is important, various options for hygiene practice might be considered. For example, one might choose to relax hygiene standards of food processing, storage and preparation and in activities such as nappy changing, care of infants and the infirm, and disposal of urinary and faecal contamination by pets. Handwashing and personal cleanliness could be discouraged. But in the absence of any guidance on how much such standards should be reduced, this would be a high risk strategy. Given the evidence of continued exposure to microbes, including pathogens, in the home and other parts of the community, outbreaks and sporadic cases of infectious disease would undoubtedly increase. On the basis of the evidence suggesting a role for non-invasive micro-organisms, a more realistic and justifiable option arises for hygiene practice. If non-invasive microbes such as environmental mycobacteria were proved to be necessary for priming a healthy immune system, the challenge would be to develop practices which maximise removal of harmful microbes, while causing minimum reduction of innocuous microbes in the environment.

The academic and popular support for the hygiene hypothesis is associated with confusion, and possibly also a loss of confidence among the public, regarding hygiene in the home. Whether the hygiene hypothesis is proved, modified or refuted, it seems that hygiene itself has been tarnished in the debate, acquiring connotations linked to the general disquiet about ‘Western lifestyle’. The beneficial outcome however is a recognition of the need to clarify what home hygiene as opposed to home cleanliness means in the modern age. Fortunately, it has already been recognised that we now need to popularise an approach to home hygiene that differs from the previous prevailing view that eradication of all sources of infection exposure is the desirable aim. Evidence from research into infectious disease transmission indicates that no particular health gain is achieved from an “over zealous” approach to cleanliness, other than that which may arise from the sense of well-being derived from living in what we perceive as “pleasing” surroundings. Infection transmission is related to high-risk activities or incidents, such as defaecation, diarrhoea, vomiting and handling raw food. The risk of spread of infection is also greater in particular areas of the home, such as the kitchen, bathroom and toilet, and in particular situations, such as when people who are infected, or are at greater risk of infection, are present. The generally agreed solution is that hygiene practice should focus on these activities and situations.
6.6 Targeting hygiene interventions

Figure 6.6 illustrates alternative propositions for hygiene in the light of three possible scenarios. Regardless of whether evidence is produced to show a decline in microbial exposure and consequent adverse effects, the logical approach is to target hygiene measures on high-risk areas and situations at relevant points in time. It is important to distinguish between cleanliness and hygienic cleanliness, the latter involving removal or destruction of micro-organisms, to a level that does not pose a significant threat of infection. Hygienic cleanliness is required only where infection risk is high, for example after possible contact with excreta. The level of risk varies according to composition of the home (e.g. presence of children, pets, ill people) and the immune status of the occupants. A targeted approach to hygiene focused on high-risk situations has the advantage of reducing the risks of infection spread, with minimal disturbance of the general microbial flora with which we have co-existed for centuries.

![Figure 6.6: Implications for home hygiene](image-url)
6. Developing hygiene guidelines

As a part of its work, the International Scientific Forum on Home Hygiene (IFH) has produced guidance documents on home hygiene practice: a set of guidelines for home hygiene (IFH 1998) and a set of recommendations on suitable hygiene procedures to reduce the infection risk (IFH 2001). The documents aim to give guidance to ‘hygiene professionals’, such as community nurses, doctors, pharmacists, Environmental Health Officers and school teachers who interact directly with the public to give advice. The key feature of the guidelines and recommendations is that they are based on the concept of risk assessment and risk prevention. The concept of risk assessment or HACCP (Hazard Analysis Critical Control Point) has successfully controlled microbial risks in food and other manufacturing environments and it is now accepted that, in order to deliver real health benefits, this approach must also be applied in the home (Bloomfield and Scott 1997, Griffith et al. 1998, Jones 1998, Bloomfield 2002). Applied to the home this has come to be known as ‘targeted hygiene’.

The guidelines start from the premise that homes always contain potentially harmful microbes (from people, pets, food, etc.) and that good hygiene is not about eradication, but about targeting measures in the places and at the times that matter, in order to limit risks of exposure. Fundamental to developing infection prevention policies is the need to recognise that the home is thus an environment where all human activities occur including food hygiene, personal hygiene (particularly hands) and hygiene related to care of vulnerable groups, all of which are based on the same underlying microbiological principles. In applying a risk assessment approach, the first step, hazard characterisation, involves identifying sites and surfaces where pathogens most frequently occur, and whether they are likely to be present in numbers that represent an infectious dose. Risk assessment depends on considering this information together with an assessment of the probability of human exposure to the hazard.

A risk assessment approach can be achieved by grouping sites and surfaces in the home into a number of categories: reservoir sites, reservoir/disseminators, hands, contact surfaces, laundry and “other surfaces” (Bloomfield and Scott 1997, Bloomfield 2002). For reservoir sites such as sink U-bends or toilets, although the probability of contamination is high, under ‘normal’ conditions, the risk of significant transfer, and thus exposure, is actually relatively limited. Thus expert opinion tends to the view that, unless there is e.g. an outbreak of diarrhoeal infection the benefit of hygienic intervention is limited. By contrast it is imperative that reservoir sites with a
high risk of disseminating contamination, such as wet cleaning cloths, are decontaminated in a suitable manner. Similarly for hands, and hand and food contact surfaces, although the probability of contamination is less in relative terms, it is still significant, particularly following preparation of contaminated food or when someone in the home is infected. Since there is constant risk of cross contamination, preventative measures are critical for these surfaces. For other surfaces there is little case for hygienic decontamination, except where there is known risk, for example soiling of floors by pets.

The targeted hygiene approach is logical and justified on its own merits, but the evidence supporting a link between microbial exposure and atopy also makes it desirable. This approach also acknowledges the general disquiet about overuse of chemicals in the home, including the tendency to view them mistakenly as uniformly unnatural and contrary to nature, and concerns about pollution of the environment. The targeted hygiene approach presents a realistic and rational option for promoting hygiene practice, addressing both real concerns and the impact of the popular dissemination of idea of over zealous cleanliness. At the same time however the targeted approach demands continued promotion of essential infection control measures, such as hand washing.

The targeted approach has the potential to improve the prevention of infection, as well as addressing some of the anxieties about modern lifestyles. Since there is evidence that cleaning is often done in an inappropriate or insufficient way, the next question is how to take forward a targeted approach.

6.8 Implications for training and hygiene education

Measures likely to prevent or control infection hazards in the home are well documented and the evidence supports continued efforts to improve hygiene-related behaviours (Larson 1999, Aiello and Larson 2002). Human beings are notoriously resistant to attempts to modify their behaviour, but behavioural science has made considerable advances in the ways of promoting health-related behaviour and of measuring behavioural changes (McQueen 1999). Realistic and focused guidelines would appear to be a cornerstone of the targeted approach: there are also implications for hygiene education and training, from schools through to specialist courses. As well as presenting simple and practical routines, training needs to include discussion about lifestyle concerns and the underlying values about nature, dirt and hygiene.
While there is much to unravel and understand about infection transmission and the importance of microbial stimuli for the immune system, the path for hygiene is clear. There is no need to return to previous eras when invasive infection was a daily risk and fear, nor should we forget that many people in the world do not have the luxury of clean water and safe sanitation, two key foundations of the civilisation in the developed world. The hygiene hypothesis caused alarm to infection specialists when first proposed, with its inference of the benefits of infection. Nevertheless, it has done a great service to those concerned with infection control guidance, by revealing assumptions and concepts of hygiene practice rooted in the early attempts to eliminate the scourge of infection epidemics. It may also have helped to promote scientific interest and an evidence-based approach in the neglected field of home hygiene (Scott 2000, Aiello and Larson 20002). Infection control requires clear guidance, and by focusing on the evidence-based risk areas, the role of hygiene will be clarified and advanced.
The hygiene hypothesis is only just over a decade old, but the microbial hypothesis for allergic diseases has a longer vintage, for example in the concept that atopic disease is a necessary price for relative freedom from infectious disease (Gerrard et al. 1976). The central question examined in this review is whether there is a need to modify home hygiene practices, in the light of evidence relating to the hygiene hypothesis. This evidence, and that examined for other potential causes of atopy, does not lead to the conclusion that hygiene practices are responsible, or that unhygienic environments are beneficial to health. The review concludes that the relationship of the hypothesis to hygiene practice has not been proved. It does however indicate a pressing need to change our approach to hygiene practice. Trends in infection and new data showing how infection is transmitted in the home suggest that the traditional approach to hygiene is unfocussed, with insufficient regard to where infection transmission risks are highest, or to concerns about ‘artificial’ exposures in the modern environment.

While there is significant evidence regarding the possible influence of microbial exposure on atopic disease that merits further research, there is little or no justification for continuing to cite hygiene practices as a major influence on the level of this microbial exposure. Thus, an important conclusion, as discussed in subsection 5.6, is that the hypothesis should be renamed as the microbial exposure hypothesis in order to prevent further misinterpretation of the hypothesis. This conclusion is based on the following points:

- The existing epidemiological evidence of ‘unhygienic contact’ is based on use of surrogate or non-specific markers rather than ‘direct’ microbiological evidence;

- The existing microbiological evidence suggests that routine cleaning and disinfection has little effect on the day to day microbial exposure in the home. Targeted hygiene can reduce exposure to pathogens but the evidence suggests that compliance with targeted hygiene practice is relatively poor in the home;

- There is no convincing temporal relationship, in terms of markedly different hygiene practices, to explain the rapid rise in asthma and other atopic disorders observed during the last 30 years. If less overcrowding at home, better hygiene and personal cleanliness were causative factors, one would expect a continuing upward rise in atopic asthma. Two recent studies sug-
gest that the incidence may be levelling off or even falling (Fleming et al. 2000, Ronchetti et al. 2001);

- If poor hygiene is protective, then the epidemiological evidence should abound with evidence of a link between atopy and exposure to gastrointestinal pathogens, to the exclusion of other types of infection less directly related to hygiene practice, such as respiratory infections transmitted by the airborne route. Respiratory transmission is to some extent associated with overcrowding and closed environments, rather than hygiene practices as implied in the hygiene hypothesis;

- Strachan’s hypothesis was based on the need to explain an inverse association between family size and hay fever, skin prick positivity and specific IgE: there is no consistent evidence that this inverse association applies to clinical atopic asthma. Redefining the hypothesis as a microbial exposure hypothesis and being more specific about the conditions to which it relates would help to clarify further research.

The connotation of the term ‘hygiene’ in naming the hypothesis has had a considerable influence on research and debate in this field: this review suggests that there is a powerful ‘hygiene rhetoric’ that in some ways is a proxy for disquiet about the artificiality of modern life. The popular interpretation that ‘dirt is good for us’ (e.g. Hamilton 1998) touches on deeply held cultural beliefs. By contrast, our attitudes to infectious disease have become more relaxed in the last 50 years. This has been nurtured by the irrefutable evidence that improved quality of water, sanitation, housing and nutrition have produced a marked decline in infection mortality and the incidence of traditional scourges such as typhus, typhoid fever and cholera. Antimicrobial therapy and advances in immunisation have supplemented these general trends in society. The result is that people are not as frightened of infection as in previous generations, and death from infection is rare in developed countries. If infection is not seen as a serious threat, it is understandable that rises in non-infectious diseases, such as atopic and auto-immune disease, are likely to excite more concern. The transition (Omran 1971), from the age of pandemics to the age of chronic and debilitating disease, has accentuated these concerns. As Porter (1997) observed, we not only expect to live longer, but also to be free of disease and health anxieties.

The implications of the hygiene hypothesis for hygiene practice

While patterns of infection exposure and outcome have changed in developed countries over the last century, infection remains a major cause of mortality in world terms and, for two billion or more people, poor sanitation
makes them vulnerable to a wide range of pathogens (WHO 2000b). Any suggestion that we should relax hygiene and sanitation in the developed world is irresponsible from the global perspective. While none of the hygiene hypothesis supporters suggest a literal return to squalid surroundings, vague references to the microbiological/immunological advantages of such surroundings continue to appear in the scientific literature and media. In putting forward the concept of ‘Give us this day our daily germs’ (Rook and Stanford 1998) were referring mainly to exposure to commensal, non-invasive organisms, rather than to disease-causing micro-organisms, yet it remains unclear how hygiene practice can avoid the former whilst achieving the latter.

The definition of microbial exposure in the hygiene hypothesis is still poorly understood: the concept of allowing a bit of beneficial dirt back into the environment is sustainable only from an academic viewpoint. In practice, any attempt to provide ‘controlled dirtiness’ in the environment would inevitably raise the risk of invasive infection. ‘Controlled dirtiness’ is not a feasible concept, raising questions such as: how often should people wash their hands or clean chopping boards; or how long washing should be delayed after exposure to dirty environments? If arguments about the need for ‘a bit of dirt’ were, for example, coupled to concerns about the possible carcinogenicity of chlorinated hydrocarbons in treated water supplies, this could have a significant impact on public hygiene policy. Failure to take a balanced view of the infection risks associated with ceasing chlorination of public water supplies was cited as the cause of the 1991 cholera outbreak in South America, involving 350,000 cases (Anderson 1991).

The evidence supporting a link between increased atopic disease and reduced microbial exposure in childhood ignores the ongoing presence of infection as a cause of disease, and tends to promote an impression that the infection battle is over. The hypothesis can thus too easily be viewed as a call for a retreat against the army of infection, to allow some of it back in, now that the major killers have been controlled. The evidence cited in this report suggests that the fight is far from over, with continuing proof of cross contamination, poor compliance with basic measures such as hand washing and widespread presence of micro-organisms in our home environment. Higher levels of chronic disease, immune system impairment and the proportion of old people all suggest that infection transmission in the home will continue to be a major problem for the foreseeable future.

Why then, does the ‘hygiene’ hypothesis continue to receive so much support? For example, Strachan’s statement that “10 years have not changed my view that infections remain the most promising candidates for the under-
lying protective factor [against allergic disease]” (Strachan 2000), or Warner’s assertion that the hygiene hypothesis is “the most credible hypothesis [for atopic disease] that has been mounted in recent years” (Warner 1999). Even those referring to the hypothesis as an ‘infection’ or microbial exposure hypothesis also refer directly to assumptions of the link with poor hygiene:

“One of the appeals of the infection hypothesis is its undoubted capacity to explain the increase in allergic disease in developed societies over recent years as an inevitable consequence of improved hygiene standards, increased immunisation, and reduced contact between children owing to declining family size and less crowded homes.”

(Lewis 1998)

Nevertheless, in his comprehensive review of the first decade of the hygiene hypothesis, Strachan (2000) acknowledges that the infection evidence is unhelpful regarding the hypothesis:

“The totality of current evidence from cross sectional and longitudinal studies of common specific and non-specific infectious diseases in infancy and childhood offers no support for the ‘hygiene hypothesis’.”

(2000)

Atopic disease and its relationship to microbial exposure

Although there seems to be no justification for continuing to cite hygiene practices as a major influence on the incidence of atopic disorders, there is significant evidence regarding the possible influence of microbial exposure on atopic disease that merits further research. The role of hygiene has been insufficiently studied. The intriguing recent findings from the analyses of ALSPAC (Subsection 3.3.4.VI), regarding cleanliness/hygiene scoring and subsequent wheezing or eczema also need to be evaluated by direct-prospective measures of cleanliness and possible confounding by socio-economic status and level of education.

The continuing strength of the hypothesis appears to lie mainly in its ability to help in the understanding of the complex development and interactions involved in the immune system. Immunological research and associated theories about its function has given the hypothesis enormous strength in terms of biological plausibility, in an era when we expect laboratory biological science to provide answers to most of our questions about disease and its aetiology. One attraction of the hypothesis, particularly to immunologists, is that it appears to offer a parsimonious explanation for atopic disease: that it assumes no more causes or forces than are necessary to account for the facts. While the hypothesis still fits known general facts about immune system pathways, emerging immunological data suggest that the ‘parsimony’ of the hypothesis is now in question.
Strachan draws on the criteria proposed by Bradford Hill (1965) in order to assess the strength of association between a postulated cause and a disease, referring to the criteria of coherence and biological plausibility as evidence of the strength of the hypothesis (Strachan 2000). Application of the Bradford Hill criteria to the evidence in this review (subsection 5.4) suggests that the credibility of the hygiene hypothesis now rests almost entirely on immunological, rather than epidemiological evidence of a link with microbial exposure or a specific type of infection. Relatively consistent findings, such as the association between family size/sibship order and atopic disorders other than asthma, have not been proved to be associated with specific infectious disease or other microbial exposure. The reliance on biological plausibility in terms of immunological research uses a posteriori reasoning, extrapolating back from the laboratory to assumed exposures that may explain the mechanisms involved. Incorporating operational measures of essential constructs, such as the difference between infection exposure and assumed infection exposure, would make the microbial exposure hypothesis a far less parsimonious construct, but could help to address the many inconsistencies presented by the evidence.

In evaluating the hypothesis and its relationship to microbial exposure, the criterion of temporality, in terms of trends in atopic disease and trends in infection is particularly pertinent. There appears to be no convincing temporal relationship in terms of falling infectious disease and rising atopy to explain the rapid rise in asthma and other atopic disorders observed during the last 30 years. Two recent studies suggest that the incidence may be levelling off or even falling (Fleming et al. 2000, Ronchetti et al. 2001). At the same time, some types of infection, such as viral gastroenteritis, are rising, and other infections are re-emerging, notably tuberculosis.

The facts on infection trends would appear to support a changed pattern of infection exposure, rather than reduced exposure. Even reworded as the microbial exposure hypothesis, account must be taken of both persisting and changing trends in infection. Bradford Hill’s criteria for causation are often taken in isolation to justify links made on the basis of statistically significant results, ignoring his advice about the need to look not just at the ‘P value’ of a statistical test, but to critically evaluate the methodology and other aspects of the evidence:

“I suspect we waste a great deal of time, we grasp the shadow and lose the substance, we weaken our capacity to interpret data and to take reasonable decisions whatever the value of P.”

(Bradford Hill 1965)
7. CONCLUSIONS

The increasing interest in the role of gut flora and gut associated lymphoid tissue represents an attempt to link the immunological plausibility with the changing patterns of intestinal colonisation. The epidemiological evidence supports the possibility that the nature of the organisms that first colonise the gut is important. Early antibiotic treatment may play a part in determining the composition of gut flora (Strachan 2000) and the tendency of the gut to revert to early colonisation patterns may be pertinent to the links with atopic disease. The protective role of commensal organisms in the gut is well recognised (Shanson 1999). It is hoped that the current focus on intestinal flora will serve to unite the fields of atopic disease and infection, providing the cross-fertilisation of ideas and studies that may clarify important variables and the mechanisms involved. The need to bridge specialties within medicine, and between science and medicine, is one of the specified attractions of the hygiene hypothesis (Settipane and Settipane 2000).

Other explanations

In Section 3 a range of alternative explanations for the recent rapid rise in atopy was considered. Of these, the dietary and exercise hypotheses look most promising in terms of explaining some of the epidemiological inconsistencies, while not affronting the knowledge base of either atopic or infection specialists. Since a lifestyle factor must be responsible for the rapid change in atopic diseases, the evidence of changing diets and their implications for health would appear to meet criteria of causality and, as Warner (1999) observes, “controlled intervention studies are awaited with great interest”.

Lessons for home hygiene and further research

This review suggests that there is no justification for a relaxation in hygiene procedures aimed at preventing infection transmission in the home. Nevertheless, the findings of this report suggests a much-needed impetus to develop a more focused approach to hygiene practice in the home. We can be clean, hygienic and healthy without attempting to create a sterile environment in our homes or a sterile ‘cocoon’ around our infants. Our modern lifestyle has left us confused about the nature of real threats in our environment and has also raised awareness of the possibility that over-zealous hygiene activities are out of tune with nature or may expose us unnecessarily to chemicals. Prospective investigation of microbial exposure is also required to provide more specific data on the nature and type of infection that may be important in the early years of life (Broide 2001). The hygiene hypothesis may be untenable in its original form, but as with other excursions into new ideas and research, the debate may have done a great service to hygiene. It
has promoted discussion and a new focus on a topic that hardly excites people, even scientists and physicians. If the hygiene and atopy debate has finally closed the ‘golden age’ of hygiene, untroubled by doubts about too much cleanliness, perhaps we are now entering, at last, a golden age of infection prevention in which we can acknowledge the myriad interactions between genetics, exposures, behaviours and disease.
REFERENCES


Bauer TM, Ofner E, Just M, Just H, Daschner FD. (1990) An epidemiological study assess-
ing the relative importance of airborne and direct contact transmission of microorganisms in a medical intensive care unit. *J Hosp Infect* 15, 301-309.


REFERENCES


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Coggon D, Barker DJ, Cruddas M, Oliver RH. (1991) Housing and appendicitis in Anglesey. *J Epidemiol Community Health* 45(3), 244-246.


Department of Health. (2002). Health effects of climate change in the UK. Report by the Expert Group on Climate Change and Health in the UK. London: Department of Health. [22452.2P.1K.APR 01(WOR)]


REFERENCES


REFERENCES


202


Glover JA. (1920) Observations of the meningococcus carrier rate and their application to the prevention of cerebrospinal fever. Special report series of the Medical Research Council (London) **50**, 133-165.


REFERENCES


REFERENCES


REFERENCES


REFERENCES


REFERENCES


REFERENCES


References


REFERENCES


UKCPI (United Kingdom Chemical Products Institution) (2002). Fact sheets on Detergents and the environment; Biodegradation; Hygiene and Health http://www.sdia.org.uk


Waterman SR, Small PLC. (1988) Acid sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2.5 when they are inoculated into certain solid food sources. *Appl Environ Microbiol* 64, 3882-3886.


family sizes on the increase in the prevalence of asthma and hay fever in the United Kingdom and New Zealand. *J Allergy Clin Immunol* 104, 554-558.


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