The Hygiene Hypothesis and its implications for home hygiene, lifestyle and public health

September 2012

Rosalind Stanwell Smith[1], Sally F. Bloomfield[2], Graham A. Rook[3]

[1]Honorary Senior Lecturer, London School of Hygiene and Tropical Medicine, London UK; Scientific advisor, Royal Society for Public Health, London UK.
[2]Honorary Professor, London School of Hygiene and Tropical Medicine, London, UK; International Scientific Forum on Home Hygiene.
[3]Centre for Clinical Microbiology, UCL (University College London), London, UK.

This review was prepared by the authors and was then submitted for detailed review by Dr René Crevel and Mr John Pickup.

Further recent data on the hygiene hypothesis and its implication for hygiene can be found in the IFH Library of Recent Publications, Topic 5 Hygiene Hypothesis. This library is updated every 6 months with new publications related to the hygiene hypothesis. These papers can be found at: http://www.ifh-homehygiene.org/IntegratedCRD.nsf/IFH_Topic_Hygiene_Hypothesis?OpenForm

Details for citation of the review: Stanwell-Smith R, Bloomfield SF, Rook GA. The hygiene hypothesis and its implications for home hygiene, lifestyle and public health. International Scientific Forum on Home Hygiene. http://www.ifh-homehygiene.org

Acknowledgement of sponsorship: This review was funded by an educational grant from Reckitt Benckiser. We would also like to thank the United Kingdom Chemical Products Industry Association (UKCPI) for a financial contribution to this work.

Copyright © International Scientific Forum on Home Hygiene, 2012

CONTENTS Page

SUMMARY 5

1. INTRODUCTION 9

2. TERMINOLOGY AND CURRENT TRENDS IN LEVELS OF ATOPY AND OTHER CHRONIC INFLAMMATORY DISORDERS **11**

2.1 What are Chronic inflammatory disorders? 11

2.2 Trends in allergy/ CIDs 11

3. EPIDEMIOLOGICAL STUDIES OF THE RELATIONSHIP BETWEEN PREVALENCE OF ALLERGY/ CHRONIC INFLAMMATORY DISEASES AND MEASURES OF INFECTION AND MICROBIAL EXPOSURE **14**

3.1 Family structure and allergy 14

3.2 Child care: day nurseries and bed or room sharing 16

3.2.1 *Day nurseries* 16

3.2.2 *Bed sharing and other early lifestyle factors* 16

3.3 Breast feeding and allergy 16

3.4 Farm and other rural exposure 17

3.5 The urban environment, 'population mixing' and the migration effect 18

3.5.1 *Allergic asthma and inner cities* 18

3.6 More direct measures of microbial exposure and/or infection 19

3.6.1 *Childhood infections* 19

3.6.2 *Studies on general microbial exposure* 21

3.6.3 *Gastrointestinal infections* 21

(i) *Helicobacter pylori* 21

(ii) *Hepatitis A (HAV) and Hepatitis B (HBV)* 22

(iii) *Enteroviruses* 23

(iv) *Salmonella* 23

(v) *Toxoplasma* 23

(vi) *Endotoxins* 24

3.6.4 *Gut microbiota* 24

3.6.5 *Antibiotics and loss of gut microbiota diversity* 25

3.6.6 *Helminths* 26

3.6.7 *Respiratory infections and atopy* 27

i) *Bacterial infections* 27

ii) *Respiratory viruses* 27

(iii) *Dust mites and other inhaled allergens* 28

(iv) *Fungi* 28

3.6.8 *Skin microbiota and allergy* ... 28
3.6.9 *Respiratory microbiota and allergy* ... 29
3.6.10 *Caesarean section and birth canal microbiota* ... 29
3.6.11 *Urinary tract infections* ... 30
3.6.12 *Immunisation/vaccination* ... 30
4. WHAT IS THE NATURE OF THE MICROBIAL EXPOSURE REQUIRED FOR 'PRIMING' THE IMMUNE SYSTEM? ... 30
4.2 The 'Old friends' (OF) hypothesis ... 31
4.3 Epidemiological transitions and the environment of evolutionary adaptedness ('EEA') ... 31
4.4 Defining the 'missing' microbial inputs ... 32
4.5 Evidence for the 'heirloom' species ... 33
4.6 Other 'old friends' ... 34
a) *Gut commensals (microbiota)* ... 34
b) *Skin commensals (microbiota)* ... 34
c) *Commensal microbiota of other areas of the body* ... 34
d) *Environmental organisms* ... 35
4.7 Other infections that may not be 'old friends' ... 35
4.8 What level of microbial exposure and intensity of interaction with immune regulation systems might be required? ... 36
4.8.3 *Route of exposure* ... 37
4.9 Can the necessary microbial exposure can be achieved by more than one microbial species? ... 38
4.10 Critical timing for microbial exposure to affect immune system priming and regulation ... 39
4.11 Microbes as treatment or preventatives in allergy and CIDs ... 40
i) *Probiotics and prebiotics* ... 40
ii) *Worm (helminth) therapy* ... 41
iii) *Helicobacter pylori* ... 41
iv) *Therapeutic use of Mycobacterial and other actinomycete strains* ... 42
4.12 Autoimmune disease and immunoplausibility of the Old Friend's hypothesis ... 42
5. FACTORS WHICH MAY HAVE CONTRIBUTED TO REDUCED OR ALTERED MICROBIAL EXPOSURE ... 44
5.1 The key questions regarding exposure ... 44
5.2 Water, sanitation and food quality ... 44
5.3 Filth and poverty ... 46
5.4 Antibiotics and immunisation ... 46

5.5 Household hygiene practices. including personal hygiene 46
5.5.1 *Our recent concerns with hygiene?* 46
5.5.2 *Home cleaning and hygiene trends* 47
5.5.3 *Personal hygiene – Showering, bathing and laundering* 48
5.6 Trends in infectious disease rates 49
5.7 Discussion of these trends 51
6. OTHER, NON-MICROBIAL, EXPLANATIONS FOR THE RISE IN ALLERGIES AND CIDS 52
6.1 Pollution 52
6.2 Diet and nutrition 53
6.3 Obesity, asthma and other CIDs: the 'obesity hypothesis' 54
6.4 Physical activity 54
6.5 Socioeconomic factors 55
6.6 Climate change 55
6.7 Genetic susceptibility 55
6.8 Concluding remarks on non-microbial exposures 56
7. THE HYGIENE HYPOTHESIS AND ITS IMPLICATIONS FOR HYGIENE – OVERALL CONCLUSIONS FROM THE UPDATE REPORT 57
7.1 Implications for acquiring healthy immune balance 57
7.2 Summary of the implications of Old Friends and biodiversity hypotheses 57
7.3 What are the implications for hygiene? 60
7.3.2 *Too clean? – a question of what that means* 61
7.3.3 *Hygiene practice 'out of the frame'?* 61
7.3.4 *Home cleaning and microbial exposure* 61
7.3.5 *Personal cleanliness and personal hygiene* 62
7.4 Why hygiene is so important in home and everyday life 62
7.5 Final points 63
APPENDIX I: Outline of current theory on the immune system 65
APPENDIX II. Childhood infections and subsequent protection or risk re: atopy in epidemiological studies 67
APPENDIX III: Summary of epidemiological evidence relevant to the hygiene hypothesis 69
APPENDIX IV: Organisms considered protective in the Old Friends' Hypothesis 71
INDEX 72
REFERENCES 75

SUMMARY

During the late 20th century, increasing levels of allergic disorders [atopy] prompted much research and debate about possible causes. The predominant explanation, postulated as the 'hygiene hypothesis' by Strachan in 1989, implicated smaller family sizes, higher standards of cleanliness, and presumed less contact with childhood infections. The idea was widely taken up by the media as the engaging notion that developed societies have become 'too clean'. The concept of modern life being cleaner than is good for us was always an over-simplification and misinterpretation of informed medical or scientific opinion, but as a simple idea to grasp in a very complicated story, it has remained very persistent. Possibly its continued popular repetition relates to a general disquiet about many of the changes that have occurred in human society. It has taken only a century or so to transform our societies from mainly rural communities to urban concrete and plastic environments, with cleaner water and safer sanitation. Our home and working environments, diet and other aspects of lifestyle have changed beyond recognition in a very short time. The concept of the hygiene hypothesis has now been extended to the increase in other chronic inflammatory diseases (CIDs), including autoimmune diseases such as type1 diabetes (T1D) and multiple sclerosis, inflammatory bowel disease (IBD), and some cancers.

We previously reviewed the research associated with the hygiene hypothesis in 2004, concluding that 'microbial hypothesis' would be a better name, since the reference to hygiene in the term gave inappropriate emphasis to the 'too clean' notion, which was not supported by evidence of continuing infection threats in the home and elsewhere, or by evidence that modern domestic and personal cleaning habits had reduced microbial exposure. This updated review is necessary because of new research and analysis, such as the extension of variants of the hygiene hypothesis to the larger group of CIDs and also to a wider range of postulated causes. While contemporary microbial exposure is no longer the sole focus of such research, an update is also needed regarding the implications for preventing invasive infectious disease by hygiene practices. The continued reference to 'hygiene' as a possible cause of allergy or other disease is confusing and potentially dangerous, if it makes people ignore the consequences of poor food hygiene, lack of hand washing and cleaning to remove possible pathogens from our homes and other environments.

This updated review further examines the evidence for changed microbial exposure, or the lack of it, as a cause for allergy and the chronic inflammatory diseases that have increased in recent decades. Newly important concepts such as the 'Old Friends hypothesis' and the related 'Biodiversity hypothesis' are examined: these suggest that microbial species beneficial for immune system development have become less common in the modern environment or have been replaced by other species, including invasive pathogens. These new concepts are linked to increasing recognition of the role of our inner ecosystem – the millions of commensal bacteria within the body that greatly outnumber human cells – and how it reacts with the changing ecosystems outside the body. The expanded hygiene hypothesis now covers a large range of medical specialties and scientific disciplines, with almost daily publication of new ideas or research. This review is not intended to cover all of these in depth: our primary focus is to identify evidence relevant to how we might sustain the necessary microbial exposures, whilst at the same time protecting ourselves against infectious diseases through appropriate domestic cleaning and hygiene practices. While the influence of declining biodiversity, or how to replace microbes identified as 'old friends', has yet to be resolved, it is possible to conclude quite firmly that relaxing domestic cleaning and hygiene practices is not necessary and is certainly not wise. We do not yet fully understand how the modern environment interacts with population or individual susceptibility to disease: but the evidence gives us confidence that domestic cleaning and hygiene practices are not significant factors. Rather than being 'too clean', it seems we may

well be losing contact with the right kind of dirt. As for the 'hygiene hypothesis', there seems little hope at present of replacing this misleading name. It appears in countless research papers as lazy shorthand for the influence of presumed dirty environments and/or the effect of unspecified cleaning or contact with others. Eventually, interest will turn elsewhere and more appropriately named hypotheses such as 'Old Friends', 'biodiversity' or 'microbial exposure' will take its place.

The review has seven sections, summarised below:

1. **Introduction**: how the hygiene hypothesis was formulated, other emerging hypotheses, a summary of the findings of the previous review, and the aims of the current review.

2. Terminology and current trends for 'atopy' and other chronic inflammatory disease. 'CID' is becoming an umbrella term for the large group of diseases associated with disorders of how the immune system is regulated. **Appendix I** explains some of the terms used in describing the rapidly evolving knowledge concerning immunology and immune regulation. This section also summarises the trends in disorders of immune regulation, to cover the shift from rural to urban societies.

3. Epidemiological studies of how infection or microbial exposure might be related to atopy. While covering the original limited range of atopic disorders implicated in the hygiene hypothesis, a broader range of the chronic inflammatory disorders is also considered. New epidemiological studies confirming the protective effect of large family size are assessed regarding increasing evidence that the effect cannot be ascribed to any single microbial species or group of species and difficult to define more specifically in terms of birth order, gender or particular diseases. The 'farm effect' – the protective role of being brought up in a farming environment – has similarly proved robust, as has the influence of early mixing with others in day care nurseries, but newer studies have failed to indicate which microbial exposures might be the root cause. Where epidemiological studies have looked at the role of specific clinical infections and their potential as protective, against atopy or the wider range of chronic inflammatory diseases (CIDs), the evidence is much less persuasive than suggested by earlier studies. A few micro-organisms and parasites have proved exceptions, such as the bacterium *Helicobacter pylori* and helminth (worm) infestations. These are discussed again later in the review with regard to the 'Old friends' hypothesis. Measures of clinically diagnosed infections, such as episodes of respiratory or intestinal infections in infancy or childhood, have not been shown to be generally protective against allergies/ CIDs. 'Microbial load' or burden – high level exposure to micro-organisms – has been studied to try to find if quantity or diversity is the important factor. Research has increasingly identified the role of the microbiota, the commensal organisms in the human gut, skin and other sites: the early establishment and diversity of these microbial ecosystems within the body appears to be very important in the development and later maintenance of the immune system.

4. **What is the nature of the microbial exposure necessary for immune priming?** Building on the results of these epidemiological studies, this section examines the Old Friends (OF) Hypothesis, as well as the related Biodiversity Hypothesis and other concepts in the expanded 'hygiene hypothesis'. The 'Old Friends' are those microbes that surrounded us during mammalian evolution and in the Paleolithic period, when it is thought that our immune system evolved in what has been called the environment of evolutionary adaptedness [EEA]. This concept and the biodiversity hypothesis hold that we are no longer exposed to the range of microbes or other organisms appropriate for our current stage of evolutionary development. If we are no longer exposed to the right mix of organisms, this is a particular challenge for

individuals with genetic predisposition to allergies and CIDs, although clearly there must be sufficient microbial exposure for those without this predisposition, or these diseases would affect everyone. The section summarises the particular species that may provide the necessary microbial exposure for healthy development of the immune system, as well as those that are not. It also explores the optimal or critical timing of microbial exposures. While the immune system reacts - and may adapt - to exposures throughout life, the most important period of exposure is very early in development. Maternal exposures in pregnancy and infant exposures in the first few days or months of life, appear to be especially important to setting the composition of microbiota and determining how the immune system develops. This does not mean that exposures after early infancy are unimportant: trials of therapy with probiotics/'old friend' microorganisms or helminth eggs have shown that symptoms of established chronic inflammatory disease, such as multiple sclerosis, can be reduced, even well into adult life and current approaches for therapy are reviewed in this section. This deliberate, therapeutic exposure to the OF should be distinguished from casual exposure to dirt, which is unlikely to contain helpful organisms. The section concludes with an examination of how the OF hypothesis may be plausible for autoimmune disease, now classified as a range of chronic inflammatory disorders [CIDs].

5. Factors that may have contributed to reduced or altered microbial exposure.

Here we explore possible reasons for the decline in microbial exposure or other alterations, such as a different pattern and range of exposures. Changes over the last two centuries in water, sanitation, food quality, poverty and medical interventions, such as antibiotics, are evaluated and trends in domestic cleaning, personal hygiene (bathing, showering and laundering) are re-examined. The impact of domestic cleaning and hygiene practices in preventing exposure to microbes is also reviewed, as well as trends in infectious disease. The conclusion to this section is that microbial exposure in the modern environment is different from that of our predominantly rural ancestors 200 or so years ago. A combination of factors is responsible for this change, but the time scales for various interventions differ, such as widespread introduction of piped, clean water supply around the beginning of the last century, while the trend to more frequent showering and bathing, a much more varied diet and the trend to more indoor lifestyles are mainly later 20th century phenomena. Meanwhile, infections are still a major cause of disease and it seems likely that, in our complex relationship with the microbes that surround us, the wrong kind of exposure, rather than reduced exposure, may be the problem, due to loss of 'Old Friends' and declining biodiversity in our environment.

6. Other, non-microbial, explanations for the rise in allergies and CIDS

Microbial exposure has never been the only factor implicated and this section briefly summarises recent research on pollution, diet and nutrition, obesity, physical activity, socioeconomic factors, climate change and genetics as possible risk factors in development of allergies and other CIDs. Our diet has changed at least as much as our exposure to rural micro-organisms – which is more important? Or is the rising epidemic of obesity to blame? Or is the combination of several factors making the expression of genetic risk more likely? Many of these factors are likely to play a role and the Old Friends and Biodiversity hypotheses recognise a multifactorial influence on immunoregulation. There are still more questions than answers, but the role of well-conducted epidemiological studies is discussed regarding how we are going to unravel the many contributing factors to the modern pattern of diseases.

Discussion and Conclusions.

Implications for acquiring healthy immune balance are brought together here, as well as a discussion of what all of this means for hygiene practice. Since the previous IFH reviews were prepared, support for the role of microbial exposure in immune regulation has continued to

increase, to such an extent that a 'microbial exposure hypothesis' is now widely accepted by the scientific community. New evidence also confirms that it makes no sense to claim that the increase in allergies and other CIDs is caused entirely by lack of exposure to microbes, or entirely by Vitamin D deficiency, or entirely by diet, obesity or whatever. Many of these factors are likely to play a role, but the essential underlying problem is immunoregulation, and our changing microbial exposures are fundamental aspects of the immunoregulatory deficit.

This report focuses on new information regarding the implications for hygiene, relative to the other factors that are likely to have altered our exposure to microbes over the last two centuries. If we define the microbial exposure aspects of 'hygiene' as the practices we use to protect us from exposure to infectious disease agents, the evidence does not support the idea that the extent to which we suffer from infectious diseases is the key to immune regulation. This puts hygiene practices as an unlikely cause of allergy and CIDs. We can also challenge the idea that being 'too clean', in relation to the general domestic cleanliness of our own homes, is the cause of the reduced microbial exposure. It's perhaps more likely that we're lacking exposure to the right kind of dirt. So, if this factor contributes at all, its contribution is likely to be very small relative to factors such as clean water, good sanitation, cleaner environments and cleaner food. More frequent showering and bathing has occurred during the last half-century: while we still cannot rule this out as a contributing factor, there is no good evidence of a link. We suggest that hygiene has been such a persistently cited risk factor because it is all too easy to blame.

As a public health measure, the need for infection prevention through hygiene is as great as it ever was, and if the burden of infectious disease is to be contained in an economically sustainable manner, it is a responsibility that must be shared by everyone. Infectious gastrointestinal, respiratory and other diseases circulating in the community continue to exert a heavy toll on health and prosperity, in addition to rising problems of antibiotic resistance which compromise our ability to treat these infections, and greater numbers of people more vulnerable to infection.

Therefore we need to address how the two issues – reversing the trend in CIDs and reducing the burden of infectious disease - can be tackled at the same time. As far as allergies and other CIDs are concerned, it appears that there is no single 'magic target' within the immune regulatory system, and unlikely that there is a single 'breakthrough' clinical solution. The proponents of the microbial biodiversity hypothesis suggest that declining diversity in microbial populations as well as of plant and animal species on the planet is an important contributory factor, related to loss of green spaces and the concentration of population into urban environments. This argument implies a need to consider measures that will preserve the natural environment and reconnect us with nature, along with changes to food production and transportation. We know that some plant and animal species have become extinct, but the extent of extinction of microbial species is not known: at present, their loss in some environments may still be a local, predominantly urban, phenomenon.

In order to address the need to sustain exposure to the necessary microbes both within and outside the home, the IFH has developed the concept of targeted hygiene. This involves identifying the critical points in the chain of infection transmission, and targeting effective hygiene measures at these points and at critical times to prevent the ongoing spread of pathogens. The targeted approach to hygiene allows a focus on preventing exposure to infectious doses of pathogens, but is more relaxed about domestic cleaning and recognises the value of maintaining exposure to our environment. Persuading people to develop lifestyles that reconnect with the natural environment, while also using targeted hygiene to protect themselves from infectious diseases, is a challenge for our time. One of the problems we have in trying to communicate more clearly with the public, in order to change attitudes and behaviour, is the fact that the terms 'hygiene' and 'cleaning' convey different meanings to different people, and are

often used interchangeably. Although the term hygiene has been used in this report to define practices which protect us from infection, we recognise it can cover a whole range of concepts from prevention of disease to personal freshness [personal hygiene]. The public health definition of this term is 'protection of health', which includes factors such as smoking, alcohol, diet and exercise, as well as measures to prevent infectious disease.

To make practical progress, we need to re-focus thinking around three key concepts:

1. The expanded hygiene hypothesis is an increasingly important issue for health. It is not confined to issues of day-to-day home and personal cleanliness, but rather to a broader range of lifestyle choices and measures introduced to protect us from infectious diseases. Together, these have inadvertently also reduced exposure to the microbial friends that regulate our immune systems.
2. The organisms identified as Old Friends that protect against CID are distinct from the pathogens causing most infectious diseases in the modern world. Relaxing home hygiene would NOT increase our exposure to the protective Old Friends, but would increase exposure to the pathogens. The problem is not one of being too clean: it's one of reduced contact with the right kind of dirt.
3. We thus need to distinguish between routines associated with cleanliness, in the sense of absence of dirt, appearance, social acceptability and freshness, and those practices required to protect us from exposure to infectious disease. This indicates a need for clearer guidance about how to target hygiene practices effectively where and when they are required to reduce infectious disease risks.

1. INTRODUCTION

A previous (2004) review commissioned by the International Scientific Forum on Home Hygiene (IFH)[1] and a subsequent 2006 peer reviewed publication (Bloomfield 2006[2]) examined the 'hygiene hypothesis' as an explanation for the increased prevalence of allergic and autoimmune diseases (chronic inflammatory diseases, CID) in the modern industrialised world, with a focus on its implications for control of infectious disease. While the association of declining infection in childhood with increasing immune system disorders in modern society was not new (Gerrard 1976[3], Golding & Peters 1986[4]), the coining of the term 'hygiene hypothesis' to explain an observed inverse relationship between family size and allergic disorders such as hayfever (Strachan 1989[5]) received wide attention.

In his formulation of the hypothesis in 1989, Strachan proposed that a lower incidence of infection in early childhood, transmitted by unhygienic contact with older siblings or acquired prenatally, could be a cause of the rise in the atopic [allergic] diseases hayfever and eczema[5]. He also suggested that sufficient microbial exposure no longer occurs because of "improved household amenities and higher standards of personal cleanliness": in effect, due to cleaner homes. Despite clarification with further research that the hypothesis had "*little relationship with 'hygiene' in the usual meaning of the word*" (Björkstén 2009[6]) and proposals that a less misleading term would be 'microbial hypothesis' or 'microbial deprivation hypothesis', the 'hygiene hypothesis' has remained the umbrella term for the various aspects that have been investigated.

Although the concept began with hayfever, eczema and general measures of atopy, it was later extended to allergic asthma (re: allergic and non-allergic types, see **Section 2.2**) and now includes other chronic inflammatory diseases (CIDs), including autoimmune diseases such as type1 diabetes (T1D) and multiple sclerosis (MS), inflammatory bowel disease (IBD), neuroinflammatory disorders, depression associated with raised inflammatory cytokines, and some cancers. Recently attention has focused on the theory that the microbial exposure

required to 'programme' or 'prime' the way immune system develops, should be examined in evolutionary terms. The 'old friends' hypothesis' (Rook 2010[7]) claims that this important microbial exposure is no longer readily available in the modern world, being based on the primaeval environment, when the human immune system evolved. Loss of microbial species in the modern urban environment has also been implicated in the related 'biodiversity hypothesis' (von Hertzen 2011[8]). Both the OF and biodiversity concepts express a similar concern about effects on immunoregulation. Together, these hypotheses provide a different perspective to the wide range of factors that might have altered microbial exposure over time. There is also growing consensus that the aetiology of CIDs is multifactorial and that the role of microbial exposure would be only part of the story.

The 'hygiene hypothesis' has thus extended in scope and interpretation, but in the media the idea has persisted that immune system disorders have resulted from being 'too clean'. Concerns from infectious disease specialists that this popularised notion could have a detrimental impact on the public's perception of infection risks in the home and elsewhere (Larson 2002[9], Bloomfield 2006[2], Bloomfield 2008[10]) have received less attention. The previous reviews[1-2] addressed two distinct questions:

- How clear is the evidence of a causal link between a decline in microbial exposure and the recent rises in atopic [allergic] disease?

- To what extent might cleaning and hygiene, as distinct from other influences on microbial exposure, be a significant factor?

The main conclusion was:
"Use of the term 'hygiene hypothesis' has led to several interpretations, some of which are not supported by a broader survey of the evidence. The increase in allergic disorders does not correlate with the decrease in infection with pathogenic organisms, nor can it be explained by changes in domestic hygiene. A consensus is beginning to develop round the view that more fundamental changes in lifestyle have led to decreased exposure to certain microbial or other species, such as helminths, that are important for the development of immuno-regulatory mechanisms".

The basic concept of a link between microbial exposure and allergic disease is now generally accepted. The idea that "*poor hygiene in itself would be protective*" against atopy has been refuted (Björkstén[6]), although it is still discussed in the popular media. This short updated review sets out to review recent evidence and thinking about the hygiene hypothesis, in particular its relationship to infection and the role of hygiene in preventing spread of infection, aiming:

(1) to examine the type and intensity of microbial exposure required to maintain optimal functioning of the immune system, for example in terms of whether pathogenic [harmful] organisms are relevant, or whether it is limited to non-invasive species; and how any helpful subclinical or clinical [symptomatic] exposure may conflict with current public health strategies aimed at reducing the burden of infectious diseases;

(2) to consider what public health strategies should/could we adopt in order to help to reduce the burden of atopic diseases, while maintaining hygiene as a cornerstone of cost effective infectious disease prevention.

2. TERMINOLOGY AND CURRENT TRENDS IN LEVELS OF ATOPY AND OTHER CHRONIC INFLAMMATORY DISORDERS

2.1 What are Chronic inflammatory disorders?

The early influence of environmental exposures has been described variously as priming, programming or conditioning of the immature immune system. While genetic factors play a part, current immunological theory focuses on the interaction with early exposures, which in some individuals results in 'faulty' regulation of the immune system [immune system dysregulation]. The hygiene hypothesis holds that reduced exposure to microorganisms is a cause of such dysregulation and hence to increases in certain inflammatory disorders. The concept began with the atopic allergic disorders, but now extends to asthma, most types of arthritis, IBD such as ulcerative colitis and Crohn's disease, neuroinflammatory disorders such as multiple sclerosis (MS), atherosclerosis, type 1 diabetes (T1D), depression associated with raised inflammatory cytokines, and some cancers. Since the disease process is one of chronic inflammation, the term '**chronic inflammatory disease**' (CID) has been applied to the group and will be used throughout this report. A summary of recent thinking about immune system components, which includes a short explanation of the immunological terms referred to in this report, can be found in **Appendix I**.

The term atopy is used to distinguish those allergic disorders (asthma, hayfever and eczema or 'atopic' dermatitis) for which the inflammatory response is associated with production of Immunoglobulin E [IgE]. Such diseases are part of a spectrum of 'immune dysregulatory' ill health, where the immune system does not function appropriately or is engaging in activities that are irrelevant to its primary role as a defence against invasion. Such invasion can be by infectious organisms or non-infectious molecules (collectively known as allergens), for example in dust or pollen. A recent survey estimated that at least six detectable allergens are present in over 50% of American homes (Salo 2008[11]). In many diseases the 'invader' is not identified, hence the concept of 'autoimmune disease' where the immune system attacks the body's own components. The range of diseases now considered 'autoimmune' is growing, currently comprising around 100 diagnoses, many of which have also been shown to have a genetic susceptibility (Heap & van Heel 2009[12]). Genetic susceptibility does not mean that a disease will definitely develop: a complex interplay of genetic and environmental factors is involved. Gene-environment interactions are well established and probably apply to several of these diseases, although the epidemiological methods to tease out the different factors are still being investigated (Tan 2007[13], Weinberg 2009[14]). Because these are chronic conditions, most studies measure prevalence [number of old and new cases] as opposed to incidence [rate of new cases]: this makes it hard to assess recent trends in new cases.

2.2 Trends in allergy/ CIDs

Some CIDs have been known for centuries, for example osteoarthritis and diabetes, and have only recently been considered as chronic immune system disorders. A recent report estimated that 50 million Americans suffer from autoimmune disease (AARDA 2011[15]). Accurate diagnosis of the majority of human diseases was not possible until scientific advances starting in the 19th century and the recent rapid development of precise diagnostic techniques: thus we cannot be exactly sure which CIDs existed in earlier times. But several diseases, for which diagnosis has been established during the last century, have shown a recent increase in western societies. While the early interest in relation to the hygiene hypothesis was on allergic disorders, these apparently parallel increases in more generally defined CID have widened the focus of the hypothesis (Rook & Dalgleish 2011[16], Rook 2011[17]). Examples include the doubling of multiple sclerosis in Italy between 1979 and 1999 and a near trebling of T1D since 1930 in Norway, while during the last 30 years T1D has doubled in Finland[15]. While CID in general has been diagnosed more frequently in developed countries than in those with slower

socioeconomic advance, the epidemiology is changing. For example, ulcerative colitis (UC) emerged in developed countries during the 20th century, followed by Crohn's disease, which has overtaken UC in the last 20 years. This pattern appears to be emerging in countries such as India, with a falling ratio of UC to CD rates, although CD still presents at younger ages (WGO 2009[18]). Both CD and UC were almost unknown at the beginning of the 20th century, rising from essentially zero in 1900, to 400-500/100,000 by the 1990's in rich northern developed countries (Elliott 2005[19]). Similarly MS was very rare until recently: it was noted in 1966 to be more common in people who adopted the western lifestyle (Liebowitz 1966[20]), with continuing increases as countries develop economically (Koch-Henriksen & Sorensen 2010[21]).

Trends for the 'atopic' disorders: asthma, hayfever eczema.

Allergic disease has a long history, including reports from around 3000 BC, but the modern era for these conditions dates from the early 19th century, associated with both more accurate diagnosis and increased recognition of the different types, although the term 'allergy' was not introduced until 1906. 'Hayfever' was first defined in 1817 and is now described as seasonal rhinitis (runny nose) or rhinoconjunctivitis (runny nose, stinging eyes). Clinical recognition increased after a seminal work by Blackley in 1870, although 'allergy' in general was so rare in the late 19th century that doctors mentioned the difficulty of finding cases! (Wjst 2009[22]). At the start of the 20th century, allergy prevalence has been estimated at no more than 0.1% of the population in UK, continental Europe or USA[23]. By the 1920s, reports in Germany suggested a hayfever prevalence of around 1% with a possible rise to 3% by the 1930s, perhaps also associated with wider access to medical care[22]. In Switzerland, hayfever rose from 0.82% in 1926 to 5% in 1958 and 10% of the population by the 1980s: similar rising trends for the 2nd half of the 20th century have been reported for Europe, UK, North America, New Zealand and Australia (Jackson 2007[24]). Indicators of atopy in stored serum samples increased from the 1970s to 1990s (Law 2005[25]). Hayfever was estimated in 1999 to affect up to 25% of people in the UK (Sibbald & Rink 1999[26]) although higher prevalence estimates were recorded in 2001 for Europe and Australasia (35%) (Janson 2001[27]) and USA (30% of adults; ~40% of children) (Joint Task force on Practice Parameters 2008[28]).

Asthma was only recognised as a unique illness towards the end of the 19th century and its links to allergy were not established until the early 1900s (McFadden 2004[29]). The proportion of asthma with an allergic component is now thought to be approximately half the cases (Johansson & Lundahl 2001[30], Douwes & Pearce 2008[31]) although the International Study of Asthma and Allergies in Childhood (ISAAC) cross-population surveys indicate considerable geographical variation, for example only 11% of the allergic type in Ecuador (Moncayo 2010[32]) and this appears to apply in general to the non-industrialised countries. The epidemiological explosion in asthma cases occurred in most developed countries from the 2nd half of the 20th century (Cserhati 2005[33]), particularly the last quarter of that century. So a gradual rise through the 1950s and 1960s became steeper after the 1970s. Asthma prevalence in children doubled between 1980 and 1995 in the US (MMWR 2004[34], Woodruff 2004[35]) and from 7.3% in 1990 to 14.6% in 2003 for Australian children (Wilson 2006[36]). In developing countries prevalence has been lower, although with evidence that asthma in these countries is less likely to have an allergic basis (Pearce 1999[37], Asher 2002[38]). The ISAAC study team reported in 1988 that the prevalence of self-reported asthma ranged from 2-3% in developing countries to 20-40% of the responding population of 13-14-year olds in industrialised countries (Beasley 1998[39]). Global differences between countries appear to be decreasing, with an increasing asthma burden in Africa, Latin America and parts of Asia (Pearce 2007[40]). As with the CIDs such as T1D, these regions appear to be catching up with the developed regions that first saw the increasing levels. While diagnosis of both allergic and non-allergic asthma is mainly clinical, there are likely to be different causes for the two categories (Hold & Sly 2009[41]): the hygiene hypothesis applies to the allergic type.

In the 2004 and 2006 IFH review, there was early evidence of falls in the prevalence and incidence of asthma (Fleming 2000[42]). This is further supported by more recent evidence and indications that levels of conditions such as hayfever and eczema have also stabilised. For example, a review of several allergy prevalence studies by Gupta in 2007[43] showed a 260% increase occurred in GP consultations for hayfever and a 150% increase in consultations for eczema from 1971 to 1991, but stabilisation or falls in the level after the mid 1990s. By contrast, large increases were found for anaphylactic reactions and food allergy and less dramatic rises in urticaria and angio-oedema. A survey of US children in 2002 showed a peak prevalence for childhood asthma of 7.5% in 1995 (Akinbami & Schoendorf 2002[44]). UK data show a recent 'flattening' or falling off in prevalence rates, with decreased incidence of new asthma episodes since the mid-1990s (Anderson 2007[45]). Similar results have been reported from Australia (Ponsonby 2008[46]). A prevalence study of children in Hong Kong, using standardised diagnostic criteria, also showed a decrease since 1994 (Wong 2004[47]). Such findings may relate to the tendency of many children to 'grow out' of asthma symptoms when they reach adulthood. This is supported by a decrease since 1999 in both mortality and hospital admissions for asthma (American Lung Association 2011[48]), although this may be related to improved facilities for diagnosis and earlier management. The possibility that asthma and other atopy increases are largely artefacts (i.e. related to a factor such as diagnostic tendencies or criteria) have been mostly discounted, particularly for the increase in asthma up to the mid 1990s[45]. In the UK, age specific asthma mortality has declined since the early 1980s (Lung and Asthma Information Agency 1997[49]). A recent systematic review of asthma prevalence questioned whether asthma is really declining, finding an overall global rise (Anandan 2010[50]), but this in turn could be related to the 'catch up' by developing countries in acquiring the diseases now common in the 'developed' world, as in the observations for IBD disorders[18].

The changing epidemiology for asthma and hayfever – the increase up to the mid 1990s and declining levels, particularly of asthma, since then – remains unexplained. This is one of the reasons why interest in the hygiene hypothesis has been increasingly directed towards the CIDs, such as T1D and MS, for which there is no evidence of a decline. This is discussed later in the review, but meanwhile prevalence of eczema [atopic dermatitis], estimated to affect 10-20% children and 1-3% adults in the US (Leung 2004[51]) does not appear to have fallen, particularly in children (Ponsonby 2008[46]). Similarly, food allergy is still increasing (Branum & Lukacs 2008[52]). Allergies to milk and egg are the most common, but peanut allergy is reported to have doubled between 1997 and 2002 (Sicherer 2003[53]) and 55% of US citizens were recently reported to have positive skin tests to ten common allergens (Arbes 2005[54]), predominantly related to foods. It should be noted that positive skin prick tests to allergens may, but not necessarily, indicate that the clinical allergic reactions will result. While negative tests are accurate in indicating absence of allergy risk, a positive test thus has low positive predictive value. Diagnosis is aided by such tests but is still most reliable in association with clinical symptoms. For food allergy, definitive diagnosis still relies on a food challenge as well as raised levels of specific IgE. A large recent questionnaire survey of clinically diagnosed food allergy in US children found the prevalence of food allergy to be up to 9.8% (Gupta 2012[55]). Anaphylaxis (an immediate hypersensitivity reaction to a foreign substance (allergen), mediated by IgE antibodies) can occur in people with any type of atopic disorder: while still uncommon, the number of cases of anaphylaxis from foods in the US was estimated to have increased from 21,000 per year in 1999 to 51,000 per year in 2008, based on long term population studies (Decker 2008[56]). A survey based on casualty (emergency department) visits in the US (Ross 2008[57]) reported 2,300 cases of anaphylaxis and 500 related admissions during just a 2-month period in 2003.

3. EPIDEMIOLOGICAL STUDIES OF THE RELATIONSHIP BETWEEN PREVALENCE OF ALLERGY/ CHRONIC INFLAMMATORY DISEASES AND MEASURES OF INFECTION AND MICROBIAL EXPOSURE

As the hypothesis has evolved, the range of potentially significant exposures under consideration has widened beyond those that result in clinical infection. The microbes postulated as responsible include non-pathogenic types or strains (commensals and environmental strains), pathogenic strains, microbial products such as endotoxins and intestinal parasites, particularly helminths [worms]. Accurate measures of infection rates are difficult to obtain retrospectively, especially for common childhood infections. Many investigations have therefore used proxy measures of microbial exposure such as large families, farm environment and attendance at day nurseries. Breastfeeding has long been established as conferring immunity to the newborn, but almost certainly also involves microbial exposure. These studies will be reviewed first, before turning to studies using more direct measures of microbial exposure and/or infection.

3.1 Family structure and allergy

Associations between atopy and family structure, particularly of less allergic disorders with increasing family size, have been frequently reported (Haby 2001[58], Dik 2004[59], McKeever 2001[60], Goldberg 2007[61]), although the associations are less consistent for individual allergic diseases, and sub-divisions such as the order in which children are born, sibship size and gender. While associations of family size with the wider range of CID have been investigated, there is no clear indication that large family size protects against conditions such as acute lymphoblastic leukaemia (ALL), with some evidence of a higher risk with four or more siblings, but paradoxically a protective effect against ALL and other types of lymphoblastic cancer in younger siblings (Altieri 2006[62]). This section focuses mainly on allergic disorders, since they were the primary interest in formulating the hygiene hypothesis and the focus of most studies. The most consistent findings for the atopic disorders such as hayfever and eczema include:

a) decreasing risk of atopy (particularly hayfever) for subjects from families with three siblings or more;

b) decreasing risk of atopy (but not including asthma) with increasing birth order (greater 'protection' from having older siblings, particularly if they are brothers);

c) recent studies indicate no association between the number or type of childhood infections and protection against atopy (see **Section 3.6.1)**

Inconsistent findings include the influence of gender and family history of allergy on asthma as opposed to other atopic disorders. Asthma in particular appears not to relate to birth order (Jarvis 1997[63], Bernsen 2003[64], Goldberg 2007[61]). Birth order may affect the pre-natal programming / conditioning of the immune system, which has been proposed by Karmaus (2001[65]), Devereux (2002[66]) and van Gool (2004[67]). Potential antenatal determinants of this programming include diet, smoking, birth season, environmental microbial exposure, maternal age and whether the parent exhibited atopy.

Bodner (1998[68]) found that having older siblings decreased the probability of hay fever or eczema, but the risk of asthma was reduced for those with younger siblings. More recently, a large survey of pregnant Japanese women reported protection against rhinoconjunctivitis if they had one older sibling, but no sibling-size association with wheeze, asthma or eczema (Miyake 2011[69]). Larger surveys have continued to confirm the family size association while challenging relationships with family structure. A Scottish survey of student health records from 1948 to

1968 reported a consistent inverse [protective] relationship between sibship size and self-reported allergic disorders (Kinra 2006[70]). In addition, they found a lower risk of allergy for increasing birth order and low socioeconomic position in childhood, which they concluded gave support to the hygiene hypothesis. Bernsen and van der Wouden (2006[71]) questioned this conclusion, citing 20th century determinants of family size as influencing the association between family size and allergy, such as shifts in socioeconomic status and possible failure to include spontaneous abortions in the counts of birth order.

A survey of the medical records of 531,116 Israeli military conscripts included 26,833 males (8.6%) and 15,079 females (6.9%) who were diagnosed with asthma between 1998 and 2004[61]. While asthma prevalence was highest in male subjects from families up to three siblings, for larger families prevalence progressively decreased to only 0.58% in conscripts with 15-20 siblings. No association was found with birth order and asthma: the authors claimed that this exceptionally large study gave no support to the hygiene hypothesis, concluding that: "*The similar asthma prevalence for all birth orders challenges the hygiene hypothesis as an explanation for the decreased asthma prevalence in larger families.*" ([61 p.1751]). A recent systematic review of UK family size trends and prevalence of atopy also concluded that reductions in family size in the last 40 years "*account for little of the increase in atopy*" (Upchurch 2010[72]).

Birth order effects have also been reported for CIDs such as MS (James 1984[73], Ajdacic-Gross 2012[74], Conradi 2011[75]), T1D (Lammi 2007[76], Sumnik 2004[77]) and also for Crohn's disease (Hugot 2003[78], Han 2010[79]), but not consistently for this wide range of disorders. For example, birth order had no effect on MS in a large Danish study (Bager 2006[80]) or in a Canadian longitudinal cohort study (Sadovnick 2005[81]), although in the latter there was a slightly higher risk for younger siblings in families of at least seven children. Usually studies have tended to show a lower risk for younger siblings: a suggested explanation for differences in results is that it might relate to the degree of contact between siblings and age at contact[75]. In the study by Lammi of over 2000 Finnish patients with diabetes, children born second or fourth had a lower risk to type 2 diabetes (T2D), but there was no association of birth order with T1D diagnosed after the age of 15. A higher risk of T2D with either very young or older maternal age has prompted the proposition that low birthweight could be an important factor. This has been confirmed in a systematic analysis of studies on birthweight and diabetes (Whincup 2008[82]). Because of the family history risk of disorders such as Crohn's disease, twin studies have yielded interesting results, such as a higher risk of Crohn's in the first born dizygotic (i.e. not identical) twin (Spehlmann 2008[83]). Sibling clusters, i.e. siblings born close together, have also been found to have a higher risk of Crohn's, attributed to an environmental factor such as smoking or "*hygiene*"[78]. IBD is strongly influenced by genetic factors, more so for Crohn's than for ulcerative colitis (UC) (Khor 2011[84]), so that birth order/twin effects on UC are correspondingly weaker.

Without a consistent explanation, the protective association from larger family size, or the less clear birth order effects, may be spurious or due to other lifestyle, socioeconomic influences or factors such as maternal age. Questions remaining to be answered include why the association applies only to larger families (but not very large families) and the gender differences in the family size effect. The wide-ranging case definition of allergic/ atopic disorders in these studies suggests that further research must use precise diagnoses as well as definition and measurement of other possible co-factors. Karmaus and Botezan proposed in 2002[85] that causal factors to explain the sibling effect must satisfy two criteria: they must vary with sibship size and they must protect against clinical atopic disease. They concluded that the hygiene hypothesis failed to explain different sibling results in 53 studies reviewed at that time.

3.2 Child care: day nurseries and bed or room sharing

3.2.1 *Day nurseries*

The increased frequency of infections in children attending day centres is well established, for example 70% more infections for infants aged one year and 10% more for three year olds (Louhiala 1995[86]). If childhood infection prevented atopy, further day care studies should have shown a consistent protective effect. This has not occurred. Some studies at the time of the previous review reported reduced risk of atopy (von Mutius 1992[87], Krämer 1999[88], de Meer 2005[89]) while others showed no protective effect (Backman 1984[90], Infante-Rivard 2001[91]) and the trend is for more negative findings. Early day care attendance increased atopy in 10,851 children followed up to six years of age (Hagerhed-Engman 2006[92]). Although a study following–up 3,693 Dutch children from birth for 8 years showed less use of steroids between 4 and 8 years, children with a history of day care had more wheezing in early infancy and there was no evidence of a protective effect by the age of 8 years (Claudri 2009[93]).

The negative findings regarding a protection from atopy contrast with several studies on the effect of day care, or their social mixing outside the home, and childhood leukaemia. In a 2012 study, both day care and increased population mixing were associated with a higher risk of developing acute ALL, probably due to higher rates of clinical infection (Wiernels 2012[94]), but previous large studies have shown a protective effect from increased social contact in the first year of life, including day care (Greaves & Buffler 2009[95], Gilham 2005[96], Ma 2002[97]). Gilham (2005[96]) suggested that inconsistencies, regarding protective or causative effects, could be due to varying or imprecise quantifying of the age and duration of exposure to social contact. Both the Gilham and Ma studies indicated a dose response protective effect based on quantified measurements; and timing of the exposure early in life appears to be a key factor, as discussed in **Section 4.8**. A German case control study of MS patients reported a protective effect from attending a day-care centre and also a possible population mixing effect in that growing up in a large urban community of more than 100,000 people was also protective (Conradi 2011[75]).

Studies examining specific day care-related infection and allergy/ CID are reviewed in **Section 3.6.1**.

3.2.2 *Bed sharing and other early lifestyle factors*

Bed sharing after infancy appears to have declined in most industrialised countries, with no recent research into allergy risk, but it may be worth noting that bed sharing was reported for 23% of 9-year-old children in China (Li 2007[98]), although a later study did not identify this as protective or harmful for atopy risk (Li 2011[99]). Sharing a bedroom in childhood is more common: this may facilitate spread of infections, for example as reported for transmission of *Helicobacter pylori* among siblings in Northern Ireland: confirmed infection was more common both for room sharing and where a parent was *H.pylori*-positive (Farrell 2005[100]). Familial transmission of *H.pylori* is typical of urban society, whereas horizontal spread is more common in rural environments, particularly in the developing world (Schwarz 2008[101]). Overcrowding within households and other proxy infection exposures have been studied also in relation to CIDs, such as leukaemia and T1D.

3.3 *Breast feeding and allergy*

Several studies have shown that breastfeeding protects the infant against infection, by transfer of maternal antibodies and by constituents affecting the infant's gut (Okuda 2001[102], Mahmood 2001[103], Ehlayel 2009[104], Bulkow 2002[105], Arifeen 2001[106]) and this is confirmed by a recent meta-analysis (Ip 2007[107]). Its effect on allergic disease is less consistent, with little or no protective effect against allergic disease for short periods of breastfeeding such as 3-6 months (Ronmark 1999[108], Oddy 2009[109], Tanaka 2010[110], Bener 2007[111], Ehlayel 2008[112]). A recent

follow-up study suggests that at least six months exclusive breastfeeding is required for protection against either respiratory infection or wheezing at 1-2 years of age, in comparison with non-breastfed babies: the authors concluded that atopic mechanisms did not explain the results (Sonnenschein 2012[113]). Data trends appear to be against a protective effect on allergy (Kemp 2004[114], Yang 2009[115]) and there is also a need to take account of parental history of allergic diseases (Stabell Benn 2004(i)[116]). A recent international study of 51,000 children showed no protection for eczema from breastfeeding of various durations (Flohr 2011[117]) and a review in 2008 challenged the " '*conventional wisdom' that breastfeeding is protective against allergy"*, criticising study design, such as lack of appropriate controls and failure to adjust for different durations of breastfeeding (Duncan 2008)[118]. An important factor could be exposure to known triggers of atopy, such as human rhinovirus (HRV) and respiratory syncytial virus (RSV), which may be transferred from the mother. Inconsistent results may relate also to the differing components of breast milk: low CD14 in amniotic fluid or breast milk has been reported to be associated with increased risk of atopic eczema and allergic sensitisation (Jones 2002[119]).

While breastfeeding has not been specifically studied as a proxy microbial exposure in the context of the hygiene hypothesis, it is established that it may transfer active maternal viral infections such as HIV and Hepatitis (Jones 2001[120]) or methicillin-resistant *Staphylococcus aureus* from the mother's skin flora (Kawada 2003)[121]. A larger study of *S.aureus* transmission confirmed colonisation in breast fed infants (Chatzakis 2011[122]). It has been proposed that the commensal microbiota of breast milk may confer protection against atopy, supported by evidence that breast milk is not sterile (Michie 2003[123], Perez 2007[124]). An 'entero-mammary' circulation may be involved: translocation of bacteria from gut to Peyer's patches is increased during lactation (Rook 2010[7]) and bacteria can be seen in the glandular tissue of the healthy breast, as well as cultivable organisms from the mononuclear cells in breast milk. Bacterial transfer in breast milk may contribute to the colonisation and education of the neonatal gut.

3.4 Farm and other rural exposure

3.4.1 Several studies indicate a protective effect of growing up on a farm (von Mutius 2010[125]), at least as regards incidence of asthma and allergies, although specific protective factors remain a puzzle. This includes the absence of such protection from a general farming environment, for example in children of farm workers (Braun Fährlander 1999[126], Gassner-Bachman 2000[127], von Ehrenstein 2000[128], Riedler 2001[129], Radon 2001[130]). Apparently protective factors include prenatal and continuous farm exposure to adulthood and close contact or proximity with farm animals, recently confirmed for French farms by Varraso[131] and in Eastern Europe by Kramer 2009[132]. Consumption of raw [unprocessed] cow's milk has also been reported as protecting non-farm populations (Remes 2003[133], Perkin & Strachan 2006[134]). A study of 800 children in rural Crete showed high (24%) prevalence of atopy as indicated by a skin prick test, but few symptoms: those from farming families had more frequent contact with farm animals, mainly goats, but were not less likely to be atopic: investigators wondered if it was the "wrong sort" of farming (Zekveld 2006[135]). This is supported by a lack of allergy protection in children of crop farmers (Downs 2001[136]). Livestock exposure appears to be a key factor (Riedler 2001[129],Wickens 2002[137], Perkin 2006[134]), with pig and cattle farming reported as particularly 'protective', including for adult farmers, although not consistently across studies (Smit 2007[138], Monsó 2003[139], Monsó 2000[140]). In a large-scale study of 24,341 mother-child pairs (Stabell Benn 2004(ii)[141]), decreased risk of atopic dermatitis at 18 months was associated both with farm living and household pets, as well as other factors. Although pet ownership has been found to be protective in some studies (Aichbhaumik 2008[142]), study results are inconsistent and pets are in general recognised as a sensitising factor in allergic asthma and other atopic disorders (Simpson 2010[143]). Inconsistencies may relate to interruption of exposure, as prolonged

exposure to a pet can desensitise, so long as contact continues. Recent findings, that pet cats and dogs protect against respiratory symptoms and infections in the first year of life, appear to further confirm this explanation (Bergroth 2012[144]).

3.4.2 From an epidemiological viewpoint, the farm effect needs greater definition in terms of the type and size of farm, as well as the nature of exposure. Clearly there would be differing exposures on a large modernised farm compared with a small-holding, or organic methods compared with use of antibiotics or 'non-organic' feeding practices. The timing and nature of exposure also needs more precise definition as the relationship of immune response to the environment is still being researched: key features from research include the probable need for farming exposure up to adulthood (Lauener 2002[145], Ege 2007[146]), perhaps even during the mother's pregnancy (Ege 2006[147], Schaub 2009[148]). A recent meta-analysis of the farm effect on asthma confirmed a general protective effect in 39 studies up to mid-2011, but no consistent detection of a specific protective factor (Genueit 2012[149]).

3.4.3 Farming studies seem to indicate that it is not just any 'mud, glorious mud' that is important but the type of mud found where livestock are farmed. Mud direct contact or aerosol may increase microbial exposure, particularly to gastrointestinal bacteria, bacterial components (such as endotoxins), parasitic organisms such as *Cryptosporidium spp* and helminths. As discussed in S**ection 3.6.3**, none of these specific types of organism have yet explained the 'farm effect', lending support to the idea that diverse rather than specific farm yard exposure is important. A protective effect from consumption of milk 'straight from the cow' in childhood is supportive of the influence of non-pathogenic bacteria in milk. Individual genetic make-up must be important, as is timing: the protective effect holds only for farmer's children who have continuous farm exposure up to adulthood. The mechanism or optimal mix of organisms remains unclear.

3.5 The urban environment, 'population mixing' and the migration effect

3.5.1 *Allergic asthma and inner cities*

Allergic asthma is rising in 'unhygienic' inner cities in USA but is less common in migrants' children in European cities, possibly related to differences in exposure to indoor allergens such as cockroaches, rodent urine or foodborne infections (Matricardi 2010[150], Cullinan 2003[151]), as well as to different types of organisms, for example few of those found in a rural environment. These migrant families live in much the same environment as the native children, but there are some lifestyle differences, which may include diet as well as more general socioeconomic differences such as poverty or access to health care. The shift from traditional rural environments to modern urban living has been studied in Greenland Inuits. In just a few decades, this population has transformed from dependence on hunting and fishing to a society working in trade, administration and services. The frequency of allergic disorders has increased: specific IgE sensitization to inhaled allergens doubled in both children and adults between 1987 and 1998 (Krause 2002[152]). The proportion of adult obesity also doubled over this period (Andersen 2004[153]), a factor linked to higher risk of allergy (see **Section 6.3**). By contrast, studies of risk factors for MS have shown a protective effect from growing up in a large urban centre such as Berlin or Paris (Conradi 2011[75]): the lower risk in Paris was attributed, possibly, to lower rates in immigrants (Fromont 2010[154]). No such protective 'urban' effect applies to food allergy, according to a recent study of 38,465 US children (Gupta 2012[55]). Children up to the age of 18 were randomly selected and results based on questionnaire reports were adjusted for race/ethnicity, age, household income and latitude. Food allergy was highest for children in densely populated urban areas (9.8%), compared with a prevalence of 6.2% in rural areas. Milk and soy allergy were not related to geographic area, milk in particular being an

almost universal infant exposure, but allergies to other foods, particularly peanuts, showed this apparent urban effect. The authors suggested a link to the different type of microbial exposure in urban and rural areas: they also found unadjusted differences related to ethnicity/race, household income and latitude of residence, but the study did not include information on whether the children were recent or later generation migrants.

3.5.2 Increasing interest in the effects of population mixing - the movement and interaction of people over time and space – has prompted migration studies on allergy and CID. Such movements represent a 'natural experiment' in which to explore microbial exposure and other contributory factors influencing immune dysregulation (Söderström 2012[155]). People migrating from a low-incidence to a high-incidence country tend to acquire immune disorders with a high incidence at the first generation (Okada 2010[156]), for example diabetes in Pakistani immigrants to the UK or multiple sclerosis in Asian immigrants to the US: Söderström[155] reported that, for T1D, this applies also to 2nd generation immigrants, as might be expected if the important exposures for priming immunoregulation occur during pregnancy or infancy. Matricardi[150] suggests that the migrant families with paradoxical lower levels of atopy may retain a 'protective microbial ecosystem' typical of their country of origin. He suggests this explains why urban studies of asthma in "*unhygienic American cities*" challenge the microbial exposure tenet of the hygiene hypothesis. As Platts-Mills and colleagues observed[157, 158], the increase in urban asthma in deprived inner city populations can hardly be ascribed to cleanliness. The possible 'red herring' here is that a dirty urban environment may include several pathogens, but not the mix of organisms that seem to protect on a farm. Socioeconomic group has proved an unhelpful proxy for microbial exposure. Although there is a positive correlation between gross national product and incidence of asthma, T1D and multiple sclerosis in Europe (Bach 2002[159]), or with family income and incidence of atopic dermatitis (Werner 2002[160]), areas such as inner cities or particular regions do not follow this pattern (Patterson 1996[161], Asher 2011[162]). The increasing emphasis on disorders such as T1D, rather than asthma, is partly due to the difficulty of excluding non-atopic asthma: such cases represent about half of asthma patients (Pearce 1999[37]). The growing focus on peri-urban/rural differences in these population and migration studies have also drawn into question the proportion of rhinitis and eczema attributable to atopy (Asher 2011[162] Weinmayr 2008[163], Flohr 2008[164]).

3.6 More direct measures of microbial exposure and/or infection

Some of the studies exploring 'proxy' exposures such as farming have also examined particular infections. Direct measures include serology and microbial culture, usually in association with clinical diagnosis, although the less readily ascertained subclinical infection may be more important to the concept of microbial deprivation. Infections by some types of organisms may result in an immune bias against the development of clinical allergy, while others may predispose to allergic disease and asthma (Sevin & Peebles 2010[165]). Investigation of foodborne, gastrointestinal, respiratory and other infections are considered below, as well as the role of the commensal gut bacteria [microbiota]. The general trend of recent studies is to indicate a protective benefit from non-specific microbial exposure at undefined levels, but with harmful effects from exposure to high levels.

3.6.1 *Childhood infections*

Children are at risk from most of the infections that also affect adults, but infections mainly confined to childhood include measles, mumps, rubella and chicken pox: these are the most studied since they are usually accurately diagnosed, although vaccination has made them less common. Childhood infections of the ear, throat, upper chest and gut are often not traced to a specific organism, unless infection is severe and involves hospital admission. In some studies, the number of reported infections is recorded rather than type, since investigators were

exploring the idea of multiple exposures having an effect on immune system development or regulation.

Despite the strength of the 'family size' effect, the number or type of childhood infections does not appear to explain it: for example, a survey of infection records of 2,111 Aberdeen schoolchildren showed "*conflicting relationships with atopic disease*" with the risk of atopy increasing with the number of childhood infections[68]. A study of Danish children followed from birth showed no protection from childhood respiratory infection and no association of atopy with birth order (Nafstad, 2005[166]). In another Danish cohort study, the risk of atopic dermatitis increased for each childhood infection (such as common cold, diarrhoea or ear infection) recorded before 6 months of age, although having other siblings, owning a pet and living on a farm were protective (Benn 2004[167]). While showing a birth order effect for "allergic disease", a large (n24,690) English follow-up study showed no association with maternal clinical infection, except for a small increased risk of atopy associated with prenatal infection or antibiotic treatment (McKeever 2002[168]). A recent large case control study using UK general practice records has provided the most conclusive evidence yet of no convincing protective association between 30 clinical infections in the first year of life and hayfever (Bremner 2008[169]). The wide range of infections also included conditions such as lice or threadworm infection. The researchers found the now established protective inverse relationship of hayfever with the number of older siblings, but this did not relate to incidence of infections. They concluded: "*supporters of the hygiene hypothesis need to look beyond acute infectious illnesses*". Similarly a large British survey based on sera taken in routine medical examinations showed no association between tests for specific allergens or specific IgE levels and presumed higher exposure to childhood infections, for example due to attending boarding school or sibship size (Law 2005[25]). More detail on the infections studied is in **Appendix II**.

Day care studies have yielded similarly negative results. Respiratory infections reported by parents and allergy in 3,766 Texan children were both found to be higher in day care attenders (Sun & Sundell 2011[170]). A study of Finnish children attending child day care centres, using controlled hygiene intervention over 15 months, showed a 27% reduction in the 'hygiene' group of both clinically confirmed ear infections and requirement for antibiotic therapy (Dunder 2007[171]). The authors commented: "*the magnitude of the reduction in infections and the duration of the [hygiene] intervention should have led to an increase in asthma rates if the hygiene hypothesis were to apply to common childhood infections*". Later questionnaire follow-up showed no difference in the hygiene and control groups on rates of asthma, allergic rhinitis or atopic dermatitis (Kim 2008[172]).

Chicken pox [Varicella zoster] is mainly airborne. Some studies have shown that early natural infection may protect against atopic dermatitis and asthma, confirmed recently by a study comparing vaccinated and infected children in New York (Silverberg 2012[173]). Children infected with the wild virus also had lower risk of allergic rhinoconjunctivitis, although there was no difference in the risk of food allergy. Chicken pox vaccine contains a live attenuated strain of the virus, so this result needs further research, particularly as other studies have not shown a protective effect from chicken pox or other viruses (Gibb 2004[174]), including a Danish study reporting an increased atopy risk following natural infection with chicken pox, mumps measles or rubella (Bager 2002[175]).

Measles: a possible protective effect was intensively researched after the work of Shaheen[176] in Guinea-Bassau, which suggested that children who recovered from natural measles infection had half the risk of atopy, in comparison with vaccinated children. Early studies such as by Bodner and colleagues[68] appeared to confirm the benefit of natural measles infection. The opposite effect, i.e. aggravating atopy, was reported by Paunio 2000[177], Bernsen & van der Wouden 2008[71] and Bager 2002[175]. The latter study showed an increased risk for measles acquired in the

first year of life but no association either way for later ages of measles infection. A 2006 study on Steiner school children found a lower risk of IgE mediated eczema following natural measles infection, with no such protection following Measles-Mumps-Rubella (MMR) vaccination (Flölstrup 2006[178]). Because of controversy about the MMR vaccination, such as the disproved link with autism, studies have focused on this vaccine in recent years. MMR vaccination was associated with fewer hospital admissions with an asthma diagnosis and also less prescription of anti-asthma medicines (Hvlid & Melbye 2008[179]), but the 2008 study by Bernsen and van der Wouden[71] found no effect from MMR on atopic disorders (see also studies on childhood infection in **Appendix II**).

3.6.2 *Studies on general microbial exposure*

Lack of evidence for specific infections has led to research into the general microbial burden in childhood, such as numbers of microorganisms in dust or levels of C-reactive protein (CRP), a general marker of clinical or subclinical infection exposure and of inflammation. High infant microbial exposure (based on maternal reports of diarrhoea or respiratory infection) was found to predict low CRP levels in adulthood (McDade 2010[180]). In a recent update from the McDade group, CRP was studied in people of the Ecuadorian Amazon at four week internals: while CRP levels varied over time, the background levels were very low in the absence of episodes of infection, indicating intact immunoregulation that terminated inflammation when it was not needed (McDade 2012[181]). In contrast, the persistently raised CRP levels frequently reported in affluent urban areas in the US (Gurven 2008[182], [181]), even in the absence of infection, suggest failing immunoregulation and susceptibility to CID and cardiovascular disease.

The biodiversity hypothesis is relevant to levels of microbial exposure (von Hertzen 2011[8], Hanski 2012[183]). This hypothesis holds that the important factor is the range and diversity of microbes, rather than measures of quantity, related to a decline in microbial biodiversity akin to the threat or extinction of several animal or plant species. A wider range of microorganisms has been reported for the dust in children's rooms on farms than in non-farming environments, including *Listeria monocytogenes*, bacillus and corynebacterium species (Ege 2011[184]). This greater diversity for farm than for non-farm exposure was associated with less risk of asthma and atopy, although no particular species was identified as protective. The home environment was examined for US school age children, finding that higher levels of both gram-negative and gram-positive microorganisms were associated with fewer asthma symptoms, with greater apparent protection from endotoxin exposure (Sordillo 2010[185]). Investigation of the personal colonisation by microorganisms has been proposed as the next step in explaining these findings and defining how "*an environment rich in non-pathogenic microbes*" may reduce the risk of asthma (Gern 2011[186]).
See also endotoxins (**Section3.6.3 (vi)**) and inhaled dust (**3.6.7(iii**)).

3.6.3 *Gastrointestinal infections*

Gastrointestinal infections relevant to this review include those caused by HAV virus (HAV), enteroviruses, *Helicobacter pylori*, *Salmonella,* endotoxins associated with Gram-negative organisms, *Toxoplasma gondii* and helminth infestations. Few infections have been shown to have a consistent effect: for example clinically diagnosed gastroenteritis showed no protective effect for hayfever in the large study on UK GP records mentioned re: family size (Bremner 2008[169]). While some infections have not lived up to earlier optimism of protective effects, there is increasing evidence for the importance of the emerging pathogen and gastric commensal *Helicobacter pylori,* helminths (worm infestations) and some evidence for *Toxoplasma gondii* infections in immune priming or atopy. The role of the gut commensal bacteria is also discussed in this section.

(i) Helicobacter pylori

H. pylori bacteria are mammalian gut commensal organisms, asymptomatic in over 80% of humans, although persistent colonisation of the stomach is associated with higher risk of peptic ulcers, gastric cancers and other disorders. *H. pylori* is associated with contaminated water and food, but also spreads from person to person and is a cause of gastritis in children as well as adults (Harris 2008[187]). It may also cause or contribute to chronic urticaria [hives] (Greaves 2002[188], Hernando-Harder 2009[189]).

An inverse association between atopic disorders and positive serology to *Helicobacter pylori* is well established (Jarvis 2004[190], Kosunen 2002[191], Matricardi 2000[192]). This association applies particularly, or perhaps only, if acquired in childhood (Chen & Blaser 2007[193]). A strong protective relationship between *H.pylori* seropositivity and less risk of childhood asthma and other types of atopy in children aged 3-13 supports this (Chen & Blaser 2008[194]). The relationship may be more pronounced in men (Shiotani 2008[195]) and applies also to food allergy (Konturek 2008[196]). Children with atopic dermatitis had lower levels of *H.pylori* antibody than controls in an Egyptian study (Zuel-Fakkar & Girgis 2011[197]): the authors concluded there would be no benefit in eradicating *H.pylori* for such cases. A recent study of 878 Ethiopian infants recorded *H.pylori* infection in 41%, as well as high levels of commensal bacteria such as enterococci and lactobacilli: *H. pylori* was associated with "borderline" protection against eczema (p=0.07, i.e. not statistically significant) but no protection against other forms of atopy (Amberbir 2011[198]). Other bacteria measured in this study were not associated with atopy.

The inverse relationship has been observed across different populations and geographical areas (Sevin & Peebles 2010[165]). But in a recent study of 2633 UK adults, there was no association between seropositivity and clinically confirmed atopy (Fullerton 2009[199]). Similarly, a 2008 study of 90 patients with gastric pain and indigestion showed no difference in *H. pylori* positivity for atopic and non-atopic participants (Baccioglu 2008[200]). A large study of specific IgE levels in British businessmen showed no association with evidence of *H. pylori* infection (Law 2005[25]). Nor has an inverse (protective) association been confirmed in two other relatively small studies of adult patients with clinically diagnosed asthma (Tsang 2000[201], Jun 2005[202]). *H. pylori* seropositivity is more common in children with food allergy (Corrado 2000[203]), which fits with its tendency to damage the gastric barrier, permitting more food allergens to pass through[200].

Such conflicting results pose the question that the protective association may be coincidental, possibly linked to removal of *H. pylori* through antibiotic therapy, other treatments or population trends. *H. pylori* appears to have co-evolved with humans and persists during the life of the host (Blaser & Atherton 2004[204]). Transient colonisation can occur but the usual pattern is chronic *H. pylori* acquisition, associated with no change in antibody titres over decades, indicating an ability to reduce recognition by immune sensors. A combined protective effect against allergy and other CIDs may depend on the presence also of helminth infestation, which would have been common in our evolutionary past. For further discussion of a possible evolutionary role, see **Section 4**.

(ii) ***Hepatitis A (HAV) and Hepatitis B (HBV)***

Positive serology to HAV has been used as a marker of past clinical or subclinical exposure but with inconsistent results regarding development of allergy. Some studies, as covered in more detail in a previous review[2], showed an inverse correlation with atopy (Matricardi 2002[205], Matricardi 1997[206], Linneberg 2003[207]) but others did not confirm this (Jarvis 2004[190], Bodner 2000[208], Cullinan 2003[151]) and another large serological study also has shown no association between IgE levels and HAV positive serology (Law 2005[25]). An explanation for such inconsistency has been suggested by Umetsu and colleagues (2005[209]): they tested 375 individuals for antibodies to HAV and for serological evidence of atopy. They found that HAV

was protective against atopy only for those with a particular variant of the gene TIM-1, The way in which HAV infection and TIM-1 alter the immune system to protect against atopy is not yet clear, although the evidence of very high levels of childhood HAV infection prior to around 1970 emphasises the possible importance of exposure to this virus during the early development of immunoregulation (Umetsu & DeKruyff 2010[210]). A study of HBV carrier children in Turkey showed they had more frequent symptoms of asthma, eczema and atopy than children with T1D or healthy controls (Cakir 2008[211]). By contrast, another Turkish study showed significantly lower atopy in both HBV and HAV seropositive children, compared with healthy controls (Kocobas 2006[212]). A recent review of studies investigating the risk of inducing atopy through immunisation for HBV, tetanus or diphtheria concluded that there was no influence on atopy or other modified immune response (Friedrich 2007[213]). Von Mutius has argued (2010[125]), that HAV should stay in the frame, since the receptor for the virus and its ligand [co-factor] belong to a family of proteins concerned with regulation of CD4 T-cell differentiation, airway inflammation and airway hyperresponsiveness.

HBV, normally acquired via blood or other body fluid transmission, has aroused interest because of the large number of asymptomatic carriers, an estimated 400 million people worldwide. While it has not been studied in detail in the context of atopy, it has been suggested that the immune changes in chronic carriage (towards Th-2 response) would be expected to increase the risk of allergic disease (Kocobas 2001[214]).

(iii) *Enteroviruses*

An enterovirus is a virus that enters the body via the gastrointestinal tract and thrives there, although often moving on to attack the nervous system. Examples include the polio, coxsackie and echoviruses. In a study of two genetically related, but culturally very different, groups of schoolchildren living in Karelia and Finland, antibodies to enterovirus infections in the Karelian children were associated with a lower risk of allergic sensitization (Seiskari 2007[215]). The difference was attributed to poorer, possibly more overcrowded or presumed less hygienic living conditions in the Karelian group, which would have favoured acquisition of these infections at a younger age than in the Finnish group. Animal studies indicate a possible protective effect of enteroviruses against T1D (Tracy 2010[216]). Where human clinical enterovirus infection has been diagnosed, there is little evidence of a protective effect against T1D, rather the reverse. For example, enterovirus RNA was found to be more common in the blood of children with T1D than in non-diabetic children (Oikarinen 2010[217]); another recent study also showed a risk of developing diabetes after enterovirus infection (Stene 2010[218]). A dual role, i.e. either protective or causative of T1D, has been proposed for these viruses, depending on whether the exposure is before the first year of life[216]. This is supported by research into the effects of rotavirus infection: childhood rotavirus infection is associated with damage to insulin-producing cells in the pancreas, causing T1D (Graham 2008[219]) but animal studies have produced conflicting results, suggesting a possible protection against T1D from very early infection (Filippi 2008,[220] Harrison 2008[221]).

(iv) *Salmonella*

An Italian study reported lower prevalence of allergic rhinoconjunctivitis and asthma in children hospitalised for salmonellosis (Pelosi 2005[222]). As yet this has not been confirmed by other research: it should be noted that most cases of *Salmonella* infection do not require hospital admission and that incidence of many *Salmonella* species has declined with increasing standards of food production. Also there could be a social class/ socioeconomic confounder, for example in differences in diet, food storage or poor quality food consumption.

(v) *Toxoplasma*

Toxoplasma gondii is a parasite hosted predominantly by cats, but other warm-blooded animals and humans can be carriers. It invades the cells and this intracellular existence allows it to avoid the host's immune system. While mainly causing self-limiting disease or asymptomatic carriage, infection during pregnancy can have serious effects on the fetus. In a study examining positive serology (i.e. previous exposure) in young Italian adults, positive tests for *Toxoplasma,* in addition to HAV and *Herpes simplex type 1(HSV-1)*, were associated with reduced risk of hayfever, asthma and atopic sensitisation (Matricardi 2002[205]), but this was not confirmed in a 30 year follow up study in Scotland: there was no association between seropositivity (for *T.gondii, H.pylori, HAV*), and atopic status (Bodner 2000[208]). A later Scandinavian study of 1249 adults also found that positive *Toxoplasma* serology was not protective, although other infections, including *H.pylori* and *HSV-1,* showed a possible protective association (Janson 2007[223]). *Toxoplasma* infection has been linked to the hygiene hypothesis because it is more common in unhygienic environments: Roma children living in "*unfavourable*" conditions were recently compared with others in the same area in Greece: antibodies to 13 infections were measured. *T.gondii*, along with *HAV, H.pylori, cytomegalovirus, HSV-1* and *Hepatitis B* virus were all more common in Roma children, but there was no protective effect for allergic disease, and Roma children in fact were found to have a greater risk of atopy (Michos 2011[224]). Another recent study of children in poor neighbourhoods in Brazil illustrates a key problem in these studies: while the children with raised sIgE (allergen specific IgE, a marker of atopy risk) were less likely to have antibodies against *T.gondii*, this did not relate to diagnosed atopy (Alcantara-Neves 2012[225]). Thus a clinical diagnosis is a more important indicator than serology tests in examining possible causation or prevention of atopy. Several of these studies showed an increased level of atopy in children who experienced the clinical effects of *Toxoplasma* and other infections, which is relevant to discussion of the type or level of exposure required to promote a healthy immune system (see **Section 6**).

(vi) *Endotoxins*

Exposure to endotoxin, a cell wall component of the outer membrane of gram-negative bacteria, was previously thought likely to explain the 'farm effect', but further studies have indicated a complex relationship depending on level of exposure and how genetic susceptibility is expressed [phenotype] (Simpson 2006[143], Martinez 2007[226]). Measurement of endotoxins is fraught with methodological difficulties: measuring endotoxin in indoor and outdoor environments does not necessarily predict daily personal exposure (Delfino 2011[227]). Braun-Fahrlander and colleagues 2002([228]) reported an inverse relationship between endotoxin in children's mattress dust and prevalence of hay fever, atopic asthma and atopic sensitization, yet high levels of endotoxin were positively associated with non-atopic wheeze. Douwes 2006[229] took mattress and floor measurements for infants followed up to the age of 4 years: mattress microbial levels showed no association with wheezing, but high floor microbial contamination, especially endotoxin, appeared to be protective for persistent wheeze. Mattress dust in farm households contains more gram-negative bacteria and their components than that from non-farm households (Schram-Bijerk 2005[230]). Muramic acid, a bacterial cell wall component, indicates levels of bacteria rather than specific endotoxin and was associated with lower frequency of wheezing and asthma in rural children in the ALEX study (van Strien 2004[231]). Endotoxin exposure was also associated with lower allergic sensitization in a study of school-age children (Sordillo 2010[185]). Studies of occupational exposure to endotoxin in adult farmers have shown protection from allergic rhinitis, but higher levels of endotoxin led to increased asthma prevalence (Smit 2008[232]) or increased airway hyper-responsiveness (Portengen 2005[233]). In a recent large study on endotoxin exposure in adult workers, current 'low mediate' levels were associated with least risk of allergic sensitisation and hayfever, showing an inverse dose response relationship: 'high' levels (above 100 EU/m3) increased the risk of chronic bronchitis and organic dust syndrome: agricultural workers were the most likely to have protective effects from the

exposure (Basinas 2012[234]). Inhalation of endotoxins in dust is also likely to be accompanied by inhalation of other components such as dust mites and pet hairs, discussed in **Section 3.6.7(iii).**

3.6.4 *Gut microbiota*

The microorganisms that typically inhabit the gut have been called its flora, commensal organisms or microbiota (terms that apply also to the bacteria present on skin (**Section 3.6.8**) or in other parts of the body). Sterile at birth, the gastrointestinal tract is colonised within a week by bacteria and after weaning develops an adult-type pattern of indigenous microflora. The microbiota constituents include the bacteria, their genes, as well as proteins and metabolites, collectively known as the microbiome (Korecka & Arulampalam 2012[235]). Trillions of bacteria are present in all human guts (approximately 10^{14}, that is, ten times more than the number of nucleus containing cells in the body (Berg 1996[236])). Early colonisers include *E.coli, streptococci*, and anaerobic organisms such as *Bacteroides, Bifidobacteria* and *Clostridium*. Human milk is probably the main source of gut bifidobacteria, (Martin 2008[237]). Formula-fed infants harbour a mixture of several bacteria in faecal microbiota, whereas bifidobacteria and lactobacilli predominate in breast-fed babies (Harmsen 2000[238]). An important role of the gut microbiota in developing the mucosal immune system has been established (Brandtzaeg 1996[239], Sudo 1997[240]) and it arguably comprises the most abundant source of early immune stimulation (Björkstén 2005[241], Björkstén 2009[6]). Germ-free mice have poorly developed lymphoid systems and increased susceptibility to inflammatory disorders (Rook 2011[17]). Thus the microbiota are not simply colonisers, but are actively involved in many aspects of metabolism, including immune system regulation: the relationship between the microbiome and the human immune cells has been described as a balancing act that has taken some 200,000 years to calibrate (Ackerman 2012[242]). This co-evolved host-microbe interaction provides mutual benefits. Recent research also suggests that a host-specific gut microbiota (for example, different between mice and humans) is required for appropriate maturation of gut immunity, in turn considered critical for a healthy immune system (Chung 2012[243]).

Evidence that the calibration of microbiota is now challenged comes from studies comparing the microbiota in traditional, rural environments and in city environments. Babies from European cities have been shown to have dramatically different microbiota compared to those born in Burkina Faso, Africa (de Filippo 2010[244]). Reduced diversity of faecal microbiota was reported for Swedish infants at one week who went on to be diagnosed with atopic eczema at 18 months, in comparison with babies not diagnosed with eczema at that age (Wang 2008[245]). Similarly, an increased risk of allergic disease was associated with less gut intestinal microbiota diversity in infancy in a Danish cohort of 411 children (Bisgaard 2011[246]). Another study of infant faeces showed an association between eczema at 6 months and reduced microbial diversity of the flora compared with controls (Forno 2008[247]). While these studies indicate a potential role for microbial diversity as regards atopy, there are also some inconsistent findings. For example, a study of faecal microflora in 300 infants born in European cities showed no evidence of acquisition of particular bacterial groups during the first year of life, or any association with eczema or food allergy by 18 months (Adlerberth 2007[248]). Regarding IBDs, gut commensal organisms have been reported as protective and associated with loss of tolerance reactions (Matricon 2010[249]), suggesting that the driving factors for the immune dysregulation are more likely to be commensal bacteria, or their products, rather than pathogens (Xavier & Podolsky 2007[250]). This is intuitively logical, as a necessary function would be more likely to depend on safe, ubiquitous organisms rather than on sporadic, dangerous microbes.
(Diversity is further discussed in **Section 4.6**).

3.6.5 *Antibiotics and loss of gut microbiota diversity*

Björkstén (1999[251]) proposed that antibiotic use in infancy might reduce the diversity of gut microbiota, which may in turn predispose to immune system disorders (Mazmanian 2005[252]).

An association of antibiotic prescription with later development of asthma or allergy has been reported in several studies over the last decade, as reviewed by Sevin and Peebles in 2010[165] and a more recent cohort study (Risnes 2011[253]). Kozyrskyj and colleagues, confirming this effect in a report of a follow-up study in 2010[254], suggest that inconsistent results in some of the early studies may have been due to bias from the use of antibiotics to treat lower respiratory tract infection, which is more common in asthmatics. Frequent antibiotic use in early life is more common amongst asthmatic children (Celadon 2004[255]]. In the study by Kozyrskyj, a significant association of antibiotic use with atopy risk was observed when children with asthma diagnosed before 6 months were excluded and those without lower respiratory infection were analysed separately. The association appeared to be strongest for broad-spectrum antibiotics or multiple prescriptions. Confounders for this effect could include the use of paracetamol [US acetaminophen] to treat fevers, associated with increased asthma and allergy in some studies, as well as exposure to antibiotics via breastfeeding. Inappropriate prescribing of antibiotics for young children may also complicate interpretation of study results and overuse of these agents remains a persistent problem (McDonnell Norms Group 2008[256], Paul 2011[257]).

The most conflicting results for antibiotic effects are for asthma as opposed to hayfever and other types of atopy (Sevin & Peebles 2010[165]). A recent meta-analysis concluded that there has been overestimation of an association between antibiotic use and subsequent wheezing or diagnosis of asthma, possibly due to varying definitions of disease (Penders 2011[258]). Assessment of potential effects is also hampered by exposure to antibiotic residues or by-products, which may persist in treated water, as well as the presence of preservatives (e.g. ethyl parabens) and antibacterial products, such as triclosan, which have been added to some personal care products, such as soap, since around the 1950s in US hospitals and from the 1990s in a wider range of products, such as toothpaste. A 2012 Johns Hopkins survey of US children aged 6 to 18 found that levels of IgE antibodies correlated with concentrations of antimicrobial agents, including propyl parabens and triclosan, in urine samples (Savage 2012[259]). The highest levels of triclosan were associated with a 2-fold higher risk of environmental allergies, as measured by IgE levels. IgE antibodies are associated with response to allergens such as pollen or pet dander: while generally higher in those with clinically diagnosed allergy, raised levels do not necessarily indicate that allergy will develop (see **Section 2**). Elevated IgE levels have also been reported following oral antibiotic therapy, attributed to alteration of the gut commensal bacterial populations (Kummeling 2007[260], Hill 2012[261]). Meanwhile, the finding of an allergy risk associated with antibacterial products needs further research and examination of the possible confounders, such as those implicated in antibiotic studies: the team at Johns Hopkins are planning a long term birth cohort study to assess antibacterial exposures.

3.6.6 *Helminths*

Helminths comprise a large range of parasitic worms: nematodea (round worms such as the hookworm or pinworm) and platyhelminths (flat worms such as trematodes (flukes) and tapeworms). Since they date back at least to the time of the dinosaurs (Weinstock 2009[262]), their colonisation of humans was, until recently, widespread. It has been estimated that around 50% of the young children of Europe and N.America were infested with pinworm (*Enterobius vericularis*) up to the mid 20th century (Gale 2002[263]). Pinworms often cause little or no symptoms and such helminths have developed the ability to evade and control the host's immune system. In return, the 'old friends' hypothesis' maintains that these organisms have co-evolved a role as inducers of immunoregulation, associated with less likelihood of autoimmune or atopic reactions (Rook 2009[264]). Elevations of anti-inflammatory cytokines, such as interleukin-10, occur during long-term helminth infections (Yazdanbakhsh 2002[265]) as well as eosinophilia and a specific immunoglobulin (IgG) response. Case control studies in Ethiopia and Vietnam have shown that helminth infection protects against allergy[262]. While most helminths infect via the oral route, immunoregulatory effects have been described for a filarial worm species (*Litomosoides sigmodontis*) that enters through the skin (Taylor 2005[266], Taylor

2007[267], Taylor 2009[268]). Studies of this organism in mice have shown inhibition of allergic sensitization and of airway hyperreactivity (Dittrich 2008[269]). A recent review of the aetiology of atopic dermatitis concluded that helminths are the only pathogens to show a protective effect (Flohr & Yeo 2011[270]). This effect appears to apply to a wide range of immune dysregulatory disorders, including IBD such as Crohn's disease and ulcerative colitis and MS. Anti-helminth treatment, while needed for symptomatic infection, is unpleasant and appears to be detrimental to atopy risk. In a trial of an anthelmintic albendazole [antihelminth] in pregnant women in Uganda, the risk of infantile eczema was increased (Mpairwe 2011[271]). This confirmed the expectations of the investigators, who had found hookworm and schistosomiasis in about two thirds of the women. Trials of worm therapy for CID are reviewed in **Section 4.11**.

3.6.7 *Respiratory infections and atopy*

i) *Bacterial infections*

Respiratory infections, excluding tuberculosis, have been estimated to cause 6% of the global burden of disease, with 4.2 million people dying each year of lower respiratory infection (Hilty 2010[272]). *Streptococci* have been implicated in influencing the phenotypic expression of asthma, for example as part of bacterial intrauterine contamination in a Finnish study: infants from these mothers had an increased risk of asthma and allergic sensitisation 15-17 years later (Keski-Nisula 2009[273]). Bacterial colonisation in utero may be a manifestation of an immune defect that later predisposes to asthma, rather than a direct bacterial effect, although studies have identified associations with particular respiratory bacteria. Neonatal colonisation of the lower pharynx with *Streptococcus, Moraxella* and *Haemophilus* were associated with increased risk of asthma and allergy at the age of five (Bisgaard 2007[274]). By contrast, a rich microbial environment of relatively non-pathogenic organisms in the airways may protect against asthma (Eder 2006[275]). *Haemophilus spp* were more frequent colonisers of the bronchi in asthma patients than in controls, while Bacteroidetes, particularly *Prevotella spp*. were more frequent in controls: the investigators suggested that microbiota are disturbed by more allergenic bacteria in asthmatic airways[272], possibly related to an increased risk of colonisation when the function of microscopic cilia [hairs] in the respiratory tract is impaired.

Tuberculous infection and other Mycobacteria

Mycobacteria are aerobic, Gram-positive bacteria and are unusual in having a thick cell wall that prevents drying out. They prompt particularly strong Th1 immune responses (associated with less risk of allergy) and bind to macrophage receptor cells known as Toll-like receptors, TL-Rs: these and other effects have made these microorganisms of great interest regarding the causes and prevention of atopy (Hopkin 2000[276]). Many strains are harmless and useful in that they degrade organic matter in soil. Pathogenic strains have long caused disease in humans, such as tuberculosis (*M. tuberculosis*) and leprosy (*M. leprae*). Animal models have shown that mycobacteria suppress atopic symptoms, but the relationship of mycobacterial infection to atopy in humans remains unclear. A systematic review of properly conducted studies of mycobacterial infection found only 40% showed a protective effect (Obihara 2007[277]). Positive tuberculin skin tests were inversely associated with wheeze, asthma and atopic eczema in a study of 5717 Japanese schoolchildren (Miyake 2008[278]), although no relationship was found for Bacille Calmette-Guérin (BCG) vaccination and allergic disorders.

Atypical respiratory bacteria

These include *Chlamydia* and *Mycoplasma* species, both of which have been particularly implicated as causing asthma, as well as exacerbating this disease(Sevin & Peebles 2010[165]). *Mycoplasma pneumniae* infection has been shown to maintain or enhance the Th2 cell responses associated with asthma (Wu 2008[279], Kraft 2008[280]).

ii) *Respiratory viruses*

Respiratory viruses have been increasingly identified as having an allergenic, rather than any protective, role, including the common cold (*Rhinovirus*) (Jackson 2008[281], Miller 2009[282], Lee 2007[283]), *Influenza A* (Riese 2004[284], Bordon 2011[285]) and *Respiratory syncytial virus* (RSV)[165]. RSV is the most studied, partly because of its ubiquity: it is thought that nearly all children are infected by RSV at least once by the age of 2 years[165] and children born four months before the RSV winter peak are particularly likely to develop asthma (Wu 2008[286]). Also, children who develop severe bronchiolitis are at higher risk of asthma and allergic sensitisation (Sigurs 2000[287]). A twin study in Denmark concluded that the relationship between RSV and asthma may be due to a shared genetic predisposition to both (Thomsen 2009[288]). Severe RSV infection may be a marker of underlying genetic predisposition to asthma: epidemiological studies are hampered by the difficulty of defining the phenotype [clinical presentation], for either RSV or asthma (Kuehni 2009[289]). Viruses other than RSV can cause bronchiolitis in infancy: a recent Finnish study found an association of pre-school asthma in children hospitalised for bronchiolitis before 6 months of age (Koponen 2012[290]). While the direction of causation remains unclear for respiratory viruses, a protective role against atopy and asthma seems increasingly unlikely.

For comment on chicken pox and measles, which are mainly airborne, see **3.6.1**.

iii) *Dust mites and other inhaled allergens*

Allergies to dust are common, much depending on the content of the dust, for example, house dust mites, endotoxins, pollen or pet hair, or the level of exposure. An inhaled allergen can cause sensitisation in susceptible individuals and thus lead to allergic symptoms on a later exposure. Sensitisation and subsequent triggering of allergic reactions should be distinguished from the underlying causes of impaired immunoregulation, which make an individual vulnerable to these allergens. Apart from endotoxins (see also **3.6.3vi**), dust components have not been implicated as causing immunoregulatory disorders. Inhalation of dust containing dust mites or cat salivary proteins is associated with increased risk of sensitisation to these allergens and to persistent wheeze (Brussee 2005[291]). A review of the contribution of pets to the infant immune response reported that in addition to pet allergens, homes with pets have higher levels of endotoxins (Simpson 2010[292]). It has proved difficult to determine the specific contribution of such allergens and of associated social factors such as education and household income.

(iv) *Fungi*

Fungal spores can trigger an allergic reaction and fungi also produce proteases, linked to allergic airway disease. Fungal walls contain chitin, an abundant polysaccharide also present in the microfilarial sheaths of helminths and insect exoskeletons. Animal models have shown that infection might induce host chitinases, which break down chitin and enhance the asthmagenic effect. Chronic infection may thus cause persistent airway inflammation and contribute, more than previously thought, to the aetiology of allergic asthma (Denning 2006[293], Sevin & Peebles 2010[165]). Markers for fungal exposure are also more common in farming households (Von Mutius 2010[125]), and a recent study of farming versus non-farming environments found that exposure to some fungal species was inversely related to asthma risk (Ege 2011[184]), although only as part of general microbial exposure.

3.6.8 *Skin microbiota and allergy*

The skin is rapidly colonised after birth. *Staphylococci* and *Streptococci* are early colonisers, with a more balanced population evolving over the first year of life. A normal adult has a vast diversity of skin flora, with 150 species on the surface of the hand alone, including *Staphylococci* and *Streptococci* but also bacteria such as *Lactobacilli, Propionibacteria* and *Corynebacterium*. A core set (about 13% of the range) appears to be common to most individuals, with more diversity in women than in men (von Hertzen 2009[294]). The diversity of bacterial communities

on the skin is similar with that of the gut, implying that the skin microbiota plays an important role in the immune system (von Hertzen 2011[8]). As in the intestinal flora, the skin community includes permanent and transient members (Grice 2009[295]), suggesting that at least part of this microbiota interacts continually with the environment. In a recent study in Finland (Hanski 2012[183]) the skin microbiota of teenagers with atopy was compared with non-atopic teenagers, as measured by atopic sensitization: the environmental diversity around homes influenced the composition of bacterial classes on the subjects' skins. The environmental biodiversity was measured in gardens around the home as well as in major land use within 3 km of the study subjects' homes. Gram-negative gammaproteobacteria were found to have particular allergy-protective potential, because of an association with production of anti-inflammatory cytokines and greater abundance on the skin of healthy subjects than those with atopy. This correlated with greater environmental biodiversity near the healthy subjects' homes, although it is pertinent to the 'farm effect' that living on a farm was unrelated to atopy risk in the study. Gammaproteobacteria is a large bacterial class that includes pathogens such as *Escherichia coli, Salmonellae, Yersinia pestis, Vibrio cholerae* and *Pseudomonads*, in addition to the non-invasive species found on skin and in soil, flowers, grass pollen and marine environments.

While species of both *Staphylococci* and *Streptococci* cause skin and other infection, both may also be involved in contributing to homeostasis [balance] in the skin environment, particularly *Staphylococci*, which have been shown to modulate the early inflammatory response (Lai 2009[296]). A study of skin swabs taken from infants aged 1 to 12 months showed early dominance of *Streptococci* and *Staphylococci* up to 6 months, but greater diversity in older infants and the authors commented that timely establishment of a balanced skin flora may protect against both infection and later inflammatory responses (Capone 2011[297]). A recent study of the skin microbiota in adults indicated that staphylococcal colonisation (*S.aureus, S. epidermidis*) in adults may be a sign of increased susceptibility to atopic dermatitis (Dotterud 2007[298]), confirming an earlier study on *Staphylococci* (Dahl 1983[299]).

The skin of babies is generally better hydrated than in adult life: the abundance of *Staphylococci* in infants corresponds to moist areas of adult skin (Grice 2009[295]). Site specific differences in bacterial communities begin within the first 3 months, but they are unstable compared with adult skin, which may allow abnormal skin development, for example in predisposition to eczema or acne (Capone 2011[297]). A recent increase in infantile atopic dermatitis has been reported (Manicini 2008[300]), attributed as possibly due to changes in the development of the skin microbiota or in lifestyle choices that affect the integrity of the skin barrier, such as excessive washing or exfoliation (Callard & Harper 2007[301]). Immune pathways in the skin, as in the gut, may be linked to the development of allergy ([301], Spergel & Paller 2003[302]), or asthma (Benn 2002[303]) as well as in assisting with the education and adaption of the immune system (Grice & Segre 2011[304]).

3.6.9 *Respiratory microbiota and allergy*

The role of the commensal organisms in the respiratory tract has been considered regarding later allergy risk or protection, particularly as earlier ideas that the lungs were sterile have been disproved (Huffnagle 2010[305]). This is an active research area but so far it has not been possible to obtain an uncontaminated sample of normal respiratory microbiota. It also remains to be seen whether the latest research on the role of skin microbiota (as described above re: Hanski 2012[183]) may involve atmospheric dust which also acts via the respiratory route. Current thinking still gives the gut microbiota the predominant role in influencing the development and maintenance of immune responses (Noverr 2004[306], Blaser 2006[307]).

3.6.10 *Caesarean section and birth canal microbiota*

Delivery by Caesarean section has increased in many countries in recent decades. The rise has been driven by several factors, for example the one child policy in China, where two thirds of babies in urban areas are now delivered in this way (Ackerman 2012[242]). In the US, Caesarean deliveries have increased from only 4.6% in 1962 to 32% in 2009 (Decker 2011[308]). During a natural birth, infants are colonised by microbes from the mother's birth canal and faeces, while Caesarean section babies are colonised by environmental microbes from the mother, ambient air and delivery staff. Caesarean delivery has been linked to a greater tendency to develop allergic disorders, leading to suggestions that transfer from the bacterial flora of the birth canal, in addition to greater exposure to faecal organisms, is important in the early colonisation of the baby's gut (Ly 2008[309], 2006[310]). Caesarean section babies have been found to have fewer *Bifidobacteria* and *Bacteroides spp*. in their gut microbiota, with associated greater risk of colonisation by the pathogen *Clostridium difficile*, in comparison with vaginally delivered infants: such differences have been cited as giving a greater risk of obesity and diabetes (Musso 2010[311]). Caesarean delivery has also been associated with a greater risk of developing Coeliac disease (an autoimmune disease of the small intestine), although not with the two major IBDs, Crohn's disease and ulcerative colitis[308]. Epidemiological studies on an association between mode of delivery and atopic disease, such as hayfever, have produced inconsistent results, possibly because of failure to adjust for a family history of atopy: a US study, restricted to children up to 9 years with a parental history of allergy, found a 2-fold higher risk of atopy for Caesarean section, compared with vaginal delivery (Pistiner 2008[312]).

3.6.11 *Urinary tract infections*

A recent Australian study examined exposures during pregnancy and later atopy in the infants (Algert 2011[313]). Maternal antenatal admissions for urinary tract infection (UTI) were associated with increased risk of childhood asthma; rupture of the membranes before labour was also a predictive risk factor. This study also showed higher risk for autumn and winter conceptions, which the investigators concluded suggested winter flu or low Vitamin D exposure, rather than any specific organism.

3.6.12 *Immunisation/vaccination*

Since vaccination involves the administration of attenuated or killed microorganisms, or selected components from them, in order to induce an immune response, the immunological effects could possibly influence susceptibility to atopic disease. Epidemiological studies as reviewed in the IFH 2004 and 2006 reviews provided no consistent support for either a beneficial or adverse effect of vaccination/immunisation on atopic tendency. This is confirmed by other research (Koppen 2004[314], Sanchez-Solis & Garcia-Marcos 2006[315]) and a review of vaccine studies (Rottem 2008[316]). Vaccination remains a controversial topic because of occasional or postulated side effects and so it has received detailed attention, for example a summary of randomised trials on influenza vaccine, which concluded no effect on existing asthma (Cates 2008[317]), although because influenza infection appears to predispose to atopy, 'flu vaccine has also been proposed as a preventive measure, based on beneficial effects in animal studies (Minne 2007[318]). A study focusing on the MMR vaccine showed no effect on atopy risk (Bernsen & van der Wouden 2008[319]), although there was evidence of protection against some forms of atopy in children vaccinated against pertussis and who later acquired this infection (Bernsen 2008[320]) (see **Appendix II**). A recent meta-analysis of studies on childhood vaccination against pertussis (and BCG) also concluded that neither vaccine affected atopy risk in childhood or adolescence (Balicer 2007[321]). The Old Friends hypothesis implies a need to focus on vaccines involving microorganisms no longer common in the modern environment, as well as those thought to be beneficial in gut microbiota (Björkstén 2012[322]). BCG vaccination is relevant to the OF hypothesis and is further considered in **Section 4.8**.

4. WHAT IS THE NATURE OF THE MICROBIAL EXPOSURE REQUIRED FOR 'PRIMING' THE IMMUNE SYSTEM?

4.1 The epidemiological evidence, reviewed in **Section 3**, shows increasing support for the role of microbial exposure as a key contributory factor for immune regulation in relation to risk of CID and allergic disorders. Despite the considerable amount of new research generated since the previous IFH reviews, the results do little to elucidate the precise nature of the exposure required for immune priming and development. The data suggest that both clinical infection and reduced microbial exposure may predispose towards development of CIDs and allergic disorders. The inconsistent results from studies suggest that, for any individual, there are many additional factors that determine whether a particular type of CID will develop. The lack of consistency also suggests that no single type of organism, no one single source (environmental, zoonotic, human) and no single route of exposure is likely to be the underlying cause. For example, whereas the recurrent observations of a protective effect of farm living suggests that exposure to zoonotic species from farm animals (including consumption of unpasteurised milk), and/or environmental strains, are important, the link to family size indicates possible protection from 'subclinical' exposure to some microorganisms. As we have seen, there is little evidence of a protective effect from particular recorded types of infection, such as ear infection, respiratory infection, diarrhoea and other infections occurring in childhood.

Since 2006, a number of hypotheses have been put forward which help to explain the inconsistencies and provide clearer and more unifying insights. In this section these are discussed in relation to the epidemiological data in **Section 3** and data from other sources, such as trials of therapy and experimental models. Here we try to distinguish:

1) the groups, types or properties of organisms most likely to be protective against allergies and other CIDs (including the role of multiple exposures) from those which are less likely to perform this role;

2) the source/s and routes of the necessary exposures that may be protective against development of allergies and other CIDs and those less or unlikely to perform this role;

3) the optimal timing for microbial exposure for correct immune system development.

4.2 The 'Old friends' (OF) hypothesis

The OF hypothesis, proposed by Rook and colleagues in 2003 (Rook 2004[323]) has gained recognition because it addresses the problems encountered in identifying 'protective' microbial exposures, but also provides a unifying explanation for the impact of other factors such as modern diet and the urban environment. The hypothesis is reviewed in more detail in a number of recent reviews (Rook 2009[264], 2010[7], 2011[17]). The **OF hypothesis** holds that the important microbial exposure for immune system development is no longer readily available in the modern world, but was more common in the primaeval environment over 50,000 years ago, when the human immune system evolved.[7]. The first mammals encountered life forms, such as bacteria, in a world in which there were already at least a million bacteria in every millilitre of water, tens of millions in every gram of soil and probably similar numbers of microbes in other parts of their environment, as well as on their skins and in their guts. As a result, a dependence evolved between the immune system of mammals and some microorganisms. Evolved dependence is where an organism has adapted to the presence of a partner, through loss of genetic material, and can no longer function without it. If correct, this would mean that some functions of the immune system were not encoded in the human genome, because continuing exposure made this unnecessary. The most relevant microorganisms are those that

accompanied early 'hunter gatherers' in the Paleolithic period and during the development of farming in the Neolithic period around 10,000 years ago (**Figure 1**).

4.3 Epidemiological transitions and the environment of evolutionary adaptedness ('EEA')

The term 'epidemiological transition' describes the major watersheds in human development (Caldwell 2001[324], Armelagos 2005[325]). An epidemiological transition occurred between the Paleolithic and Neolithic periods, with a second transition occurring during the modern industrialised age from around 1800 (**Figure 1**). The Paleolithic stage of human development was termed the 'environment of evolutionary adaptedness' (EEA)[7] . A related concept is that human behaviours, such as instincts, evolved during the hunter-gatherer existence in pastoral and agricultural environments and that the human adaptation to more recent environments, such as cities, has been cultural and technological rather than genetic. Since the immune system does not produce conscious awareness that it is receiving inadequate microbial stimuli, we have until recently been unaware of this possible misfit with our physical environment.

Figure 1. Aspects of human immunological history relevant to the hygiene hypothesis (adapted from Rook 2010[7])

Time period	Human activity	Microbes in environment
Paleolithic (from 2.6 million years ago to around 10,000 years ago)	The human 'EEA'. Hunter gatherer small groups by lakes and rivers; scavenging; early fermented drinks	Environmental saprophytes such as **Mycobacteria**, Dietzia, Tsukumurella, Gordona etc. Microbial molecules: lipopolysaccarhides (LPS), peptidoglycans, etc. **Gut microbiota**. 'Heirloom species' inherited from primate ancestors: pinworms and other **helminths**, viruses such as herpes, papovaviruses, adenoviruses, parvoviruses, **HAV**, some enteroviruses, perhaps *Hepatitis B*. ***Helicobacter pylori, Salmonella***, *Staphylococcus*, early forms of **tuberculosis**, *Toxoplasma*, *Pneumocystis*. Also fermenting **lactobacilli.**
1ST EPIDEMIOLOGICAL TRANSITION		
Neolithic (from about 10,000 years ago to ~3,000 yrs BCE)	Larger social groups, animal husbandry, prolonged animal contact, domesticated cats & dogs + rodent pests. Increased orofaecal transmission	Calicivirus, rotavirus, coronavirus, Influenza B & C, measles, mumps and parainfluenza viruses, smallpox. Rapid microbial evolution in this new ecological niche. Modified human-animal helminth cycles. Bacterial infections include cholera, plague and typhus. Increase in orofaecal microorganisms.
Bronze age (2-3,000 yrs BCE)	Larger cities	Influenza B & C, mumps, smallpox, measles, plague (including endemic infections).
Iron age to pre-industrial age (about 1500 BCE to 1800)	97% still living in rural environment e.g. farms, animal & mud contact, untreated water	Plagues and epidemics but little change in everyday exposure since Paleolithic period.
2nd EPIDEMIOLOGICAL TRANSITION		
Modern age (from early 19th century)	Urban spread with concrete and tarmac so less mud. Clean chlorinated water, washed food. Soap & detergents. Diminished orofaecal transmission. Less animal contact. Antibiotics. De-worming	Loss of exposure to environmental saprophytes. Disappearance of helminths. Less orofaecal spread of H.pylori, HAV, Salmonella. Less *Toxoplasma*. Loss of ammonia-oxidising bacteria (AOB) from skin flora. Restricted exposure to gut microbiota of other individuals. Intermittent disturbance of gut microbiota by antibiotics.

Notes: BCE denotes Before Common Era (a.k.a. BC). *Microbes shown in bold are those implicated in epidemiological studies of the association between microbial exposure and atopy*.

4.4 Defining the 'missing' microbial inputs

If the human EEA is the hunter-gatherer environment of the Paleolithic era, we should be able to define the microbial inputs that our immune systems require. As suggested in **Figure 1**, this includes microbes that were abundant in the EEA but scarce in the modern environment. According to Rook (2009**Error! Bookmark not defined.**), Paleolithic populations would have carried organisms inherited from their primate ancestors ('heirloom' species). The heirloom species would have included helminths, herpesvirus, enteroviruses, HAV, *Pneumocystis, Salmonella, Staphylococci* and *H.pylori*. Scavenging would have exposed early humans to zoonoses via carrion and in addition, they must have consumed countless harmless environmental saprophytes daily from soil and water. Before the industrial age, these 'pseudocommensals' were continuously present in the environment. They seem relevant to the hygiene hypothesis because of their extremely long association with man, often in harmless carrier states.

The first epidemiological transition associated with the shift to agriculture and animal husbandry, around 10,000 years ago, would have had little effect on exposure to the 'pseudocommensals' or heirloom species. Meanwhile, a more sedentary lifestyle increased orofaecal transmission and caused prolonged contact with animals, associated with increased infection by viruses, for example rotavirus, caliciviruses – including noroviruses. Increase in population size to communities of a few hundred thousand would have given opportunity to acquire orthomyxoviruses (Influenza B and C), smallpox, mumps and measles. Such communities did not occur until the appearance of cities some 3,000 years ago. From that period to ours represents only 100–150 generations: extremely strong selection pressure would have been required for evolved dependence to appear, which seems improbable. This may explain the lack of a protective effect in studies on viral infections, apart from HAV. The important point is that dramatic changes to our microbial environment since the first epidemiological transition did not appreciably diminish exposure to the 'old friends', because the majority of the population still lived in rural environments, close to mud, animal and human faecal sources of these organisms. The 2nd epidemiological transition dates from the early or mid 19th century for most industrialised countries: improvements in sanitation and hygiene reduced or delayed exposure to many of these 'heirloom' organisms, an effect increased by the introduction of antibiotics. These missing organisms are thus thought to have contributed to the increases in allergies and CIDs.

4.5 Evidence for the 'heirloom' species

The OF hypothesis is supported by epidemiological evidence regarding protection against allergies and other CIDs for some of the 'heirloom' species (indicated in bold in **Box 1** below) as reviewed in **Section 3.6.** It must be borne in mind that there are inconsistencies in the data, with findings of both protective and harmful effects.

Box 1: 'Heirloom species' (from Figure 1)

Pinworms and other **helminths**, viruses such as herpes, papovaviruses, adenoviruses, parvoviruses, **HAV**, some enteroviruses, perhaps ***Hepatitis B***. ***Helicobacter pylori, Salmonella***, ***Staphylococcus*****,** early forms of **tuberculosis**, ***Toxoplasma***, *Pneumocystis*.

There is an association between chronic **helminth infection** and protection against allergies and other immune system disorders (**Section 3.6.6**). As discussed, this protective effect is clear in areas where these parasites are endemic, but may not apply if the infection is acquired after childhood. Trials of helminth therapy have nevertheless produced promising results, as reviewed in **Section 4.9**. ***H. pylori*** is also a strong contender for an immunoregulatory role (**Section 3.6.3.i**). Studies have also shown an association between *H.pylori* infection and protection against allergies and some CIDs. Blaser and Falkow (2009[326]) have claimed that such immune system effects support the concern with the loss of the ancient commensal microbiota, in other words, the 'old friends'. **Hepatitis viruses (HAV and HBV) (3.6.3.ii)** have not shown a consistent protective effect against allergy/ CID, but positive HAV serology in adults, especially without current evidence of clinical infection, may be a marker for childhood exposure in general to microbial orofaecal spread. **Salmonellae** were mentioned as possibly protective in one study and with immunological credibility (**Section 3.6.2iv**) but studies on this aspect have been so far confined to animals. ***Toxoplasma gondii*** has been shown to be protective against atopy in a few studies, with negative or inconsistent results in others (**Section 3.6.2v**). It is identified as a potential 'Old Friend' because its characteristics (intracellular parasite, high levels of carrier state) fit with a possible role in the immune system priming required to reduce the risk of allergy/CID.

4.6 Other 'old friends'

If the OF hypothesis is correct, then, in addition to the heirloom species, other groups of organisms, present from Paleolithic, and possibly also Neolithic times, might be expected to also exert an immunoregulatory role, including microbes or parasites readily spread by the **orofaecal route**, especially those with ability to establish carrier states (which increase the likelihood of transmission), micro-organisms permanently resident as **gut commensals** (also normal skin microbiota/ flora or other sites) and **environmental organisms** that serve as '**pseudocommensals**' if consumed regularly.

a) *Gut commensals (microbiota)*

The list of microbes that meet the criteria as potential immunoregulators, as proposed by the OF hypothesis, include the gut microbiota (**Figure 1**). Some of these commensal bacteria have been used as probiotics: living microorganisms that may be expected to exert health benefits when ingested. The potential roles of species such as *E.coli* and anaerobic organisms such as *Bacteroides, Bifidobacteria* and *Clostridium* and *Lactobacilli* is supported by evidence on differences between the microbiota of the gut and faeces (**Section 3.6.4**). Rook (2011[17]) has also cited studies indicating an immunoregulatory role for several of these organisms, concluding that, at the very least, these experiments emphasise the very close relationship between the microbiota and the human immune system. Breast-feeding transfers *Lactobacilli* and other organisms and is thus presumed to have a role in colonising the neonatal gut (**Section 3.4**). Raw (untreated) cow milk may perform a similar role, as suggested from the studies on the 'farm effect' (**Section 3.3**). As discussed, antibiotics and other aspects of modern life have been implicated in reduction of diversity of commensal microbial populations in the body, particularly the gut: this has been called the 'microflora hypothesis' (Shreiner 2008[327]). The use of probiotics in attempts to address this and lessen the risk of atopy is discussed in **Section 4.9**.

b) *Skin commensals (microbiota)*

Staphylococci have been cited as 'old friends' and recent studies appear to confirm a role in promoting a balanced skin microbiota and associated immune response (**Section 3.6.8**). Staphylococci as well as other skin commensals may also be transferred via breastfeeding (**Section 3.4**) and other close skin contact between mother and infant (Chatzakis 2011[122]). Rook[7] argues for the importance of ammonia-oxidising bacteria (AOB) on the skin. These are widely found in the soil and on skin convert urea and ammonia into nitrite and nitric oxide, with

a possible evolutionary role as part of the nitrate cycle in the body. It has been suggested that they may be readily removed from skin, for example by certain surfactants, and that the modern human is 'nitropenic' i.e. deprived of this natural nitrite source (Whitlock 2009[328], Rook 2010[7]). Hanski and colleagues (2012[183]) have described the effects of gram-negative gammaproteobacteria on immune system regulation, with observed differences in the skin microbiota in healthy and atopic individuals. The emerging importance of the skin flora is an area of active research, with possible implications for skin cleansing as well as for concerns about declining diversity of organisms in the natural environment.

c) *Commensal microbiota of other areas of the body*

While the gut and skin flora have been most studied regarding a role in regulating the immune system, the microbiota in other parts may also be important, for example in the lungs[7]. Babies born by Caesarean section have been found to be more prone to allergic disorders/ CIDs, underlining the importance of transfer of gut microbiota as well as microbes in the birth canal, as discussed in **3.6.10**.

d) *Environmental organisms*

Actinomycetes are the most important group of environmental saprophytes. This group includes Mycobacteria, Dietzia (e.g. *D. maris*), Tsukumurella and Gordona. Because of their branching growth pattern, they were thought to be fungi for many years but are now classed as higher bacteria. They are found in the environment, such as in soil or untreated water. Although most are slow growing and not harmful, some cause disease such as abscesses or pneumonia in immunocompromised patients. As discussed (**Section 4.2**), these organisms meet the 'old friends' criteria[7] because paleolithic man would have consumed large amounts of the harmless environmental saprophytes, a daily exposure that continued until our modern age. This is supported by immunological research on saprophytic strains of *Mycobacteria*, including increased levels of messenger RNA encoding CD14 and TLR2 (outer cell membrane receptors) in the children of farmers (Rook 2003[329]). Therefore, saprophytic *Mycobacteria* present in soil, water and dust in a natural environment may have an immunoregulatory role, associated with less tendency towards allergic disorders. Animal studies have yielded some intriguing associations. Environmental strains of *Mycobacteria* have been found to suppress some allergic features in studies on mice (Abbas 1996[330], Herz 1998[331] , Zuany-Amorim 2002[332]) and such organisms are more likely in a farm environment, at least those conforming to a traditional farm. *Mycobacteria* include the bacteria that cause tuberculosis (TB) and leprosy, neither of which have been shown to protect against allergy and CIDs, although a more pertinent point may be that humans probably passed infections to their animals during the Neolithic period, such as bovine tuberculosis (Gagneux & Small 2007[333]) and an inverse (protective) association has been reported between TB rates and atopy at the country level (Von Mutius 2000[334]). Trials of these organisms for immune system disorders (**Section 4.11**) have provided further insights into the potential role of the mycobacterial species.

4.7 Other infections that may not be 'old friends'

4.7.1 At the outset, apparent protection against allergic disorders for younger siblings led investigators to conclude an association with infections made uncommon by modern hygiene. But, as discussed in **Section 3**, protective effects for specific infections have not been confirmed in epidemiological studies of infection rates and family size. The OF hypothesis helps to clarify this lack of evidence in studies of infections in childhood, family size, the 'farm effect' and day-care nurseries. The more recently evolved childhood viral infections such as **mumps, measles, rubella** and **chicken pox** did not spread widely until community size increased in the populations. This did not occur until after the 2nd epidemiological transition and these viruses are unlikely to have evolved a co-dependent, immune regulatory role. This applies also to most other viruses (**Figure 1**). Further insights, as to whether pathogens might be involved as critical protective exposures can be gained, by looking for temporal correlations between rising

rates of CIDs and infectious disease (particularly the very rapid rise since the 1970s), as well as declining infection rates from the late 1800s in developed countries. Infectious disease trends are discussed in more detail in **Section 5.6**, whilst trends in CIDs are discussed in **Section 2**. These data show little evidence of a relationship between trends in allergies and other CIDs and trends in infectious disease. This includes trends in dangerous infections such as cholera and TB, endemic diseases such as HAV and helminth infections, childhood infections such as measles and diphtheria, and food and water borne diseases. Although they are only just beginning to be addressed, epigenetic effects are relevant to deductions based on temporal correlations. This is discussed further in **4.7.2** and **Section 6.7**.

4.7.2 As reviewed in **Section 5.2,** although the sanitation revolution started in the mid-19th century, provision of safer water and sanitation did not reach significant levels until the late 1800s and the population explosion and growth of cities increased infectious disease in the early 1800s. This included many of the infections spread by the oral-faecal route and notably the emergence of cholera. Cholera, previously unknown in Europe, arrived there in the 1830s. Cholera, as with other dangerous infections, has not been identified as an OF. The impact of the sanitary revolution on mortality rates from infection was not apparent until the turn of the 19th century, but then showed a continuous decline in the 20th century (**Section 5.6)**. Although this suggests a possible link between the gradual rise in allergies and CIDs up to the post WWII period and decline in infections such as **typhoid fever (***Salmonella enterica, serovar Typhi***), TB and rheumatic fever**, their decline occurred too soon to be associated with the very rapid rise in atopic disease in the late 20th century and too late to act as the trigger for the emergence of atopic diseases during the 1800s. The same applies to infections such as **whooping cough, diphtheria and measles,** which also showed a rapid decline over the same period (Aiello 2008(i)[335]). The decline in helminth and HAV infections occurred later and may be more relevant to the late 20th century increase in atopic disease. Since these infections remain common in some parts of Europe, as well as the developing world, they are the subject of current studies on inheritable changes in gene expression (epigenetics), which may yield insights as to how the consequences of the loss of Old Friends could be delayed by a couple of generations. Although there was a decline in mortality from infections during the first half of the 20th century, with more rapid decline from the 1950s, Velimirovic (1986[336]) concluded that the main change was in morbidity and mortality from infectious diseases rather than frequency. In other words, infectious diseases are still common, but with less morbidity and mortality, due to medical advances. While many infections with high mortality, such as cholera and diphtheria, have declined, this has been offset by emergence of new infections or infectious strains, and those that have re-surfaced, for example TB. Both World Wars were associated with infection epidemics, such as Influenza in WWI and typhus and dysentery in WWII. In the US, the number of respiratory illnesses per year has been estimated at around 1.5–3.0 in adults, and around 3.5-5.5 in children under 5 years of age (Monto & Sullivan 1993[337]).

4.7.3 Improved control of water and **food-borne pathogens** led to a decline in enteric infections, through the 20th century, despite emergence of new strains, such as in the **Salmonella** species. In the UK, the general trend has been an increase in food poisoning notifications since the early 1970s, particularly in the 1980s/1990s and peaking in 1997, with a decline of some types since then partly attributed to improved livestock control (Parliamentary Office of Science and Technology, 2003[338]). As reviewed in more detail in **Section 7,** food-related, waterborne, and non-food-related infectious intestinal diseases remain common. A recent UK community based study (Tam 2011[339]) estimates that the number of cases of IID due to norovirus is 3 million, whilst the estimated annual number of cases in the US is 21 million[340]. Viral infections, such as rotavirus or norovirus have remained common, with norovirus only being identified (as Norwalk virus) in 1968, but now estimated to account for around 90% of epidemic non-bacterial gastroenteritis worldwide (Lindesmith 2003[341]). Rotavirus is the leading cause of gastroenteritis in children under 5 years of age (Soriano-Gabarró 2006[342]).

Since exposure to pathogens does not necessarily lead to illness, it seems highly likely that we are constantly exposed to pathogens in the food we eat and are regularly exposed to low numbers of noroviruses via surface to hand to mouth contact. Skin, wound, eye and ear infections are common, particularly in children but, since most of these infections go unreported unless a laboratory isolate is confirmed, little data is available on the burden of these infections. A 2008 UK study (Hayward 2008[343]) indicated a general increase in community-onset staphylococcal disease over the last 15 years, although the OF hypothesis cites staphylococci as possible OFs because of their long association with man.

4.8 What level of microbial exposure and intensity of interaction with immune regulation systems might be required?

4.8.1 One of the factors discussed in the earlier IFH reviews was how to define the 'intensity' of the necessary microbial exposure (how many units and how reactive), in relation to whether prevention of infection through hygiene might also prevent the necessary immune priming. Earlier proposals that microbial exposure might need to be "*intense*" (von Hertzen 2000[344]), or at least produce clinical disease, no longer appear to be confidently applied to the hygiene hypothesis. The only tenable possibility is a protective role for 'background' exposure to subclinical doses of pathogens, i.e., microbial exposure that does not cause illness or a lasting and measurable immune response, or to commensal or environmental microbes, particularly those with low invasiveness or virulence such as the rapid growing saprophytic strains of *Mycobacteria,* or perhaps endotoxins. Matricardi (2010[150]) discussed three possibilities:

(i) commensal, not dangerous, bacteria;
(ii) mild pathogens;
(iii) potentially fatal pathogens.

He dismissed (iii) as a protective effect dependent upon highly virulent, potentially fatal infectious agents was improbable from an evolutionistic viewpoint, but was not convinced that evolution would depend on a few species of non-pathogenic commensals. Matricardi commented that the focus should be on whether an organism can stimulate immune regulation, regardless of whether it results in disease. Although Matricardi supported the OF hypothesis as being "*the strongest one at present providing an explanation for the hygiene hypothesis"*, he argued that the regulating influence on the immune system would be exerted mainly by bacteria which induce a strong immune response, rather than by commensals. He cited the most typical example of this category as the mycobacteria that contaminate the soil, water and food. Since these bacteria exert a mainly transient influence on the immune system, he proposed that humans should be exposed continuously, mainly through ingestion, to the beneficial influence of these bacteria from food and environmental sources. He suggested that the somewhat broad range of 'old friends' could be restricted to infectious agents that are able to survive as intracellular pathogens, such as mycobacteria, listeria, salmonellae and *Toxoplasma gondii,* although this restriction may not be tenable: organisms do not need to survive in antigen presenting cells to induce an immune response. Support for the OF hypothesis comes from the observation that infection by Salmonella species early in life is associated with a lower risk of hay fever and asthma (Pelosi 2005[345]). Salmonellae and mycobacteria share many characteristics in the way they stimulate the immune system[150].

4.8.2 Matricardi's answers to the current dilemmas do not provide a clear message regarding the type of microbial exposure required; and his view that listeria, salmonellae and *Toxoplasma* are 'mild' pathogens is contestable. Matricardi's rejection of a major role for commensal organisms also contradicts the large literature on the influence of the gut microbiota on immunoregulation, as reviewed by von Hertzen 2011[8]. The answer may lie in subclinical or asymptomatic microbial exposure, i.e. exposure that does not cause illness or a lasting and measurable immune response. The important factor is likely to be tolerance of the microbe by

the body, so that it can induce immunoregulation. Such 'subclinical' exposure is much harder to detect in epidemiological studies, which means that, if it is required for immune priming, proxy exposure studies may be more valuable in unravelling this, for example in the studies on farming or day nursery environments. Rapidly developing knowledge about the gut microbiota is now helping to explain how these organisms may shape immune responses both in health and in disease (Round & Mazmanian 2009[346], Ackerman 2012[242]).

4.8.3 *Route of exposure*

Another aspect that could shed light on the nature of the missing microbial exposure, and why it is missing, is by identifying the route of exposure of key microbes, i.e. oral, respiratory or other routes. As stated by Matricardi (2010[150]), a preventive role against allergy and asthma was attributed initially to respiratory viruses, whereas infections of the gastrointestinal tract were thought to protect from food allergies. In contrast, atopy was related inversely to hepatitis A virus (HAV) and other orofaecal/food-borne infections, but not to airborne viruses. While it has been recently argued[150] that research increasingly implicates the importance of gastrointestinal infections, there is also strong experimental evidence that non-gastrointestinal worms, such as the filiarial helminth *Litomosoides sigmodontis*, can affect the regulation of the immune system by inducing Treg cells (Taylor 2009[268]). The tiny worm larvae enter via the skin, with no evidence of presence in the gut or peritoneal cavity (see also **Section 3.6.6**). Farm studies (**Section 3.4**) confirmed that exposure to cattle and livestock (i.e. to a faecally contaminated environment) and ingestion of unpasteurized milk (i.e. exposure to food-borne microbes) protect against allergic disease. This is also supported by experimental studies in animal models. This coincides with accumulating data related to the OF hypothesis (Rook 2011[17], **Appendix IV**). The probable importance of the gut microbiota to immune system development would appear to favour the oral-faecal route of exposure. The respiratory route may also be important, for example inhalation of bacteria and endotoxins in farm and other environments. Other routes, such as insect-borne infection might have been relevant during the paleolithic period (Armelagos 2009[347]) but have become rarer in the modern world.

4.9 Can the necessary microbial exposure can be achieved by more than one microbial species?

The epidemiological evidence, and the inconsistencies in the data strongly suggest that it is not one single microorganism which may confer protection, but most probably a number of different agents and mechanisms. In biology there are usually many ways of achieving a particular outcome, a concept known as redundancy. From an evolutionary viewpoint, redundancy is probable and it is well established that there is considerable redundancy within the immune system, making it feasible that the necessary microbial exposure for immune priming could be supplied by exposure to more than one microbial species. Diversity of exposure therefore seems important. The **high turnover and diversity hypothesis** (Matricardi 2010[150]) proposes that diversity and turnover of bacterial species in the mucosa of the gut and possibly other sites is a key factor for priming or regulating the immune system rather than stable colonization by a single species (Wold 1998[348], Matricardi 2000[192]). Matricardi[150] cited evidence from a European study showing significantly less faecal microbial diversity for infants with atopic eczema than for infants remaining healthy. The authors hypothesised that reduced diversity in the faecal microbiota of infants in the first week of life may predispose to atopic eczema during the first 18 months of life (Wang 2008[245]). A number of recent studies (**Section 3.6.4**) support the microbial diversity hypothesis, including a study showing reduced diversity of faecal microbiota in Swedish infants at one week associated with higher rates of atopic eczema at 18 months (Wang 2008[349]) and a Danish study showing increased risk of allergic disease associated with less gut intestinal microbiota diversity in infancy (Bisgaard 2011[246]). Björkstén 1999[350] also reviewed evidence suggesting that antibiotic use in infancy may predispose to immune dysregulation by reducing the diversity of gut

microbiota (see also **Section 3.6.5**). The loss of diversity in human microbiota has been linked by the observed loss of biodiversity in the outside environment (von Hertzen 2011[8], Hanski 2012[183]). The **biodiversity hypothesis** proposes that reduced contact of people with natural environmental features and environmental microbiota leads to inadequate stimulation of the circuits involved in immunoregulation. This could be seen as a further interpretation of the OF hypothesis, in that the decline of immunoregulatory 'old friends' in the environment is linked to the decline of many microbial species in our modern surroundings.

If the OF hypothesis is correct, and exposure to old friends is critical/a prerequisite, it is impossible to believe that our modern environment has lost all of the key species that influence immunoregulation - for how could any of us develop an immune system? If, as seems likely, the principle of redundancy is inherent in the immunoregulatory system, it seems probable that loss of most of the 'old friends', and of some diversity, has still left our modern world with sufficient natural exposure to microbes (including some old friends) that 'do the job'. It seems plausible to assume that all of us still get some of the requisite microbial exposure, but whereas for some individuals this is enough for immune priming and regulation, in other individuals, particularly where other factors play a part, CID is triggered. Redundancy in the system is a logical assumption, since some of the old friends, such as HAV, *H.pylori*, salmonella and helminths, are pathogenic species, although it is important to note that the distinction between pathogens and non-pathogens is not absolute: so called 'harmless' organisms can invade in some circumstances, while some pathogens may be harmless depending on type and timing of exposure, as in early infection by HAV or *H.pylori*. This means that increased risk of CID need not be an inevitable trade off from reduced exposure to pathogens, providing that a correct balance or mix of other exposures is sufficient to turn on immunoregulation.

In his 2010 review[7], Rook posed the question "How many functions or mechanisms relevant to setting up optimal balances within the immune system need to be triggered by microorganisms or their components?" He speculated that the striking effects of the polysaccharide from *Bacteroides fragilis* administered to germ-free mice (Mazmanian 2008[351]) might suggest that a few critical molecules are involved, but also that what else is present or not present in the environment may give importance to a particular organism.

4.10 Critical timing for microbial exposure to affect immune system priming and regulation

4.10.1 The age at which exposure occurs appears to be critical in relation to the development of the immune system. The various stages of development up to adolescence are vulnerable to outside or inner influences only at certain times (Von Mutius 2010[125]). There is recent evidence of the importance of microbial exposure during the neonatal period, when it appears to have persistent effects on T cell production and function (Olszak 2012[352]). The 'window of opportunity' for programming appears to range from pre-pregnancy to 24 months of age (Vassallo 2008 & Walker[353]). Further research since the previous review has reinforced this without, as yet, identifying the precise timing. Trials of helminth therapy for MS, discussed below, suggest that the adult immune system can respond, at least in halting the progression of disease. It would seem logical that whatever the importance of early exposure for development, the system can be partly modified later; also that the concept of redundancy must apply, in that a wide range of exposures could affect the immune system, even after infancy.

4.10.2 The importance of timing has directed recent research to two related hypotheses: the **fertile field hypothesis** regarding embryonic and fetal development, for example a higher risk of T1D resulting from Coxsackie virus infection during development of the pancreas, or a possible protective effect from other microbial exposures at this stage (Coppieters 2011[354]); and the more broad ranging **fetal programming (or fetal origins) hypothesis (**Barker

2004[355]) that also proposes pregnancy, or even pre-conception, as the key time for exposure. A recent study in mice showed that prenatal exposure to *Acinetobacter lwoffii* altered the Th1/Th2 balance in offspring, with resulting protection from asthma as measured by airway hyper-reactivity and inflammation (Brand 2011[356]). Kaplan and colleagues (2011[357]) have suggested that the most important perinatal influence is on the gut flora [microbiome] and its colonisation during the peripartum period (i.e. during and just after birth). Holt and Strickland (2009[358]), reviewing the 'farm effect' on lower risk of allergy, emphasised the evidence for transmission of protective factors during pregnancy. Fetal origins of disease have been identified for a wide range of disorders, including CIDs. The finding that people of African-origin are more prone to hypertension [high blood pressure] has been attributed to fetal programming, for example related to changes in diet, since hypertension is more common in migrant African populations in the West, as well as obesity and higher risk of some CIDs (Forrester 2004[359]), although the possible environmental factors have not been identified.

4.10.3 The possible beneficial effects of microbes and helminths as treatment for CID are discussed below: it is important to note that while these treatments can modify or temporarily suppress symptoms, they have not yet been show to cure diseases or to cause permanent changes in the immune system. Thus these effects differ from the 'critical timing' theory of how a balanced immune system develops and as yet there is no evidence that exposure later in life reduces the likelihood of CID.

4.10.4 ***Delayed exposure***

Rook[17] suggested another twist to the hygiene hypothesis, in that the 'sanitary revolution' has delayed microbial exposure, especially by orofaecal transmission. This means that viral infections occur later in life than was normal during human evolution and after decline of maternal antibodies passed to the fetus via the placenta in the first year of life. Because these exposures now occur at an inappropriate stage of maturation of the human body, the viruses may trigger or otherwise predispose to CIDs. Examples include association with immune disorders for Coxsackie virus or rotavirus infection if the infections occur after weaning, while earlier exposure may be protective (Filippi 2008[220]).

4.11 *Microbes as treatment or preventatives in allergy and CIDs*

Clinical intervention studies are producing data on the nature of the critical microbial exposures required for immune regulation. Approaches to 'putting back' the loss of microbial exposure to 'Old Friends' have included using animal models to detect optimum timing and type of exposure, and trials of therapy with organisms of low pathogenicity that may help with immune system regulation (Eifan 2010[360]). A recent commentary on progress described a "*golden age*" for the development of agents acting on the immune system (Sewell & Thomas 2011[361]). The exploration of these novel therapies shows how the concept of 'old friends', related to recreating a favourable microbial environment for immune system conditioning, is helping to unify the OF version of the hygiene hypothesis and test it in a clinical setting. Some intervention studies using bacteria are discussed in this section.

i) *Probiotics and prebiotics*

Probiotics contain viable microorganisms that may alter the microflora of the host; prebiotics are supplements or foods containing a nondigestible food ingredient that stimulates either the growth or activity of indigenous probiotic bacteria. Several microorganisms, particularly those identified as gut commensals have been used in animal and human trials, particularly for allergic disorders, including *Bacteroides fragilis, Bifidobacteria spp, Escherchia coli, Enterococcus faecalis* and *Lactobacilli*. Prescott and Björkstén reviewed probiotic trials in 2007, reporting a small, but inconsistent, beneficial effect on eczema but none on any other allergic disease[362]. They suggested that host factors, including genetic differences, diet and antibiotic treatment, could

explain the lack of evidence despite a "*sound theoretical basis for anticipating benefits*". *B. fragilis*, a prominent human gut commensal, has been shown in a recent animal study to induce T-regulatory cells (Tregs) and mucosal tolerance, hence less inflammatory response, in germ-free mice (Round & Mazmanian 2010[363]): the effect appeared to be largely due to polysaccharide A, an immunomodulatory molecule present in *B. fragilis*.

The microorganism used in the probiotic is an obvious variable factor. Trials using extracts of *Escherichia coli* and *Enterococcus faecalis* to prevent atopic eczema are in progress (Matricardi 2010[150]). Meanwhile, there was no difference in frequency of allergic disease and sensitization in a trial of probiotics (*Propionibacterium freudenreichii ssp shermaniii* and galacto-oligosaccharides) and placebo for Finnish children with high risk of atopic disease (Kuitunen 2009[364]), although a subgroup of children delivered by Caesarean section showed approximately half the level of IgE-associated allergic disease, compared with the placebo group. A 2007 Finnish study, reported significant reduction in atopic eczema for 900 infants given *Lactobacillus rhamnosus*, although with no effect on other allergic diseases by the age of 2 years (Kukkonen 2007[365]). So far only one study, a randomised controlled trial of probiotics (*Lactobacillus acidophilus*), has shown an increased risk of atopic dermatitis and allergen sensitization in high-risk children in Australia (Taylor 2007[366]). In a 2010 review of probiotic and prebiotic treatments, beneficial effects were cited for probiotics used to treat acute viral gastroenteritis in otherwise healthy children, or for necrotising enterocolitis in very low birth weight infants (Thomas 2010[367]), although no benefits have been observed for children with Crohn's disease. While **prevention** via probiotics remains unproved, the prospects seem better for **treatment** of allergy. A review of *Lactobacillus* and *Bifidobacterium* treatment of allergic rhinitis in 2011 concluded that in general symptoms were improved (Noguerira 2011[368]).

Matricardi reviewed intervention studies with probiotics in 2010[150]. Since they continue to show the inconsistencies in the data, Matricardi poses the question "*How can failures be reconciled with the hygiene hypothesis?*" He argues that the 'gut commensal hypothesis' was originally based on very small epidemiological studies but has now been tested more thoroughly through a large birth cohort study in three European cities, giving results which do not support the hypothesis that sensitization to foods or atopic eczema in European infants in early life is associated with the lack or presence of any particular intestinal commensal bacteria, including so-called 'probiotic' bacteria (bifidobacteria and lactobacilli). He concluded that the studies suggest no direct evidence that lactobacilli or bifidobacteria explain the protective effect of the 'traditional' lifestyle of a farming environment. Very few lactobacilli strains have been shown to turn on immunoregulation and trials have been restricted by intellectual property rights on some strains. The increasing role identified for the gut microbiota in immunoregulation suggests that there must be other explanations for the relatively disappointing performance of probiotic therapy to date: a recent review of clinical trials identified other reasons for inconsistent effects, such as small sample size, limited follow-up, lack of 'state of the art' analyses of the gut commensals or failure to take account of the Vitamin D pathway in gut homeostasis (Ly 2011[369]).

ii) *Worm (helminth) therapy*

Several clinical intervention studies have investigated the potential of helminth therapy for modifying or temporarily suppressing allergy and CID symptoms. The maximum load of hookworm (*Necator americanus*) tolerable without adverse affects is known (Mortimer 2006)[370], although there is concern that low 'safe' levels may not produce sufficient immunological change (Blount 2009[371]). Trials with whipworm (*Trichuris*), to assess alleviation of symptoms, are more promising: 79.3% of patients with IBD (Crohn's disease) treated with *Trichuris suis* ova (pig whipworm eggs) responded well (Summers 2005(i)[372]). A double blind trial of *T.suis* for ulcerative colitis resulted in 43% improvement in symptoms compared to 16.7% in the placebo group (Summers 2005(ii)[373]). A phase 1 clinical trial of the safety of a helminth (*Trichuris suis*) as an inducer of immunoregulation in MS has been completed (Fleming

2011[374]) and further trials are in progress. It is noteworthy that MS patients who picked up natural helminth infections (that were not treated) showed no disease progression during a 5-year follow-up, whereas those who remained free of helminths showed the anticipated deterioration (Correale 2007[375]). Trials on patients with rhinoconjunctivitis and airway hyper-responsiveness are also in progress and there seem good prospects for application to a wider range of allergy and CIDs.

iii) *Helicobacter pylori*

As discussed in **4.2.5.(i)**, evidence of past or chronic infection with *H.pylori* is associated with a decreased risk of atopy, particularly for infection acquired early in life. Acquisition later in life on the other hand is perceived as generally harmful (e.g. ulcers, cancers). A recent animal study showed that mice orally infected with *H.pylori* were **protected** against allergic sensitisation, airway hyperresponsiveness and other hallmarks of asthma (Arnold 2011[376]). The researchers suggested that the *H.pylori* acted by inducing T regulatory cells. As yet this has not been investigated as a human clinical intervention.

iv) *Therapeutic use of Mycobacterial and other actinomycete strains*

There is currently great interest in the possibility that some mycobacterial strains can be used to **treat** allergic disease and CID, reinforced by recognition that saprophytic strains are 'Old Friends'. The pathogenic strains such as *Mycobacterium tuberculosis* are slow growing, but faster growing strains such as *M. vaccae* may be more important regarding a **protective** effect. As yet, trials for conditions such as severe atopic dermatitis have not produced convincing results (Smit 2004[377], Rook 2006[378]). Saprophytes (actinomycetes) have been used to treat animal infections, for example *Gordona bronchialis* and *Rhodococcus coprophilus* were used successfully in a trial to **treat** flea allergy in dogs (Marro 2011[379]). The most supportive evidence comes from animal studies such as those showing **suppression** of allergen-induced eosinophilia in mice by infection with *Mycobacterium bovis* (Erb 1998[380]) and from promising initial results of trials with mycobacterial vaccines for the treatment of some diseases associated with immune dysregulation (Arkwright & David 2001[381], Camporota 2003[382]).

Bacille Calmette-Guérin (BCG) vaccine against TB is possibly one of the most widely used in the world and has been administered since the 1920s. It is prepared from a live, weakened strain of the bacterium that causes bovine tuberculosis (*Mycobacterium bovis*). BCG has been claimed to be an immunomodulator that could **prevent** allergy and asthma (Barlan 2005[383]). It is therefore disappointing that trials with BCG vaccination have produced conflicting results (Alm 1997[384], Aaby 1997[385], Omenaas[386], Arkwright 2001[381], Camporota 2003[382], Krishna & Salvi 2002[387], Cohon 2007[388]). A trial of BCG for 121 high risk infants reported in 2008 did not show a significant **reduction** in allergic disease, compared with control infants, although there was a trend towards less use of medication for eczema which did not reach statistical significance in this small study (Steenhuis 2008[389]). Large multicentre trials are probably needed to examine the possibilities for BCG and other mycobacterial strains: a recent meta-analysis concluded that BCG offers some protection against subsequent development of asthma (El Zein 2010[390]). The mycobacterial strain in BCG may be the reason for inconsistent results, or effects may be altered by route of administration, such as mucosal instead of through skin (Barlan 2005[383]). Veterinary studies are yielding some intriguing results, for example a *Mycobacterium avium* subspecies infection of cattle and other ruminants (Johne disease) has been successfully treated and prevented with probiotic treatment using *Dietzia* (an 'old friend' and related to the mycobacteria family of organisms), leading to the suggestion that *Dietzia* should be included in trials for human IBD (Click 2011[391]).

4.12 Autoimmune disease and immunoplausibility of the Old Friend's hypothesis

Immunologically, the rise in allergic disorders in developed countries was attributed to 'excessive hygiene', encouraging an expansion of Th1 lymphocytes and downregulation of the Th2 lymphocytes that mediate allergic disorders, thus to an imbalance between Th2 and Th1 cells. The hygiene hypothesis turns out to be incorrect in several ways. First, as discussed in **Section 3.6.1**, childhood virus infections have not been shown to protect against allergic disorders and human rhinovirus (HRV), and respiratory syncytial virus (RSV) are potent triggers of asthma (Yoo 2007[392]). Subsequent epidemiology has shown that protection from allergies is associated with exposure to organisms that accompanied human evolution, many of them transmitted via the oral-faecal route (Matricardi 2000[192], Umetsu 2010[393]), including, in developing countries, exposure to helminths (van den Biggelaar 2004[394]). Some of the microorganisms involved may be saprophytic environmental organisms found in untreated mud and water (Ricklin-Gutzwiller 2008[20]). Collectively these organisms have been referred to as the "Old Friends" to emphasise their long association with man, in contrast to the viruses whose association with man is more recent (Rook 2010[7]) (**Section 4.1**).

Secondly, the notion of Th1/Th2 imbalance is also untenable, and arose because of a narrow focus on allergic disorders. Diseases such as MS, an autoimmune disease mediated by Th1 and Th17 cells, have increased in westernised countries and with economic development (**Section 2**). Moreover, both within Europe and in the rest of the world there is a very tight correlation between the incidences of T1D (another Th1-mediated autoimmune disease) and Th2-mediated asthma (Stene 2001[395]). Similarly, there has been a parallel rise IBD, mediated by Th1, Th17 or mixed responses (see **Section 2** and **Appendix I)**. Like the increases in allergic disorders (Eder 2006[275]), and MS (Koch-Henriksen 2010[21]), these increases are real (Elliott 2005[19]), and cannot be dismissed as due to evolving diagnostic criteria, or clinical awareness. Thus chronic inflammatory disorders (allergies, autoimmunity and IBD) mediated by *all three* main arms of the immune system (Th1, Th17 and Th2) are rising in parallel in rich developed countries (Bach[396]). Whereas a distorted Th1/Th2 balance cannot account for this, an imbalance between the lymphocytes (whether Th1, Th2 or Th17) that mediate these chronic inflammatory processes, and the anti-inflammatory cells that regulate them can potentially do so. This was suggested a decade ago (Rook 1998[397], Wills-Karp 2001[398]), and reviewed in detail recently (Rook 2010[7]). The identification of the protective organisms ("Old Friends") outlined above fits this hypothesis. Humans co-evolved with organisms transmitted by the faecal-oral route, helminths, environmental saprophytes, and microbiota. These all had to be tolerated, and therefore evolved a role as inducers of immunoregulatory circuits[7]. Importantly, recent data show that some helminths (Grainger 2010[399]), and some members of the gut microbiota (Round 2011[400]), secrete molecules that directly expand the subpopulations of regulatory lymphocytes (Treg), while others cause immune cells to mature into forms that drive immunoregulation (Smits 2005[401], Le Bert 2011[402]). Twentieth century urban man has little exposure to animals, faeces or mud, few or no helminths, and grossly distorted microbiota (de Filippo 2010[244]), and is therefore susceptible to poorly regulated immune responses[7].

If this is correct, and partly responsible (together with diet, Vitamin D deficiency and other factors) for the recent rises in incidence of the CIDs, then there should be evidence for defective immunoregulation in these diseases. Such defects have been recorded in allergies (Adkis 2004[403]), food allergy (Perez-Machado 2003[404]), multiple sclerosis (Viglietta 2004[405]), autoimmune endocrinopathies (Kriegel 2004[406]), T1D (Ferraro 2011[407]) and IBD (Makita 2004[408],Veltkamp 2011[409]). There is another set of findings that links the parallel increases in these three classes of chronic inflammatory disorder to defective immunoregulation. Animal models of all of them can be treated by exposure to micro- or macro-organisms that boost immunoregulatory pathways (regulatory T cells (Treg) or IL-10 or TGF-b). For example, mouse

models of allergies, T1D, MS, colitis, and autoimmune arthritis can all be treated with helminth infections that act in this way (Osada 2010[410]).

These points suggest that the increases in human CIDs associated with modern urban life are partly attributable to defective immunoregulation resulting from diminished exposure to immunoregulation-inducing organisms from man's evolutionary past. Final proof will require rigorous clinical trials with these organisms, or with molecules derived from them. In the study cited in **Section 4.11** on MS patients (Correale 2007[375]), the helminth infected patients developed circulating regulatory T cells that specifically responded to a peptide from myelin basic protein by releasing the anti-inflammatory mediators IL-10 and TGF-b[375]. When some of the patients subsequently had to have their helminth infections treated there was rapid disease progression, and loss of these brain-specific regulatory T cells (Correale 2011[411]). Thus in this human trial-like 'experiment', immunoregulatory helminths were acting as Treg adjuvants (i.e. stimulants or accelerators of Treg expansion). Interestingly, there might be other diseases that need to be thought about in the context of dysregulated inflammatory responses. For example, depression is becoming more common, particularly in towns. It is often accompanied by evidence of decreased immunoregulatory functions and by raised levels of inflammatory mediators (Raison 2010[412]). Such mediators are known to be able to induce symptoms of depression (Musselman 2001[413]). Similarly the epidemiology of some cancers that are increasing rapidly is remarkably similar to the epidemiology of T1D or of allergies. Persistent inflammation is a risk factor for oncogenesis, and also provides growth and angiogenesis factors that encourage progression of established tumours (Rook & Dalgleish 2011[16]). Other areas of medicine in which these arguments need to be considered are outlined elsewhere[7].

5. FACTORS WHICH MAY HAVE CONTRIBUTED TO REDUCED OR ALTERED MICROBIAL EXPOSURE

5.1 The key questions regarding exposure

Two important questions, examined in the 2004 and 2006 IFH reviews[1,2], were "what changes which have occurred which might have affected microbial exposures that are vital for development of the immune system – to the extent that they no longer occur, or occur to an insufficient extent?" Also, in relation to the original synthesis of the hygiene hypothesis, "could modern trends in hygiene amenities, practice and personal cleanliness be contributory factors?" From the outset it was recognised that this was difficult unless critical exposures could be precisely identified and their extent assessed. The 2004 and 2006 reviews therefore examined the data from two angles, looking for trends or correlations between reduced microbial exposure and trends in atopy – or conversely whether the data could lead us to exclude any particular factor or factors. This was approached by examining:

1. What is known about the impact of medical and public health measures, introduced in recent years to reduce the burden of infectious diseases, on exposure not only to pathogens, but also commensals, environmental and other strains which could be candidates for priming of the immune system and its regulation.

2. Whether there are any strong temporal correlations with trends in atopic diseases/CIDs that might indicate one or more public health measures as significant causal factors.

The factors examined included improvements to water, sanitation and food quality, and immunization and antibiotic use. It also included household cleaning, use of cleaning products, and trends to more frequent showering or bathing. Two fundamental changes in thinking have

occurred since the 2004 and 2006 reviews, which dictate a need to reassess the conclusions drawn at that time:

1. The 2004 and 2006 reviews focussed on trends over the last 50-60 years, which mark the rapid late 20th century rise in atopic disease in industrialised countries. If the OF hypothesis is correct, we need to take account of trends over a longer period: the 2nd epidemiological transition, which dates back to the early 1800s in most 'modernised' countries.

2. The weight of evidence now suggests that the critical exposures for immune priming are not the modern childhood infectious diseases, as originally thought. This suggests we need to look more carefully at the trends most likely to have reduced exposure to species identified by the OF hypothesis. The difficulty here is that reduced exposure to OF species has been a silent, or at least unmonitored, by-product of measures intended to reduce exposure to pathogens. Reduced rates of notifiable or laboratory-identified infections can only provide general markers of possibly reduced overall microbial exposure.

In the following sections medical and public health trends are re-examined together with data on trends in allergy and CIDs (as reviewed in **Section 2**) and infectious disease trends.

5.2 Water, sanitation and food quality

Early water treatment comprised crude filtration, often within the consumers' homes. Sand filtration was widely introduced in the 19th century: slow sand filtration was introduced in the UK from the early 19th century but required large areas and frequent cleaning: a more rapid sand filtering system was developed in the US and used there from the late 19th century. Sand filtration removed many contaminants and large living organisms, but did not reliably remove bacteria and viruses, a risk only fully appreciated after the isolation of bacteria in the 1880s. Chlorination of water was proposed in the early 1800s but was more often used to disinfect sewers, as the main interest was in eliminating smells from sewage and waste that might transmit disease. Drinking water chlorination was introduced in England in the 1890s and became routine in municipal water supplies in the UK and many other industrialized countries from the early 20th century, as well as other types of water treatment. In the United States, the number of people receiving sand filtered water rose from virtually none in 1870 to 20 million by the 1920s[414]. Municipal chlorination of water in US dates from around 1910 (Aiello 2008(ii)[415]). Sewers predated water treatment by several centuries, although adequate sewer design was not established until the late 19th century. Meanwhile, cesspits and payment for collection of human waste inevitably exposed the majority of the population to faecal organisms, either airborne, through water contamination or via direct and indirect contact. Sewage treatment is relatively recent, such as the introduction of sludge beds in the UK in the 1890s. Flushing toilets were available in England from the 18th century but widespread introduction did not occur anywhere until the late 19th century.

For towns and cities, it can be said that these improvements reduced pathogen exposure via water or sewage from the early years of the 20th century, although in some rural areas this occurred a few decades later. Water and sanitation progress undoubtedly protected people from dangerous diseases such as cholera and typhoid fever, but also removed the saprophytic microorganisms now identified as 'old friends'. During this period, or perhaps up to the mid 20th century, we also lost the worm [helminth] infestations that appear to have beneficial immunological effects (**Section 3.6.6.**) and, as discussed, have been used with some success to treat symptoms of CIDs (**Section 4.11**). Water, even with modern treatment, is intended to be safe from pathogens but it is not sterile and contains small numbers of bacteria and viruses as well as other natural components and contaminants. Worldwide, but mainly in low income communities in developing countries, 894 million people are estimated to lack access to safe

water and 2.5 billion lack basic sanitation (WHO/UNICEF 2011[416]).

Control of the microbial content of food has developed over the same period as the progress in drinking water and sanitation. Food safety, in terms of excluding pathogenic or chemical contamination from foods, has greatly improved over the last century and a half, and it is difficult to believe that changing food technology and dietary preferences have not produced fundamental changes both in the pathogen content and environmental strains in the food which we consume. Food routinely contains millions of microbes per gram, including microbes such as lactobacilli and yeasts that are deliberate constituents of foods like cheeses and yoghurts. Despite advances in food safety in the past 200 years, the level of foodborne disease remains high (**Section 7.4**). European data from 2008 suggests that contamination rates for poultry supplied to the public ranges from 2.3-12% for salmonella, and 0-95% for Campylobacter (European Food Standards Agency 2008[417]). Although it may be possible to use surveillance data to chart the changes in pathogen content of food over the last two centuries, commensal and environmental contaminants are not monitored, nor the effect of different cooking methods.

Some studies of environmental risk factors for disorders, such as IBD, show no consistent relationship with availability of treated water supply (Molodecky & Kaplan 2010[418]). A French case control study on IBD, which included information on home hygiene, such as piped water supply and sharing a bedroom in childhood, showed a higher risk for IBD in those who had not had access to piped drinking water or who had shared a bedroom in childhood (Baron 2005[419]). Such studies are hampered by the lack of accurate long term data on all of these facilities.

5.3 Filth and poverty

As reviewed by Aiello (2008(iii)[420]), the years up to the first half of the 19th century were years of filth, poverty and disease in Europe and the US. A major component of the filth was human and animal faecal matter. Both rural and city dwellers would have been constantly exposed to both pathogens and other microbes from these sources. Animal wastes were everywhere on farms, whilst city streets were used for disposal of waste and were covered with horse manure. In most cities there were free-roaming animals. In the mid-1800s, a perceived link between this filth and disease led to a wave of disgust in Western civilisation (Aiello 2008(ii)[415]). The sanitarians of those years mistakenly believed that 'miasmas,' foul smelling emissions from decaying organic matter, caused disease. So they took steps to clean up the miasma sources in sewage, factories, streets and homes. Although their reasoning was wrong, their efforts to improve the environment paid off. Proper disposal of rubbish helped control insects and rodents, which are reservoirs and carriers of disease, while better sanitation reduced exposure to faecal organisms.

5.4 Antibiotics and immunisation

Antibiotics were first introduced in the 1930s (sulphonamides) but their use increased rapidly after the development of penicillin in the 1940s and discovery of other antibacterial agents. Widespread use preceded the rapid rise of atopic disorders from the 1970s and has continued to increase, although from the 1990s, increasing concern about antibiotic resistance has led to introduction of restrictions on prescribing some types of antibiotics (Working Party Report 1994[421]) and decline in usage in some countries, such as France and Japan (Hamad 2010[422]). Antibiotics are still the commonest medicines given to children, amounting to around 6 million prescriptions a year in the UK (Spyridis & Sharland 2008[423]) and the general trend is upwards in many countries[422]. The question for this review is whether these trends implicate antibiotic use as a contributory factor for the rise in allergy and CIDs. The usage trends show a better fit, compared with those for water and sanitation improvements, with the rise in allergies and CIDs documented since the 1970s. As reviewed in **Section 3.6.5**, Bjőrkstén[251] proposed that

antibiotic use in infancy might reduce the diversity of gut microbiota, which might in turn predispose to faulty immune system regulation. Although an association has been demonstrated between antibiotic use and later development of asthma or allergy in several studies, other studies suggest that it is due to frequent antibiotic use in early life, which is more common amongst asthmatic children. This highlights the point that the children at greater risk of developing allergic disorders may also require more treatment for infection. An analysis in 28 countries (Foliaki 2004[424]) found a positive association between per capita antibiotics sales and the prevalence of symptoms for asthma, rhinitis, and eczema, but the associations generally became negative after adjustment for Gross National Product [GNP]. Trends in the introductions of vaccines may also be relevant. Until the 20th century, this was restricted to smallpox vaccination. Immunisation against tetanus, typhoid fever and diphtheria became common during the first half of the 20th. century, followed by vaccines against several childhood infections. As discussed in **Section 3.6.12**, epidemiological studies provide no consistent support for either a beneficial or adverse effect of vaccination/immunisation on atopy rates.

5.5 Household hygiene practices. including personal hygiene

5.5.1 *Our recent concerns with hygiene?*

The hygiene hypothesis, as originally stated, proposed a link between rising atopic disorders in the previous 30 years and improved household amenities, with less opportunity for 'unhygienic contact'. Modern hygiene practices are relatively recent developments. In the absence of confirmed knowledge of pathogens, and the prevalence of vague theories of disease transmission linked to miasma prior to around 1900, the application of hygienic practice was often erratic or even altogether absent. The application of cleaning and hygiene was partly dictated by the availability of domestic water supplies. A constant water supply, much less of hot water, was still rare in mid-nineteenth century England (Hartley 1978[425]). Industrially manufactured bar soaps first became available in the late eighteenth century, as advertising campaigns in Europe and the United States promoted popular awareness of the relationship between cleanliness and health. Domestic use of soap in England was equivalent to 3.6 lbs. per person in 1801, increased to 8 lbs in 1861, and almost doubled again by 1891.[426] Soap manufacture in the USA more than doubled between 1904 (8.4 kg per capita) and 1919 (16 kg per capita) (Greene 1984[427]). In the US, soap sales continued to increase until around 1940 (Aiello 2008(ii)[415]). From 1940 to 1970, synthetic soap (detergent) sales in US and Europe rose steadily as laundry products converted from soap to detergents. The development of detergents was driven by the shortage of fat and oil supplies for making soaps during WWI and WWII. An assessment of ongoing trends (Stanwell-Smith & Bloomfield 2004[1]) shows that 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. Total product usage per capita across 12 European countries was estimated to have increased by about 50% between 1969 and 1994.

5.5.2 *Home cleaning and hygiene trends*

During the earlier parts of the 20th century there was significant emphasis on home cleanliness, with advice on regular cleaning of walls, ceilings and other areas partly prompted by the fear of infection before the antibiotic era. Social changes during the latter part of the 20thC, such as less domestic help, less fear due to availability of vaccinations and antibiotic therapy, changed the approach to housework. As women began to work outside the home and have less time available, these changes led to a more superficial approach to home cleaning, with speed and aesthetic factors more important than disease prevention.

The 2004 and 2006 reviews concluded that support for a link between atopy and domestic cleaning and hygiene is weak. The evidence, as reviewed by Bloomfield 2012[428], shows that human, animal and foodborne microbes are continuously brought into the home. Transmission from these and other sources via hands, hand contact surfaces, food preparation surfaces and cloths during normal daily activities provide ample opportunities for exposure to foodborne pathogens or pathogens from infected people or pets, as well as exposure to commensals and environmental microbes. Audit studies of the home environment[428] show that even apparently clean modern homes still abound with a rich mixture of bacteria, viruses, fungi and moulds, as well as dust mites and other insects. Audit studies also show that *E. coli* and other faecal coliforms are commonly isolated from sites and surfaces in the home (Josephson 1997[429], Ojima 2002[430], Scott 1982[431]). Judah (2010[432]) showed that organisms of faecal origin, such as *E. coli,* are quite commonly isolated from hands, suggesting that oral contact with these organisms is not uncommon. In this study, swabs taken from the hands of 409 commuters at train stations across the UK were analysed. *E.coli* was isolated from 9% of samples. Other isolates were *Enterococcus* in 22%, *Klebsiella* in 2.5% and *Enterobacter* in 0.3% of samples[432]. As time spent indoors has increased to nearly 16 hours per day according to German, US and Canadian studies (Brasche 2005[433]), our opportunities for exposure to these various organisms are quite likely to have increased rather than decreased.

Studies of levels of microbial contamination also show that routine daily or weekly cleaning habits have little effect in reducing overall exposure to microbes, beyond the levels that have probably prevailed throughout the rise in atopy, even where they involve use of an antibacterial product or disinfectant (Scott 1984[434], Rusin 1998,[435] Josephson 1997[429]). Re-colonisation of surfaces rapidly occurs and many species are adapted to survival, even on apparently dry surfaces. Contrary to perception, domestic cleaning practices can increase the distribution of microbes in the home (Cogan 1999[436], Cogan 2002[437], Barker 2004[438]. Although the pattern of microbial exposure in the home may have changed, there is no evidence that our modern preoccupation for cleanliness has resulted in a decline in overall microbial exposure.

Whilst little is established about how altered microbial exposure might negatively affect the immune system, even less is known about how the design of our living environment (architecture, design, and building materials), climate, the way we live in that environment and so on) affect indoor ecosystems i.e the types and diversity of the microbes we are exposed to during our daily lives, apart from the fact that buildings are complex ecosystems that house trillions of microorganisms interacting with each other, with humans and with their environment. Kembel (2012[439]) recently reported a study of a healthcare facility where bacterial rRNA sequencing was used to quantify relationships between building attributes and airborne bacterial communities. The diversity and abundance of bacteria varied as a function of room design, with the composition of bacterial communities "*in window-ventilated patient rooms*" found to be intermediate between mechanically ventilated patient rooms and outdoor air. Importantly, differences in composition were also associated with differences in diversity [number of kinds of bacteria]. Outdoor air was most diverse, followed by rooms with an open window and finally, mechanically ventilated rooms. Bacterial communities in indoor environments contained taxa [groups] that are absent or rare outdoors, including taxa closely related to potential human pathogens. The best predictor of the number of potentially harmful species was the room's diversity of bacteria; rooms with greater diversity of bacteria had fewer bacterial species similar to human pathogens. As discussed in **Section 4**, the hygiene hypothesis is being explored as to whether it is the diversity (range), composition (which kinds) or abundance (how many in total) of tiny life forms that matters. Kembel and colleagues' results suggest that air conditioned/heated, closed off apartments and offices may be devoid of the necessary diversity.

In another new study, Flores[440] used rRNA sequencing to explore the patterns exhibited by bacteria across ten surfaces in twelve US public toilets. Most sequences belonged to four phyla: *Actinobacteria, Bacteriodetes, Firmicutes and Proteobacteria*. The communities clustered into three categories: those found on surfaces associated with toilets, those on the restroom [toilet] floor, and those found on surfaces routinely touched with hands. On toilet surfaces, gut-associated taxa were more prevalent, suggesting faecal contamination. Floor surfaces were the most diverse of all communities, containing several taxa commonly found in soils. Skin-associated bacteria, especially the *Propionibacteriaceae*, dominated surfaces routinely touched by hands. Further data confirmed that human skin was the primary source of bacteria on restroom surfaces. Overall, these results demonstrate that public toilet surfaces host relatively diverse microbial communities, dominated by human-associated bacteria, with clear linkages between communities on or in different body sites and those communities found on public toilet area surfaces.

5.5.3 *Personal hygiene – Showering, bathing and laundering*

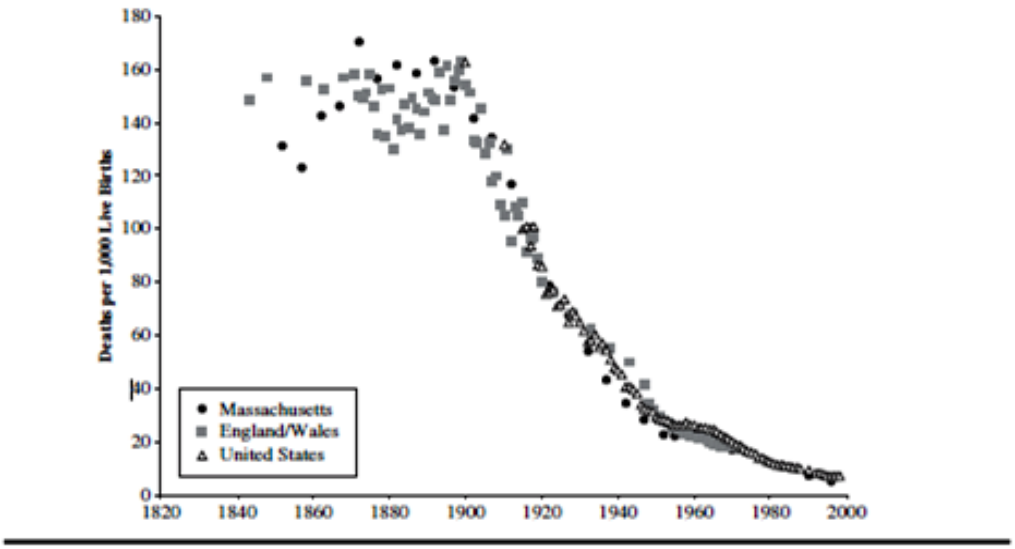
The trend towards modern habits of regular bathing and laundering in the USA and UK dates from 1890 to 1915. Late UK 19th century and early 20th century legislation, which required local authorities to provide baths and laundry facilities for people without domestic water supplies, encouraged higher standards of cleanliness. According to Aiello[415], the history of baths and bathing in the U.S. during the mid-1800s was not much different to that in Western Europe. With no running water in homes, bathing was largely left to occasional dips in ponds or streams. Washing parts of the body and bathing started to come into the home during the mid-1800s, although indoor plumbing in any building was still extremely rare. In England, home bathing and laundering became a social norm in the years immediately before and after World War II; in the U.S. it might have happened slightly earlier.

Frequent bathing, hair washing and the introduction of showers has been a further change in society over the last half-century. 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[427]). A birth cohort study in the UK reported an association between the small proportion of children with high washing scores and subsequent atopy (Sherriff 2002(i)[441], Sherriff 2002(ii)[442]), but this has not been further confirmed and a recent study from the ALSPAC group also showed no association between frequency of bathing in swimming pools and risk of asthma (Font-Ribera 2011[443]). A study of Japanese children with atopic eczema reported lower risk, compared with controls, for infants who had been bathed or given a shower at least once a day (Miyake 2007[444]). A higher risk was associated with mite allergen level in maternal bedclothes or mould in the kitchen, but lower risk for frequent vacuuming during pregnancy was also reported. Damage to the skin barrier by too frequent showering or use of inappropriate skin cleansers is an often cited problem for children with eczema (de Benedetto 2012[445]) and in epidemiological studies it is important to distinguish between such lifestyle factors, genetic predisposition to skin barrier problems and improved hygiene. Few studies have been able to assess frequency of bathing or showering prospectively: usually, a presumption is made about the hygiene status of the home, for example, a recent study of Polish children from foster homes compared with those living with their parents, based on higher measured levels of endotoxins (Stelmach 2007[446]), showed that fostered children had less atopy, presumed to be attributable to "*unfavourable environmental circumstances*", although whether this included less washing was not clear. A study by Dunder[171] showed that measures, such as making the children wash their hands more often, reduced infection rates but had no effect on atopy risk in a 12-year follow-up.

5.6 Trends in infectious disease rates

In the 2004 and 2006 IFH reviews, data covering the past 100 years were examined to identify public health and medical changes in relation to their impact on infectious disease rates, as well as any inverse correlations with trends in atopy. The aim was to find clues as to the nature of the critical exposure and why they might have been lost. Trends in infectious disease rates in the US during the period 1800 to 2000 were more recently reviewed by Aiello (2008(i)[335]). Mortality rapidly declined between 1900 and 1950. Data for specific diseases (Aiello[335]) indicate that the declining rates of whooping cough measles, diphtheria in the US and UK also dates from around 1900. The same trends were observed for India and China, but not until 1950s onwards. As shown in **Figure 2** below, the decline in infant mortality began in the U.S. around 1890. Diarrhoea mortality in Baltimore, among children less than two years old, consistently dropped every decade from 1870 (265/100,000) to 1920 (90/100,000) (Aiello (2008(ii)[415]). Similar data are available for the UK, where, for example, 50% of children aged 5-9 died from infectious diseases in 1911-15 (Hicks & Allen 1999[447]). This trend of declining infant mortality has continued in more modern times. In 1916, the top infant killers in the US were diarrhoea and related diseases and six of the top causes of infant deaths were infectious diseases. Even as late as the 1940s in the US, six of the top-ten causes of infant death (which included diarrhoea) were of an infectious nature, whereas by 1998 there were only two infectious diseases in the top-ten causes of mortality. Interestingly, Aiello (2008(ii)[415]) show data demonstrating a possible correlation between increased consumption of soap and washing powder and lower infant mortality rates in Canada, Japan, and India, although they advised caution in interpreting such "*rudimentary correlations*", which would require further investigation by more detailed prospective (follow-up) epidemiological study. Nevertheless, they commented on the possible importance on any relationship that this may suggest for decreasing infant mortality and increasing shipment or production of soaps and detergents across geographies in the same time period, across time in the same geography, and even across time and geographies.

Figure 2: Decline in infant mortality in Massachusetts, other parts of the US and England and Wales from approx.1840 to 2000



Reprinted from Am J Infect Control, 36:(10 Supp3) Aiello AE, Larson EL, Sedlak R, The health revolution: medical and socioeconomic advances, Figure 2-5, pS119 (2008), with permission from Elsevier.

As reviewed in **Section 4.5**, the decline in mortality and morbidity from infectious diseases in the last 100 years, particularly the killer diseases such as cholera, typhoid fever and diphtheria has tended to foster a view that there has been an overall decline in the incidence of infectious disease in developed countries. It is not frequency, but morbidity and mortality that have, in fact, declined (Velimirovic 1986[336]). Improved control of **water** and **food-borne pathogens** led to a decline in enteric infections, through the 20th century, despite emergence of new strains, such as in the **Salmonella** species. The incidence of food poisoning rose during the critical period of the increase in atopic disorders (1970s-1990s), making food-borne pathogens unlikely candidates to explain this increase, at least in the terms of the hygiene hypothesis. As reviewed in more detail in **Section 7**, food-related, waterborne and non-food related infectious intestinal diseases remain common. A recent UK community based study (Tam 2011[339]) estimates that the number of cases of IID due to norovirus is 3 million, while the estimated number of cases in the US is 20 million. **Viral infections, such as rotavirus or norovirus** have remained common, with norovirus only being identified (as Norwalk virus) in 1968, but now estimated to account for around 90% of epidemic non-bacterial gastroenteritis worldwide (Lindesmith 2003[341]). For children under 5 years of age, rotavirus is the leading cause of gastroenteritis (Soriano-Gabarró 2006[342]). Respiratory infection also remains common, with acute respiratory illness contributing 6% of global disability or death, including 1.5 million annual deaths of children aged under five and 3-5 million severe influenza infections (Schluger 2010[448]). It has been estimated that the average child in the US has 4-8 respiratory infections per year (Monto 1995[449]). In a more recent study of 1314 German children followed up from birth, an average of 21.9 respiratory infections was recorded by the age of 12 years, with up to 11 episodes a year the norm for infants (Grüber 2008[450]). In the US, it was estimated in 2003 that about 12 million cases of acute tracheobronchitis are diagnosed each year, accounting for a third of patients presenting with an acute cough (Fendrick 2003[451]). Community associated skin infections with Methicillin-resistant *Staphylococcus aureus* have increased (Edelsberg 2009[452]) and skin infections in general are a significant cause of morbidity in both developed and developing countries (Afsar 2010[453]).

5.7 Discussion of these trends

Gaining a clear understanding of the factors that have contributed to reduced or altered microbial exposure, and deciding whether remedial public health measures might be feasible, is unlikely unless the key microbial exposures can be positively identified. This means that we are still unable to positively include or exclude any particular public health trend as an underlying cause of the rise in allergies and other CIDs. If it is correct that no single type of exposure is the whole explanation, then it may be that several of the factors reviewed in this section have contributed to some extent. The key is to determine which factors are of most relevance and their relative past or continuing impact. The OF hypothesis proposes that the critical, absent or much reduced exposures are the 'OFs' to which we were exposed during the hunter gatherer period, but that our separation from these exposures did not happen until the start of 19th century and the later sanitary revolution. While provision of sanitation, clean water and less contaminated food are part of the modern environment and with a timescale relevant to the emergence of CID, evaluation of data trends for CIDs (**Section 2**) against trends in public health measures or infectious diseases (see also **Section 6**) does not support a temporal association between reported infectious disease and either allergy or the range of CIDs. At the same time the epidemiological studies (**Sections 3 & 4**) give little or no support to a causal link between lower infectious disease rates and allergies/CIDs: indeed, there is evidence to the contrary, in that some infections have been implicated as triggering or causing CIDs (Fairweather & Rose 2004[454], Bach 2005[455], Paccagnini 2009[456]). Many of the infections implicated as causative are viruses, such as Coxsackie virus, which were not common in the Paleolithic period, and should be distinguished from the OFs, such as helminths, that are postulated as protective.

Whereas the introduction of public health measure designed to reduce the burden of infectious disease, such as improved housing, sanitation and clean drinking water and vaccination programmes, correlate with the decline in life threatening diseases, such as cholera, typhoid fever and tuberculosis, or viral infections such as measles and mumps, these would seem to have occurred too late to have triggered the early emergence of allergies and other CIDs in the 19th century and too early to be associated with the epidemic rise in these diseases in the late 20th century. For example, childhood infections such as measles started to decline in the 1920s (Aiello 2008(i)[335]) while whooping cough was in decline from the turn of the 20th century. Reduced exposure to food-borne pathogens or exposure to enteric viruses are unlikely causes, since the incidence of food poisoning and other enteric diseases rose during the critical period of the rise in atopic disorders. The introduction of antibiotics from the 1930s onwards, with increased prescribing over the next 30 or more years, shows a possibly greater correlation with the rapid rise in allergies in the 1970s. There are conflicting results and interpretations of whether antibiotic use is a contributory cause (**Section 5.4**). Epigenetic effects (**Section 6.7**) are possibly relevant to interpreting public health trends.

So much for measurable or reported impacts of public health measures or medical treatments: but the impact on human and animal commensals, and altered exposure to saprophytes in the food, water and our environment is much harder to assess. Alterations in gut and skin microbiota provide clues to these changes, but these studies are recent. The reductions in infectious diseases over the past two centuries act as a marker, confirming that the public health and therapeutic measures over the past 200 years have been very successful in reducing or altering our exposure to pathogens and helminths. As a by-product, they must also have also reduced or changed our exposure to the mix of microbes such as the OFs that inhabit the same human, animal and other environments. A pertinent example is the decline in endemic helminth (worm) infections, probably related to provision of indoor plumbing and not going barefoot through muddy areas. The decline in intestinal diseases transmitted by the faecal-oral route over the critical period since 1800s shows how the combined effects have successfully separated us from exposure to gastrointestinal (GI) pathogens, but must have also been accompanied by declining exposure to other species that contribute to the human gut microbiota. A key question for this report is to re-examine the original synthesis of the hygiene hypothesis regarding a possible contribution from modern trends in hygiene amenities, practice and personal cleanliness. Trends in home cleaning are difficult to interpret, but the bacteriological evidence of a link with home cleaning and hygiene remains weak. Although the early 20th century of increase soap use and attention to personal hygiene (i.e washing and bathing) correlates with emergence of CIDs, again it predates the rapid late C20th rise in atopic disorders by several decades. It is interesting that Greene[427] concluded that the emerging emphasis on cleanliness probably played an essential, but generally ignored, role in the sanitary revolution and the associated control of infectious disease.

6. OTHER, NON-MICROBIAL, EXPLANATIONS FOR THE RISE IN ALLERGIES AND CIDS

A number of factors were examined in the 2006 review, such as pollution, diet, obesity, physical activity and measures of social disadvantage. This section considers new evidence for these non-microbial factors and how this might fit with a unifying hypothesis based on the OF. If correct, the modern environment has enhanced inflammatory responses, which, combined with other 'modern' factors may provide the answer to why CIDs have become more prevalent. The OF hypothesis differs from the original hygiene hypothesis in that it includes the role of factors such as diet, Vitamin D deficiency, pollution and the urban environment as additional indicators of the changed exposures since the 1800s. It is also more closely linked to the research into immune system mechanisms, such as the defective immunoregulation now demonstrated for

diseases such as food allergy, MS, T1D and IBD (**Section 4.11**). Rook (2011[17]) has emphasised the way many factors from the modern environment may interact, so that while the changing microbial exposures may be the fundamental major modulating factor, others may become important only when immune regulation has become impaired, serving to trigger or worsen the allergies or CIDs. From this viewpoint, the fundamental problem in these diseases is a disorder of immunoregulation. How the various factors interact has not been established in this emerging field of research.

6.1 Pollution

Pollution was discussed in the 2006 IFH review, for example as linking asthma with air pollution (D'Amato 2000[457], Rios 2004[458]) as well as to factors such as maternal smoking (Lødrup Carlsen 2002[459]). While a major role for pollution in causing asthma has been discounted (Charpin 1999[460], Strachan 2000b[461]), pollutants continue to be implicated as promoters of allergic disease. Some pollutants, such as diesel oil fumes, have paradoxical effects. Diesel exposure worsens respiratory infections by depressing protective immune responses and has been suggested as a cause of asthma (Pandya 2002[462]) and decreased resistance to infection (Gilmour 2012[463]). Exposure during pregnancy to benzene air pollution is associated with reduced lymphocyte numbers in cord blood, which may reduce immune competence (Baïz 2011[464]). In a recent study of 700 infants followed up to age seven, high exposure to traffic related air pollutants was found to be an independent risk factor for wheezing during infancy and early childhood (Bernstein 2012[465]). A cross-sectional study of primary school age children in Korea (Jeong 2011[466]) found higher levels of 'allergy-related diseases' and asthma symptoms in an industrial city than those in a more rural environment, although this does not necessarily implicate pollution as the active factor. Exposure to indoor environmental dust is well established as exacerbating allergic disorders, while not confirmed to have a causal role.

At least 100,000 new chemical compounds have been introduced since 1900, although these represent only a few percent of known chemical compounds: ultimately the whole planet and all life are made entirely of chemical substances. The immunotoxicity of some now widespread compounds is established (De Witt 2012[467]): some groups of such substances are reviewed elsewhere (Putman 2002[468], Galloway & Handy 2003[469], Seo 2011[470]). Relevant to this review, concern has focused on how those compounds that persist in the environment might interact with bacteria, viruses or other influences on the immune system. For example, polychlorinated biphenyls (PCBs) are known to suppress the immune system: while production has ended, it has been found that high exposure results in a greater risk of infection as well as possibly development of CID (Carpenter 2006[471]). It has been suggested that simultaneous exposure to a mixture of such chemicals and to infections may have complicated effects on the immune system, for example causing people to develop autoimmune disease (Abedi-Valugerdi 2005[472]). This could explain why more children with 'low to moderate risk' genes are developing T1D (Vehik 2008[473]). Exposure to Coxsackie virus (an enterovirus) can alter the distribution of dioxin in mice organs, suggesting that viral infection may increase the toxicity of contaminants in the body (Funseth 2000[474]). While such evidence argues for including infectious agents in the multidisciplinary investigation of environmental contaminants (Feingold 2010[475]), it does not suggest that microbial exposure in this context has any protective effect.

The possibility of some impact on atopic disease from the use of chemical products in the home has been examined in statistical analyses of data from the ALSPAC birth cohort in Bristol, UK (Sherriff 2005[476], Henderson 2008[477]). Both studies found an association between scores reflecting reported pre-natal frequency of use of a disparate range of "household chemicals" and persistent wheezing in the children. The broad list of substances surveyed included dry cleaning fluid, insect killer aerosols, air fresheners, paint and varnish, window cleaner, as well as disinfectants and bleach. In the more recent study (2008), the frequency of use score was

associated with a greater risk of persistent wheezing in the non-atopic children at ages from under 18 months to over 30 months. The authors postulated either pre-natal effects on the developing airway or irritant effects postnatally, but did not consider that increased hygiene in the home was involved, particularly as the association was only observed for children with non-allergic asthma. They suggested a link with occupational studies that have shown irritant effects of chemical exposures in domestic or office cleaners[477]. The authors examined whether use of particular product types was the source of the increased risk, but found that removing each type in turn in the statistical analysis had no effect on the results. Though some product types will contain irritants at some level, there is no obvious toxicological mechanism by which a wide array of products might have such a similar effect: other suggested explanations include over-reporting of both symptoms and "chemical" use by certain respondents. Scores based on self-reported frequency of use are, in any case, a poor indicator of product exposure even within a single category: use of different amounts applied in different ways, over different time periods and affecting different routes of exposure, can yield scores which run contrary to actual exposure. The epidemiological problems of assessing such environmental exposures have been discussed by Franklin (2008[478]) and meanwhile no link between any specific cleaning agent and atopy has been established.

6.2 Diet and nutrition

Diet has been implicated as contributing to the rise in allergies, although a recent review found no conclusive evidence about the influence on asthma prevalence of specific nutrients, food types, or dietary patterns past early childhood (Kim 2009[479]). Nevertheless, rapid changes in diet are in many ways a hallmark of westernisation, so research into dietary factors is relevant to this review. Consumption of antioxidants, such as Vitamins C, E and beta-carotene, has declined, although a recent meta-analysis did not find a consistent association of dietary intake of these factors with asthma (Gao 2008[480]). Supplementation with individual antioxidants plays a minor role in preventing asthma (Devereux 2007[481]). The case may be stronger for Vitamin D: lack of exposure to sunlight is associated with Vitamin D deficiency, in turn related to increased incident of asthma in young children[479]. This deficiency has been described as a "*pandemic*" (Rathi & Rathi 2011[482]). The current consensus is that Vitamin D appears to have a broad immunoregulatory role affecting a range of allergies and CIDs, for example Vitamin D insufficiency has been implicated in MS (van der Mei 2007(i)[483]). The large survey of childhood food allergy by Gupta (2012[43]) adds to the debate on the 'Vitamin D hypothesis' for atopy/CID since their results showed a higher risk of allergy in southern latitudes of the US: contrary to expectation, since Vitamin D deficiency is more common in northern, less sunny latitudes. Latitude differences have also been reported from Australia (van der Mei 2007(ii)[484]) but explained only around 4% of Vitamin D deficiency: behavioural differences, such as using sun block or avoiding sun exposure may account for observed lack of Vitamin D also in sunnier latitudes. More supplementation studies are needed to further investigate the Vitamin D Hypothesis (Bozzetto 2012[485]). Meanwhile better maintenance of Vitamin D levels requires "*adequate calcium intake, more exercise and less obesity*" (Mason 2011[486]). The 'lipid hypothesis' (rise in asthma due to increased consumption of polyunsaturated fatty acids) has not been supported by intervention studies (de Vries 2009[487], Kim 2009[479]), while the Mediterranean diet has been found to reduce asthma risk (Castro-Rodriguez 2008[488]), although the mechanism remains unclear[479]. Associations with low fruit intake (Patel 2005[489]) or with increased hamburger consumption (Wickens 2005[490]) may be due to confounding by social factors/ income. Poor nutrition in early life has been linked to later development of allergy, coronary heart disease and T2D: this has been described as the 'thrifty phenotype hypothesis' (Hales 2001[491]).

6.3 Obesity, asthma and other CIDs: the 'obesity hypothesis'

An association between obesity and increased risk of asthma is well established (Beuther 2006[492], Ford 2005[493], Shore 2006[494]), also multiple reports of an association between elevated body mass index and asthma (Luder 2004[495]). Overweight adolescents are more likely to exhibit both allergic sensitisation and asthma (Guerra 2004[496]). Higher levels of asthma in Australia between 1990 and 2003 correlated with increases in obesity and a decline in vigorous exercise, as well as rising diabetes (Wilson 2006[36]). The current epidemic of obesity has been linked to the hygiene hypothesis through the role of the gut microbiota on energy balance and inflammation (Musso 2010[497]), with implications for avoiding excessive fat intake. Others have also suggested that a fatty diet may encourage an 'obesogenic' gut microbiota by unbalancing the bacterial community and impairing the gut barrier (Ajslev 2011[498], Tuohy 2009[499], Serino 2012[500], Tilg 2009[501]), although other factors contributing to obesity in childhood include maternal weight, dietary composition (e.g. lack of fibre) and possibly early antibiotic therapy, which in turn may influence the gut microbiota. While Type 2 diabetes is the commonest CID implicated in these changes, allergies and other CIDs have been linked to the loss of immune tolerance associated with obesity (Hersoug & Linneberg 2007[502]). Other research suggests that the obese state is characterised by low-grade chronic inflammation: markers of inflammation decrease with weight loss (Yudkin 1999[503], Bastard 2000[504], Festa 2001[505], Engtstrom 2003[506], Esposito 2002[507], Chiellini 2004[508]). Hersoug and Linneberg[502] have proposed that these obesity-related changes suggest a new hypothesis for the rise in allergy and CIDs. They suggest in addition that obesity-induced immunological changes in pregnant women are likely to increase the risk of atopic disease in the offspring: immune tolerance has been shown to increase with successive pregnancies, which could at least partly explain the family size protective effect on allergies and CID. A review of recent human and animal research appears to confirm a very important influence of obesity on inflammation during pregnancy and the subsequent development of allergy and CIDs (Thornton 2011[509]).

6.4 Physical activity

The decline in physical activity during the last century has been linked to asthma risk, possibly through greater exposure to allergens in the indoor environment (Platts-Mills 2005[510]). The asthma risk could be enhanced by associated obesity. Physical exercise has beneficial effects on circulating immune function and has been recommended in the management or possible prevention of chronic, noncommunicable diseases (Sears & Genius 2012[511]). Exposure to outdoor air has received more recent attention, for example in the finding that there are higher concentrations of viable bacteria in outdoor air than inside office buildings (Tsai and Macher 2005[512]). Both gram-positive and gram-negative bacteria in the air can stimulate the regulatory responses associated with controlling allergic reactions (Rogers 2005[513]). As yet, little is known of the bacterial content of the ambient air but research supports the need for more outdoor activity, particularly in early life.

6.5 Socioeconomic factors

The association of social disadvantage with worse health outcomes is well documented. The GNP has been identified as a key factor in explaining the international variation in asthma symptoms, for example in showing that prevalence increases with economic development (Weinmahr 2007[514]). Socioeconomic status is related to the risk of autoimmune rheumatic diseases, although other factors include environmental pollutants, infectious agents and Vitamin D (Shapira 2010[515]). A review of asthma studies highlighted the neglect of equity issues, such as poverty, differential access to medical care and variation in environmental or occupational exposure (Greenwood 2011[516]). Most socioeconomic factors are not easily measured and frequently act as 'confounders' in epidemiological studies, that is, a factor that distorts two other factors being studied, such as a type of exposure and apparent effect, for example poor hygiene and protection against atopy, because it is mixed up with the actual exposure effect (e.g.

poverty and poor diet associated with living in unhygienic conditions). The distortion can lead to overestimation or underestimation of an effect and may even change the apparent direction of an effect. For example, investigators have queried the role of confounders in the 'family size' and 'farm effect' studies (**Section 3.4)**.

6.6 Climate change

Global changes in asthma prevalence have been linked to climate change, for example milder winters as an explanation for declining cases in England and Wales (Fleming 2000[517]). Changing pollen profiles due to global warming and/or rising carbon dioxide levels have also been implicated (D'Amato 2001[518], Ziska 2000[519], Ziska 2003[520], Beggs 2004[521], Fitter 2002[522]).

6.7 Genetic susceptibility

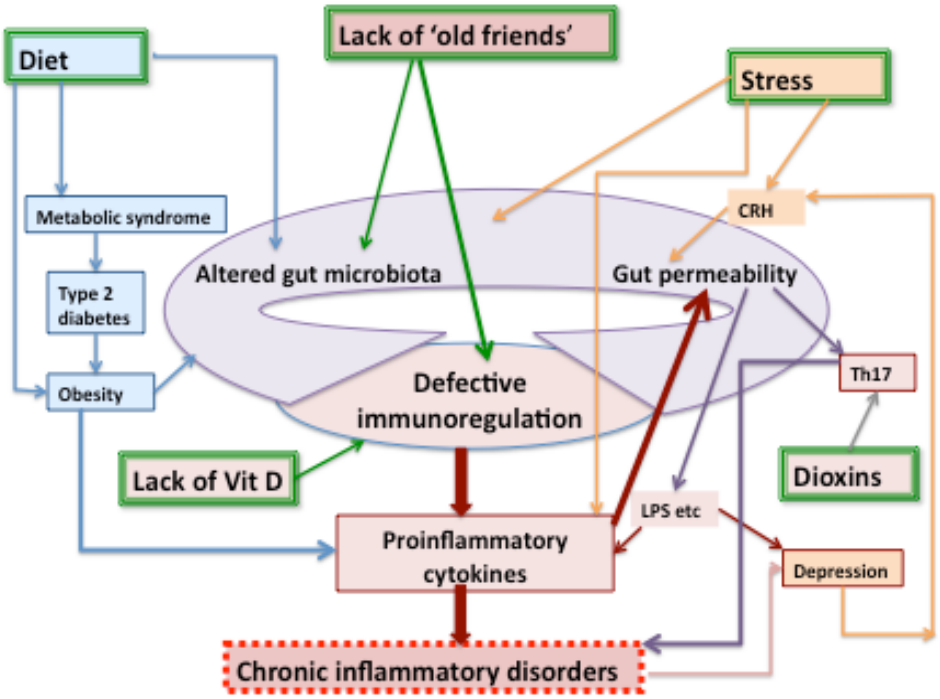
It is often stated that there has not been time for population genetic change to occur in since the main evolutionary changes of the immune system and the possibility that the rise in allergy and CIDs can be attributed to genetic factors alone has been refuted by Rook (2011[17]), arguing that the major cause must be environmental, the genetic role being to determine which individuals develop the disease after exposure to environmental factors. Inbuilt susceptibility to diseases within our genes is well established, with increasing evidence of genetic factors for the CIDs, such as leukaemia (Wiernels 2012[94]). While genetic change in human chromosomes is unlikely to have occurred, postulated 'modern environment' factors, that may have increased the expression of genetic risk as clinical disease, include declining herd immunity to less common infections, raising population susceptibility to such infections. Another possibility is that children with innate greater risk of succumbing to severe infection would have died at early ages in the past. Such susceptibility to several infections is well established and further supported by recent genome studies (Chapman & Hill 2012[523], Hill 2006[524]). Fortunately children with this risk now mostly survive, but perhaps with associated higher tendency to develop allergies and CID, for example the recent discovery of a genetic variant associated with several CIDs and also with increased risk of invasive pneumococcal infection and leprosy (Hill 2012[525]). There have been sufficient generations since the start of the decline in infant mortality for altered susceptibility to infection to be having an impact on our populations, a modern phenomenon that in Darwinian terms might be expected, once the selection pressure allowing 'natural selection' was removed. Research topics include a need to appraise "*the interplay between infectious exposures and the status of immune response in individuals*" (Wiernels[94]). There is a growing interest in epigenetics: the study of heritable changes in gene expression that occur without a change in the DNA sequence (Rodenhiser & Mann 2006[526], Gilbert 2010[527]). The effects of environmental change might not be fully manifested in the first generation: it might take two or more generations. This is relevant to causation of CIDs because disorders in the regulation of gene expression may explain the rise of such diseases in our era: it is a swiftly evolving research field, but interaction between innate factors and the environment requires further study.

6.8 Concluding remarks on non-microbial exposures

Since the IFH 2006 review, the brief outline above demonstrates that dietary factors such as Vitamin D deficiency and the rise in obesity have been more strongly implicated as significant contributory causes to CIDs. Another important emerging theme is the greater emphasis on interaction between pollution and other contributory factors, as well as new kinds of pollution. Socioeconomic factors remain rather neglected, particularly as possible confounders when not carefully controlled in epidemiological studies. The genetic susceptibility to both infection and to CIDs, and the way our genes interact with the environment, is a rapidly moving research field and may provide a clearer indication as to how the various implicated factors act and interact. The OF hypothesis helps to unify many of these seemingly unconnected factors (Rook 2010[7])(**Figure 3**). This figure links exposures such as dioxin, diet, and lack of exposure to old

friend species to the increased expression of CIDs. It also includes stress, mentioned in the IFH 2004 review but not previously referred to here: recent comment on the wide range of CIDs, susceptibility to infection and other diseases linked to 'stress' is reviewed elsewhere (Kemeny 2007[528], Stojanovich 2010[529], Marshall 2011[530]). If Rook's umbrella concept is correct, there are major implications for modern lifestyle that take us beyond considering the role of microbial exposure.

Figure 3: How modern life may interact with the lack of 'Old Friends'



Notes to figure: CRH = corticotrophin releasing hormone which affects the gut mucosa; LPS = lipopolysaccharide; Th17 = T helper cell type 17
(Based on Rook 2010[7], where these possible interactions are more fully described).

7. THE HYGIENE HYPOTHESIS AND ITS IMPLICATIONS FOR HYGIENE – OVERALL CONCLUSIONS FROM THE UPDATE REPORT

This report has aimed to review current data and thinking on the hypothesis that reduced microbial exposure has been a key factor in the emergence of allergic and other CIDs during the last two centuries, but most particularly in the past 30-50 years, and the reasons why this might have occurred. This final section covers the key findings of the report.

7.1 Implications for acquiring healthy immune balance

Since the 2004 and 2006 IFH reviews were prepared, support for the role of microbial exposure as a key contributory factor for immune regulation in relation to risk of CIDs and other disorders has continued to increase, to such an extent that a 'microbial exposure hypothesis' is now widely accepted by the scientific community. Equally importantly, there is still no clear indication of what are the key microbial exposures and why and how we have become deprived of them. This review of epidemiological studies relating to the hygiene hypothesis (and its

expanded variants) shows that there are inconsistencies in the evidence for any one factor and the results point to interrelated factors, such as changes in our diet, interactions with pollution, genetic susceptibility, climate change, less physical activity and obesity (summary of general findings in **Appendix III**). So there is no simple explanation or 'one size fits all' solution. The interaction of microbes with the immune regulation system is clearly a complex process, in which increased risk of CID for any individual depends primarily on:

- Type/s of organisms from which the individual is deprived;
- Timing and duration of deprivation: prenatal, postnatal, early childhood – onwards;
- Their genetic predisposition to CIDs;
- Impact of other lifestyle factors such as diet, obesity, or antibiotic usage, specific to that person.

This report is inevitably an incomplete summary of the wide range of diseases and factors now linked to the expanded hygiene hypothesis, but it allows us to begin to focus on what might be the important exposures and routes of exposure required for normal immune regulation. In **Table 1** the range of environments and microorganisms for which there is the most consistent data on a positive (protective) impact against allergies is summarised. This is based on a review paper (Rook 2011[17]) in which the analysis is extended to a wide range of CIDs, with a summary of environments relevant to the findings of this review (listed in **Appendix IV**).

7.2 Summary of the implications of Old Friends and biodiversity hypotheses

The wide range of organisms implicated as possibly beneficial, in terms of risk of allergies/ CIDs, suggests that our microbial exposure needs are not confined to a single species and, as shown in **Table 1**, that there is overlap between exposures. There is growing evidence that the specific exposure needs of an individual person can be met by one or more of a range of quite diverse species and that, if any of these species are missing, their role may be met by one or more other species i.e. there is considerable redundancy in the system. Although the key microbial exposures remain undefined, the following changes in thinking, prompted by new research since 2004 are beginning to give us some clearer ideas on which we can now build.

Table 1. Evidence for microbially protective environments

Types of Environments	Types of organisms likely to be present in these environments	Route of exposure	Type of supporting data‡
Large families	Childhood infections (?including at subclinical level), HAV, H.pylori, gut microbiota organisms	Oral/ airborne	E
Day care	Childhood infections (?including at subclinical level), HAV, respiratory viruses, gut microbiotal organisms	Oral/ airborne	E, I
Farm living (type of farm poorly defined but probably involves livestock)	Endotoxins, gut microbiotal organisms, HAV, *H. pylori*, Helminths, lactobacilli (and other organisms in untreated milk), saprophytic *Mycobacteria, Salmonellae, Toxoplasma*, other zoonoses	Oral/ airborne/ ?insect borne	E, Ex
Prenatal environment	Depends on maternal environment e.g. helminths & endotoxins in a rural or primitive	Oral/airborne	E, Ex, I

Types of Environments	Types of organisms likely to be present in these environments	Route of exposure	Type of supporting data‡
	environment; viruses etc. in modern Westernised environment		
Post natal environment	Gut and skin microbiota, including lactobacilli and staphylococci	Oral/ direct contact	E, Ex

‡Types of supporting data: E = epidemiological, I = intervention, Ex = experimental models.
Based on: Rook 2011[17] (table with list of organisms and supporting evidence in **Appendix IV**).

7.2.1 Since 2004, several large well planned epidemiological studies have been carried out which generally confirm a link between lower allergy risk and factors such as larger families and certain types of farm living. But studies on family size or birth order have shown no consistent evidence that childhood infections protect against allergy. The original proposition of a key role for clinical infections in early childhood is now largely discounted.

7.2.2 The OF hypothesis, which has gained considerable support since 2004, may provide an explanation. Of the many theories that have been put forward, the OF provides the most unifying and coherent explanation for many of the conflicting aspects of the hygiene hypothesis. The fundamentally new proposal of OF is that the key microbial exposures from which we have become separated are those on which our immune systems developed a dependence during the ancient paleolithic period, when the human immune system was developing, in close association with the abundant microbes present in that era. This symbiotic relationship is common to all evolved creatures: we are essentially communities of organisms, described recently as the "*ultimate social network*" (Ackerman 2012[242]). Bacteria form the majority of cells present in the human body and many important functions, as well as genetic variation, are encoded in their genomes rather than in ours, particularly in our commensal organisms (Gilbert 2010[527], Moran 2007[531], Lee & Mazmanian 2010[532]). Significant deprivation from exposure to the OF organisms did not occur in western societies until the urbanisation of the early 1800s and the introduction of clean water and sanitation in the late 1800s (the sanitary revolution), which coincided with the emergence of CIDs in these societies, although rapid increases are more recent. While the sanitary revolution acted to protect us against 'modern' diseases developed over more than two millennia of living in close societies, it also inadvertently reduced our exposure to old friends. We cannot re-establish the complete, balanced ecosystem of the distant past, when a co-dependence between humans and microbes evolved: even the most primitive ecosystems today do not provide the mix of organisms associated with Paleolithic living conditions. Interfering with ecosystems to try to restore these exposures, for example by relaxing hygiene, is not the answer: in most poor, traditionally rural, environments, the ecosystems have already changed due to influences such as economic and social crises, changing agriculture and international travel, in addition to attempts to control life threatening or debilitating disease (Patz 2005[533]).

7.2.3 One consequence of the decline in OF is that the diversity of microbial species has also reduced: it has been estimated that around a third of the 56,000 known animal and plant species are threatened with extinction (von Hertzen 2011[8]) and we may assume that many microbial species are also threatened. The environmental diversity of plant species and micro-organisms in the area around the homes of adolescents with allergy is less than that of non-atopic individuals (Hanski 2012[183]). Within the next 30 years most of us – two thirds of those in developing countries and 85% of the population of developed countries – will live in urban areas with little green space (United Nations 2008[534]). If the OF hypothesis and related biodiversity hypothesis are correct, we have a global problem in maintaining natural

environments: yet this also liberates us from the notion that CIDs are the price we have to pay for no longer suffering the burden of killer infectious diseases.

7.2.4 Another area where we now have some, although limited, clarity is the possible nature and intensity of the interaction between microbes and the immune system. The earlier idea that microbial exposure needs to be 'intense', or at least produce clinical disease, is no longer confidently applied to the hygiene hypothesis. It now seems more likely that the protective effect comes from background exposure to subclinical doses of pathogens, or to commensal or environmental microbes, particularly those with low invasiveness or virulence such as the rapid growing saprophytic strains of *Mycobacteria*.

7.2.5 It is now accepted that the original concept of a distorted Th1/Th2 balance is an over-simplified explanation for increased susceptibility to CIDs, but that the more likely explanation is defective regulation of the balance between inflammatory and anti-inflammatory lymphocytes (T cells) and other immune cell types. This allows the possibility that different microbial species may interact with the immune system in different ways and at different points. Also, the structural components of microbes involved in immune priming reactions may be quite different from cell components which are pathogenic, i.e. that cause disease. Thus, some species of microbes can interact strongly with the immune system without being pathogenic and pathogenicity is not a prime requisite for immune regulatory effects. If there are multiple receptors within the immune regulatory system, this agrees with the idea that multiple species interact with this system in different ways but still elicit the same response. There is increasing evidence for the related theory of 'microbial turnover' i.e that while the human immune system is developing, exposure to a wide range of species provides essential challenges that prime the immune regulatory system in a multiplicity of different ways, thereby protecting against a range of CIDs.

7.2.6 An important aspect of the hypothesis is "what is the critical timing for microbial exposure (ME)?" and equally importantly – "can CIDs be reversed by replacing the exposure?" As far as atopic diseases are concerned, research strongly suggests that a critical time for exposures is perinatal or in early childhood and that it needs to be maintained over a significant period. If this is lacking then the risk of atopic disease or CIDs is considerably increased. Unanswered questions include:

i. Is **ongoing microbial deprivation (MD) a factor** in the sudden development of hay fever or asthma, which sometimes occurs only in adulthood?

ii. For CIDs such as MS, which develops in adulthood, does the problem still originate from microbial deprivation and immune dysregulation in early infancy, triggered only when other factors kick in, or is it due to ongoing microbial deprivation, which causes immune dysregulation that eventually manifests as clinical disease?

iii. What is the **explanation for remission or lessening of symptoms** (as in childhood hayfever, eczema or asthma, or response of some CIDs to helminth therapy), even if the MD is continuing?

iv. At what stage does the immune system develop a '**permanent memory'**, for example to cause autoimmune disease, since CIDs appear to be irreversible?

v. If **some ME is 'critical' to immune priming**, and the OF hypothesis is correct in implicating the sanitary revolution in most westernised countries, then why don't all of us, who have been on the receiving end of this revolution, now suffer from CIDs? Genetic predisposition and the factors influencing genetic expression are important

here, for example, there are many people shown to be atopic on serological tests who do not express symptoms.

vi. What is the **critical route of exposure**? The proposed oral-faecal route is still the most likely candidate, which fits with the OF hypothesis, but study findings do not rule out other potential routes including via inhalation or through the skin.

7.2.7 The new data confirm earlier findings, indicating that it makes no sense to claim that the increase in allergies and other CIDs is caused entirely by lack of exposure to microbes, or entirely by Vitamin D deficiency, or entirely by diet, obesity or whatever. Many of these factors are likely to play a role, but the fundamental underlying problem is immunoregulation, and our changing microbial exposures are fundamental aspects of the immunoregulatory deficit. Ultimately, the key question needing an answer is **what measures could be put in place** to reverse the trend in CIDs and other disorders related to lack or exposure to microbes. Since it appears that there is no single 'magic target' within the immune regulatory system and that translation of immune dysregulation into disease involves a range of factors, then it is unlikely that there is a single 'breakthrough' clinical solution. Success will be a slow process of small forward steps. Some of the current approaches for therapy are reviewed in **Section 4.11** and also by Rook (2010[7]) and Matricardi (2010[150]). Using probiotic strategies to reintroduce the key microbes to our environment has obvious dangers until there is much better understanding of which 'old friends' are truly friendly – and safe. Meanwhile the focus of this report is to review new data and new thinking in terms of implications for hygiene. This is reviewed in the following sections.

7.3 What are the implications for hygiene?

7.3.1 Because the umbrella term remains the 'hygiene hypothesis', any public debate emphasising the growing acceptance of this hypothesis, acts to reinforce the public notion that it has been proved that we have become too clean for our own good. Despite several attempts to change the name of the hypotheses (Microbial Exposure, Microbial Deprivation, Old Friends) the media and others still refer to it as the 'Hygiene' Hypothesis. In some ways it can be argued that that the title is technically correct, because the sanitary revolution of the early 19th century is also referred to as the 'hygiene revolution'. This means that the hygiene revolution may have been a root cause of the microbial deprivation-led rise in CIDs, but not for the 'too clean' reasons put forward in the popular media.

7.3.2 *Too clean? – a question of what that means*

Where the phrase "*we have become too clean for our own good*" is being reinforced by consumers to each other, or by the media, or by experts, it would be useful to know what they understand by this. The terms cleaning and hygiene are currently used interchangeably but, although there is significant overlap, the term hygiene generally implies more than just making oneself and one surroundings visibly and aesthetically clean. In general, but not always, the word hygiene is used to imply measures designed to promote and protect health by, for example, protecting us from exposure to infectious diseases, allergens or toxic chemicals. In many cultures it is also applied to public health issues such as alcohol abuse, obesity and smoking. It is interesting that, despite modern understanding of infectious disease transmission, we have not developed modern terminology to allow us to distinguish between domestic cleaning to remove visible dirt, and decontamination to remove harmful agents or substances to a level where there is no longer a risk to health. As a result, people still tend to assume that these are one and the same, and we have no simple way to tell them it is not. This vagueness further compounds the confusion over the hygiene hypothesis and its implications for domestic cleaning and/or hygiene practices. In the 2004 and 2006 IFH reviews [see **Section 5** for a brief summary], we made a detailed examination

of the trends in consumer cleaning and hygiene practices over the past 100 years and their impact on exposure to microbes. There was no evidence of a correlation between such practices and the rapid rise in allergies from the 1970s to the 1990s. The same conclusion applies to the increase observed in CIDs such as T1D or MS. Nevertheless, it is incontestable that the last two centuries have dramatically changed our lifestyles, both in relation to safer water, food, sanitation, personal cleansing and home cleaning and to factors such as diet, urban living and less outdoor physical activity. Possibly hygiene, because it is so vaguely defined, has been the easiest scapegoat to blame amongst these many changes.

7.3.3 *Hygiene practice 'out of the frame'?*

If we define choose to define 'hygiene' (as we have done for the purposes of this report) as the specific practices we use to protect us from infectious diseases, the evidence reviewed here suggests that the extent to which we suffer modern infections is not the key factor. This puts hygiene practice as an unlikely contributor to the multifactorial mixed causes of allergy and CIDs. Since the evidence suggests that human and animal commensals, together with environmental saprophytes (and possibly also low levels of pathogens), have a role in immunoregulation, the question then is how do we best protect against infection while sustaining exposure to the necessary microbes both within and outside the home. Where should we intervene to protect against invasive infection? Here, our understanding of infection transmission is helpful: we do not need to expunge our homes of all microbes, just those in areas where faecal-oral or transmission via surface contact could be a problem. This is the basis of '**targeted hygiene'**, which seeks to sustain normal exposure to environmental microbes. Targeted hygiene is an approach developed by the International Scientific Forum on Home Hygiene (IFH) as part of its work to promote better understanding of hygiene and better hygiene practice (Bloomfield 2012[428]). Targeted home hygiene is described in a range of home hygiene guidelines and training resources produced by the IFH[535,536,537,538]. It involves identifying the critical points in the chain of infection transmission and targeting hygiene measures at these points and at critical times to prevent the ongoing spread of pathogens. Risk assessment and management are now the accepted approaches for controlling microbial risks in food and other manufacturing environments, and also in hospitals and other healthcare settings. If background exposure is proved to be the important factor, the targeted approach to hygiene allows a focus on preventing exposure to infectious doses of pathogens, but is more relaxed about other exposures.

7.3.4 *Home cleaning and microbial exposure*

We can also challenge the idea that being 'too clean' in relation to the general domestic cleanliness of our own homes, particularly in relation to caring for children, is the cause of the reduced microbial exposure. The conclusions about domestic cleaning remain unchanged since the last review: the evidence of a link between atopy and domestic cleaning is weak. If this factor contributes at all, its contribution is likely to be very small relative to factors such as clean water, good sanitation, cleaner environments and food quality. Studies show that modern homes, however clean in appearance, still contain a rich mixture of bacteria, viruses, fungi and moulds, as well as dust mites and other insects. Since we now spend more time indoors, our opportunities for exposure to these are likely to have increased rather than decreased. It could be suggested that rather than saying "we have become too clean", it would be more accurate to say that we have become "afraid of getting dirty" and to have more potentially beneficial contact with our environment, especially outside. A frequently voiced assumption is that one of the reasons for the 'increased cleanliness' has been the widespread use of antibacterial products since the 1990s (see **Section 3.6.5**). It has been shown that, although disinfectants used for a specific purpose (e.g. during food preparation) reduce the risk of infection transmission, non-targeted, indiscriminate use has little impact on overall levels of microbial contamination in our environment (Larson & Duarte, 2001[539]). This suggests that antibacterial products, used in a non-targeted way for routine general cleaning, have an insignificant effect on microbial levels in the home. In reality, routine daily or weekly cleaning habits have not reduced the overall exposure to the microbes in

our home environment, including both environmental microbes and human commensals that are shed from the body (Scott 1984[434], Josephson 1997[429]). This human contribution to the home environment includes *Staphylococci*, gut organisms and others identified as potential 'old friends'.

7.3.5 *Personal cleanliness and personal hygiene*

More frequent showering and bathing has also been implicated as a major change in society over the last half-century. While we still cannot rule this out as a factor, there is no good evidence of a link, except where excessive skin cleaning worsens conditions such as eczema. This issue is interlinked with family size: larger families give more opportunity for sharing gut, skin and respiratory microbiota: this may include interchange of microbes via close contact, or sharing towels, toothbrushes, food utensils and other personal items. Oral hygiene has improved during the last century, through better dentistry and teeth/mouth cleaning practices, but poor oral hygiene has not been implicated as a cause of allergies or CIDs, rather the reverse. Inflammation or infections associated with poor oral hygiene are associated with greater risk of some cancers and cardiovascular disease (atherosclerosis being identified as a CID) (Koren 2010[540], Meurman 2010[541]). Since microbes in the mouth (buccal cavity) usually reach the gut, the mechanisms involved may be related more closely to the gut microbiota (Forno 2008[247]), although study of the more accessible flora of the buccal cavity may provide insights into how the gut microbiome develops (Korecka & Arulampalam 2012[235]).

7.4 Why hygiene is so important in home and everyday life

7.4.1 Since the 2004 and 2006 IFH reviews, concerns about the need for increased emphasis on hygiene promotion has increased rather than decreased. A 2009 IFH report[542] shows that infectious gastrointestinal, respiratory and other diseases circulating in the community continue to exert a heavy toll on health and prosperity in both developed as well as developing countries. Food-related, waterborne, and non-food-related infectious intestinal diseases remain at unacceptable levels (Scallan (i) 2011[543], Scallan (ii) 2011[544], EFSA 2008[545]). Using data from 18 OECD countries, a 2003 World Health Organization (WHO) report concluded that about 31% of reported food-borne outbreaks occur in private homes (Rocourt 2003[546]). The recent UK community-based study (Tam 2011[339]) estimates that the annual number of cases of Salmonella and Campylobacter infection is 38,000 and 600,000 respectively. Enterovirus infections, such as norovirus and rotavirus, are common throughout the world (Widdowson 2005[547], Soriano-Gabarró 2006[342]) (**Section 5.6**). It is no longer appropriate to refer to urban environments as containing helpful dirt in the context of the hygiene hypothesis, since a plastic and concrete environment may become dirty, but does not contain the 'old friend' organisms involved in immunoregulation. We cannot restore the Paleolithic microbial exposures, but nor can we risk the infection dangers of the modern ecosystem by relaxing hygiene.

7.4.2 Evidence shows how respiratory hygiene plays a part in limiting the spread of respiratory infections (Goldman 2000[548], Monto & Sullivan 1993[337], Jefferson 2009[549]). Influenza epidemics alone cause an annual average of 36,000 deaths and 114,000 hospitalisations during influenza epidemics (Bridges 2003[550]). In response to the threat posed by emerging pandemic strains such as SARS and swine flu, hygiene is now promoted as an important first line of defence[549]. Populations with a low education level, income level or occupational class are at higher risk of infection. These factors initiate a vicious cycle of infection predisposing to malnutrition and growth faltering, which in turn leads to increased risk for further infection (Farmer 1996[551], Semenza 2010[552]). Meanwhile the financial burden of infectious disease is enormous: £745 million annually in the UK for infectious intestinal disease alone (Anon 2010[553]) and $152 billion annually in the US for food-borne illness, far exceeding previous estimates of $6.9-35 billion (Scharff 2010[554]). Tackling antibiotic resistance is a global priority (Anon 2009[555]). For the

Transatlantic Task Force on Antimicrobial resistance, one of the three component strategies for tackling antibiotic resistance is "*Prevention of healthcare and community-associated drug-resistant infections*" (Transatlantic Taskforce on Antibiotic Resistance 2011[556]). Hygiene provides a means to reduce the silent epidemic spread of Methicillin Resistant *Staphylococcus aureus* (MRSA), and the multidrug resistant Gram-negative strains (including ESBL and NDM-1-producing strains) in the community. As persistent nasal or bowel carriage of these strains in the healthy population spreads across the world, this increases the risk of infection in both hospitals and the community (Vidal-Navarro 2010[557], Poirel 2011[558], Klein 2009[559], Köck 2010[560]).

7.4.3 Governments, under pressure to fund the level of healthcare that people expect, are looking at prevention as a means to reduce health spending. Increased homecare is one approach to reducing health spending, but gains are likely to be undermined by inadequate infection control at home. Healthcare workers now accept that reducing the burden of infection in healthcare settings cannot be achieved without also reducing the circulation of pathogens such as norovirus and MRSA in the community. At the same time, people with reduced immunity to infection make up an increasing proportion of the population, currently up to 20% (Bloomfield 2009[542]). The largest proportion is the elderly, many of whom have chronic ill health, which further reduces immunity to infection. Much of the care of these vulnerable groups is carried out by family members, who therefore need an understanding of infection prevention to protect them against foodborne and respiratory infections. Infectious diseases can act as co-factors to other diseases that manifest at a later date, such as cancer and chronic degenerative diseases, or as triggers for development of allergic diseases[542].

7.5 Final points

Since 2006, the risk of disease related to poor hygiene has not diminished and the need for hygiene promotion has been further recognised, including by proponents of the hygiene hypothesis. The concern about over domestic cleanliness and hygiene practices has been shown to be misplaced, while multifactorial causes for allergic and other CIDs, including the role of obesity, physical fitness and socioeconomic influences, have gained importance. The conclusion of this review is that diminished exposure to the immunoregulation-inducing organisms from man's evolutionary past ('Old Friends') is a consequence of an accumulative series of changes in lifestyle that result in loss of contact with mud, animals and faeces, and major alteration of the microbiota. This is quite distinct from 'hygiene' in the sense of reducing the risk of infectious disease transmission. As Rook has said aptly in a recent review:

> "*Relaxing hygiene in a modern urban environment would not expose us to Old friends --- only to new enemies like E. coli O104!*" [17]

Thus the major conclusion is that hygiene, in the sense of decontaminating or disinfecting in the times and places where it matters to prevent infectious disease, is still very much needed. It seems likely that many aspects of our modern civilisation are contributing to the rise in allergies and CIDs, including several that cannot be safely changed, such as clean water, sanitation, less contaminated food and urbanised living. The term 'hygiene hypothesis' is too entrenched to expect it to be changed in the near future. We have shown that there is no evidence that domestic cleanliness has gone too far and, in particular, that it is vital to continue to promote efficient hygiene practice. For those working in the field of infectious disease prevention and hygiene, we need to develop health promotion messages that help people to distinguish between letting children play in mud in the garden but also protecting them against potentially harmful microbes at the appropriate times. Persuading the public to develop lifestyles which reconnect with the natural environment, whilst also using targeted hygiene to protect themselves from infection represents a challenge for our time. To move forward, we need to convey three essential key concepts:

1. The expanded hygiene hypothesis is an increasingly important issue for health. It is not confined to day-to-day home and personal cleanliness, but rather to a broader range of lifestyle choices and measures that have been introduced to protect us from infectious diseases. Together, these have inadvertently also reduced exposure to the microbial friends that regulate our immune systems.
2. The organisms identified as Old Friends that protect against CID are distinct from the pathogens causing most infectious diseases in the modern world. Relaxing home hygiene would NOT increase our exposure to the protective Old Friends, but would increase exposure to the pathogens. The problem is not one of being too clean: it's one of reduced contact with the right kind of dirt.
3. We thus need to distinguish between routines associated with cleanliness, in the sense of absence of dirt, appearance, social acceptability and freshness, and those practices required to protect us from exposure to infectious disease. This indicates a need for clearer guidance about how to target hygiene practices effectively where and when they are required to reduce infectious disease risks.

The Old Friends hypothesis has unified the search for causes of allergies and other CIDs and provided a basis for further research and immunoregulatory therapy. The widened hypothesis should no longer be known simply as the 'hygiene' hypothesis. Like our environments, the historical meaning of 'hygiene' has lost much of its diversity in our era. In its former use, 'hygiene' involved the protection and promotion of health in all its aspects: 'public health' would be a modern, although less comprehensive, equivalent. We suggest that 'biodiversity' or 'Old Friends' are better names for the widened hypothesis and its implications for health and disease. Further aspects of the wide range of chronic inflammatory disorders need to be unravelled but for the present, it's three cheers for hygiene again.

APPENDIX I: OUTLINE OF CURRENT THEORY ON THE IMMUNE SYSTEM

This is a very brief outline explaining the basics components of the immune system and its regulation, to help non-immunologist readers with the terms and mechanisms referred to in this review. Readers are cautioned that this is a highly simplified summary of an immensely complex system. More details on individual concepts can be found using a Wikipedia search or in basic immunology texts[561] and a recent review article gives a good summary of recent research on the role of the commensal organisms in the immune system (Ackerman 2012[242]).

.

The structure of the immune system

The immune system is a complex network of organs (e.g. thymus, spleen), tissues (e.g. lymphatic system and bone marrow), white blood cells, antibodies, complement system and hormones. These act together to defend the body against invading organisms (bacteria, viruses, fungi, helminths), other foreign matter or cancer cells. The system has **innate (i.e. inborn) functions** (cells such as macrophages which digest foreign invaders such as microbes or dust) and **adaptive functions** (which allows it to respond to exposures). Adaptive immunity, also known as 'acquired immunity', is very specific to particular antigens and the response tends to increase with repeated exposure. Immunity can be strong or weak, long lasting or brief depending upon the type of antigen, its route into the body and genetically programmed responses.

Although these mechanisms are vital to protect the body against disease, they are also potentially dangerous to the host if they are not appropriately regulated: a simple analogy would be the need for military police to stop an army going on the rampage. Good health in these terms means a good balance between belligerent T cells and more tolerant regulatory T cells. Allergic diseases, such as asthma, occur when the body overreacts to foreign proteins such as pollen causing inflammatory responses such as sneezing and irritation. **Immune tolerance** is the process by which the body prevents itself from attacking the its own cells: this starts during pregnancy with exposure to 'self-molecules' (NIAID 2007[562]). **Autoimmune diseases** such as T1D and multiple sclerosis occur when the immune system overreacts against its own cells and tissues, and IBDs are at least in part due to inappropriate immune reactions to the contents of the gut..

Regulation of the immune system

Adaptive immunity involves two important types of white blood cells, known as **B and T cells or lymphyocytes**. T-cells are produced by the thymus: those often mentioned in relation to the hygiene hypothesis include Th1, Th2 and Th17 (see below and **Table** 3). B-cells (B-lymphocytes) are formed by stem cells in the bone marrow and produce antibodies in the form of immunoglobulins/ gammaglobulins in response to specific challenges, also through interactions with other cells, in particular the **T-helper cells** described below. Varieties of immunoglobulins include **IgG**, associated with antibodies against microbes, and **IgE**, associated with both parasitic infections and the symptoms of allergy, such as sneezing or skin rash.

The immune system is co-ordinated and regulated by T cells together with hormones generated independently by components of the immune system, which are known as **cytokines** (for example, interleukins). Cytokines have distinct functions such as acting as messengers or activating other cells. T cells arise from 'Naïve' T cells, activated in response to antigen challenges, a process known as **priming, conditioning or programming**. They mature into various T-cell types including**: helper T cells** (**Th1 and Th2** cells, the more recently identified **Th-17 cells** and **Treg cells** (Schmidt-Weber 2007[563], Mesquita 2009[564]).

Antigens are processed within the **APC (antigen presenting cells)** and fragments are expressed on the surface of these cells**. R**ecognition of the antigen activates Th cells to secrete

distinct sets of cytokines that mediate the immune response. These cells co-ordinate immune responses as shown in the examples in **Table 3.**

Since the last IFH review, refinements have been made to the immunological theory of how the immune system is maintained and regulated , in particular with a shift from the emphasis on concept of the Th1-Th2 balance to a more significant role for **regulatory T cells (Treg)** (as discussed in **Section 4.9**). The Th1-Th2 balance theory was based on the observation that allergy resulted from overzealous Th2 responses. But exaggerated Th1 responses can also produce disease, such as T1D, multiple sclerosis and Crohn's disease. Since establishment that both Th1 and Th2 cells are implicated in autoimmunity, the idea that these two subsets could regulate each other has been replaced by the theory that Treg cells have an 'immune management' role, determining how the system adapts to challenges. Release of regulatory cytokines from Treg cells (**Table 3**) suppresses other responses which leads to downregulation of autoimmunity, IBD and allergies. In the absence of the immunoregulation-inducing microbial stimuli discussed above, these immunoregulatory circuits are no longer adequately induced. **Toll-like receptors (TLRs)** are proteins that recognise molecules on microbes and form a first line of defence against foreign invasion by viruses or bacteria (NIAID 2007[565]. Their specificity allows them to detect the presence of infection and activate inflammatory and antimicrobial immune responses. Recognition of microbes by TLRs initiates immune responses.

Table 3. Important components of immunoregulation Note: this table contains only a few examples of the multiple functions performed by these cells, as considered relevant to this report.

	Naïve $CD4^+$ cells			
Type of T cell produced	T_H1	T_H2	T_H17	T_{Reg}
Examples of effector cytokines produced by T cell	IFN-γ, TNF-β	IL-4, IL-5	IL-17, IL-21, IL-22	IL-10, TGF-β
Examples of responses	Activation of phagocytes; antibody production	Production of IgE	Recruitment of leukocytes	Inhibition of inappropriate T cell activation & Th cell effector functions
Examples of pathological or other effects	Prevent clinical infection; autoimmunity e.g. T1D, MS	Type 1 hypersensitivity (IgE-mediated allergy, asthma) & autoimmunity (atopic dermatitis, ulcerative colitis); killing of helminths	Autoimmunity (psoriasis, multiple sclerosis) & IBD ((Crohn's disease)	Downregulation of autoimmunity, IBD & allergies

APPENDIX II. CHILDHOOD INFECTIONS AND SUBSEQUENT PROTECTION OR RISK RE: ATOPY IN EPIDEMIOLOGICAL STUDIES

Authors	Childhood infections studied	Source of information (questionnaire etc.)	Result
Bodner 1998[68]	Measles, Pertussis, Rubella, Mumps, Varicella	Parental questionnaire 'childhood infections' before and after age 3; serology HAV, H.pylori, toxoplasma (stored samples from older participants)	Measles before age 3 associated with lower risk adult asthma. No other protective association with wheezing or other symptoms of atopy in adulthood. Chronic cough/ phlegm more common for those seropositive for HAV or H.pylori and with increase in number of any infections.
Nafstad 2005[166]	Respiratory infections e.g. croup, including common cold (before 6 months) and ear infections	Parental questionnaire at ages 6 months to 10 years	No protective effect: increase in asthma symptoms by age 10 esp. after lower respiratory infections
Benn 2004[167]	Common cold, ear infection, diarrhoea, pneumonia	Parental interview for Infections before age 6 months.	Increased risk atopic dermatitis for any reported infection; risk increased with number of infections
McKeever 2002[169]	Maternal viral and bacterial infections requiring antibiotics during pregnancy, including respiratory, ear, eye and gastrointestinal infection and Candida (other causes not specified)	Medical records during pregnancy	Small increased risk of atopy in children following prenatal viral infection, including small dose-response relationship of increasing atopy with number of infections recorded
Law 2005[25]	HAV, H pylori + childhood infection exposure assumed from boarding school or number of siblings	Sera from blood samples taken at routine medical examinations of adult business men	Earlier cohorts less likely to show markers of atopy but no association with HAV, H.pylori or the assumed increased exposure to childhood infections.
Bremner 2008[169]	Upper respiratory, diarrhoea and vomiting, acute ear infection, measles, chicken pox, rubella, influenza, impetigo, lice, threadworm, 'viral rash' – total of 30 infections studied.	GP records for first year of life.	Bronchiolitis (upper resp. infection) associated with increased risk hayfever. Other infections associated with increased risk until adjustment made for consultation frequency.
Dunder 2007[171]	Common viral or bacterial respiratory, ear and enteric infections (not specified)	Parental questionnaire re: Hygiene intervention study in day care facility – symptoms such as rhinitis or diarrhoea recorded	Hygiene intervention reduced reports of infection but no affect on later allergic illness.
Sun & Sundell 2011[170]	Respiratory infection plus assumed higher infection exposure from day care attendance	Parental questionnaire	Day care associated both with increased respiratory infections and "allergic" symptoms
Nafstad 2005[166]	Respiratory and ear infections including croup in 1st year & common cold up to	Parental questionnaire at 6 months, 1 year and 4 years (Oslo Birth Cohort Study)	Early respiratory infection (esp. lower respiratory) did not protect against asthma or allergic rhinitis up to age 10.

Authors	Childhood infections studied	Source of information (questionnaire etc.)	Result
	6 mths; also assumed exposure to infection at day care or having older siblings		Increased risk of asthma at age 10 associated with early respiratory infection.
Bager 2002[175]	Maternal history of infections in childhood including measles, mumps, rubella, varicella (chickenpox)	School records for infections before school entry at age 7 in pregnant mothers participating in birth cohort study	Measles in first year of mother's life associated with higher risk of atopy (compared with those who had not had measles). No associations (protective or otherwise) with mumps, rubella or varicella
Bernsen & van der Wouden 2008[71]	Measles, mumps and rubella (in children not vaccinated with MMR vaccine, compared with non-vaccinated children)	Parental reports for children aged 8-12	Measles in MMR vaccine group predisposed to 'any' atopic disorders (particular eczema); rubella in unvaccinated children protective against eczema and food allergy. No other statistically significant associations.
Bernsen et al 2008[320]	Pertussis (whooping cough) in vaccinated and non-vaccinated children	Parental reports for children aged 8-12	No association between pertussis and atopy in the unvaccinated group, but children in the vaccinated group who caught pertussis appeared to be protected against atopic disorders, including asthma, hayfever and food allergy.
Gibb 2004[174]	'Definite' non-specified infection by age 3 mths or 1yr, gastrointestinal infections diagnosed by GP or reported by parent, immunisation with MMR or pertussis, bronchiolitis, chicken pox, ear infections, "coughs and colds", "minor infections" as reported by parent	Case control study children aged 1-5: parental questionnaire, GP records and salivary antibodies	Increased exposure to infection did not relate to risk of atopic dermatitis. Having older sibling was protective but this was not related to type or number of infections.

APPENDIX III: SUMMARY OF EPIDEMIOLOGICAL EVIDENCE RELEVANT TO THE HYGIENE HYPOTHESIS

Exposure/ factor	Main finding	Comment
Family size	Having 3 or more siblings protective for hayfever, less consistent for other types atopy	Not explained by specific infections, number of infections, or studies examining level of microbial exposure (usually based on symptoms or reported illness). Basis of the original formulation of the **Hygiene Hypothesis**
Prenatal exposures	Associated with both increased and decreased risk of CID depending on type of exposure	Exposures include family size, farms, maternal history of allergy etc. Part of the **prenatal programming/ fetal hypothesis**
Farming environment	Protective for several types atopy, requiring exposure from early infancy (or prenatally) up to adulthood	Specific exposure not identified but milk 'straight from the cow' protective against allergy. See also Endotoxins.
Urban environment	Higher levels allergy	Influence of culture, ethnicity, geographical area of origin all seem important but levels of hygiene have not been implicated in recent studies
Day nursery care	Higher risk clinical infection; no consistent protective effect against later allergy/ CID	A few studies have shown an adverse effect on risk of allergies. Similar effects for room sharing etc.
Migrant studies	Some migrants moving from rural to city environment are protected against allergies; first generation also at higher risk.	Some migrants may retain a protective microbial ecosystem in their diet, lifestyle and contacts.
Breast feeding	Protective against infections (6 months duration of BF), results inconsistent for allergy protection.	Role of microbiota on skin and in breast milk may play role in infection prevention – role in immune system regulation needs further research
Gut microbiota	Differs according to environment at birth	Diversity of microbiota may be the most important factor for any protective effect against allergy/ CID (The **microflora hypothesis**)
Prenatal exposure	Maternal microbial exposure of certain types may protect against allergy/CID	**'Fetal programming hypothesis'**
Infections		
Childhood infections	Measles, mumps, chicken pox etc have not been shown to explain the family size effect or to be otherwise protective against allergy/ CID	In general measured from self-reports, clinical diagnoses or serological evidence of past infection: subclinical/ asymptomatic infection much harder to measure but this could be the more important type of exposure. This exposure is most often cited re: the **reduced microbial exposure hypothesis.** A variant, the **fertile field hypothesis** relates to damage caused by viruses such as Coxsackie, which could lead to CID, particularly T1D.
Gastro-intestinal infections (in general-	In theory, particularly re: gut microbiota, gastrointestinal bacteria have an important role in priming the immune system	Includes all infections transmitted by the oral-faecal route.

Exposure/ factor	Main finding	Comment
specific GI infections listed below)		
Enteroviruses	A protective role has not been identified for GI viruses	Mostly causative but may depend on timing and type of exposure, e.g. early exposure could have a 'priming' effect on immune system
Hepatitis A (HAV)	HAV studies of a protective effect against allergy are inconclusive	Positive HAV serology may be a marker for 'traditional' rural or farming exposures in childhood.
Helicobacter pylori infection	Inverse (i.e. protective) relationship with allergy; acquisition later in life associated with pyloric ulcers & gastric cancer	Eradication may increase the risk of allergy while long-term effects of infection harmful: an important organism in the **Old Friends Hypothesis,** which suggests that this organism may have been harmless when co-existing in body with helminths.
Chronic helminth infection	Associated with lower risk of allergy, possibly several CIDs	Current research is exploring treatment of allergy and CIDs with small doses or extracts from helminths
Mycobacteria	OF hypothesis suggests that saprophytic strains (actinomyces) should be protective	Trials with low pathogenic strains of *Mycobacteria* have not shown a consistent protective effect, including BCG vaccination
Respiratory viral infections	Do not protect against atopy	Rhinoviruses and RSV may be particularly allergenic
Salmonellae	Possible evolutionary role	Share similar immune system stimulating characteristics with Mycobacteria (Matricardi 2010[150])
Staphylococci	Possible evolutionary role in maintaining 'balance' of skin flora	Skin microbiota may modulate early inflammatory response
Immunotherapy and antibiotics		
Probiotic therapy for allergy and CIDs	Slight, but not consistent, benefit in probiotics trials as yet	Differences in gut microbiota between developed and developing country environments suggest an important role, also relevant to the **Rapid Turnover hypothesis (**re: diversity and rapid turnover of microbiota as defence against both infection and allergy).
Antibiotics	Prescription in early childhood has been implicated as possible cause of later allergy	Confounders such as treatment for fever and the influence of the infection itself need to be unravelled
Non-microbial factors		
Pollution	Role in worsening allergy and CIDs, e.g. via traffic pollution, although no specific cause identified.	Seen mainly as a factor that triggers or worsens existing allergic responses/CID.
Diet	Vitamin D	**Vitamin D Hypothesis**
Obesity	Increased risk of allergies in obese children & children of obese mothers	**Lipid or Obesogenic hypothesis –** may help to explain the family size effect
Physical fitness	Associated with less risk CID	Importance of outdoor air exposure

APPENDIX IV: ORGANISMS CONSIDERED PROTECTIVE IN THE OLD FRIENDS' HYPOTHESIS

Organism or location	Disease or model or effect
• Gut microbiota	
Segmented filamentous bacteria	Th17 cells
Clostridia species	Treg in lamina propria
Bacillus fragilis	IL-10 and Treg
Faecalibacterium prausnitzii	Crohn's disease
• Faecal–oral transmission	
Helicobacter pylori	Allergies
Salmonella	Allergies
Toxoplasma	Allergies
–Viruses	
Enteroviruses	Allergies
Hepatitis A virus	Asthma
–Viruses	Protective if infected very early, but trigger disease if late
Coxsackievirus B	Type 1 diabetes
Rotavirus	Type 1 diabetes
• Helminths	
Many species	Allergies
Assorted natural infection	Multiple sclerosis (MS)
Trichuris trichiura	Multiple sclerosis (correlation)
Enterobius vermicularis	Type 1 diabetes (correlation)
Various species	Inflammatory bowel disease
–Animal models treated with helminths	
Heligmosomoides polygyrus	Allergy, T1D, colitis
Schistosoma mansoni	Allergy, T1D, EAE, colitis, arthritis
Strongyloides stercoralis	Allergy
Fasciola hepatica	Experimental autoimmune encephalitis (EAE)
Trichinella spiralis T1D,	EAE
Hymenolepsis diminuta	Colitis, arthritis
–Clinical trials with helminths	
Trichuris suis	Multiple sclerosis (MS)
Trichuris suis	Inflammatory bowel disease (IBD)
Necator americanus	Asthma
• Other natural microbial flora	
Skin flora; ammonia-oxidising bacteria	Nitrite, nitric oxide
Lung flora	Asthma
Oral and periodontal flora	Inflammatory bowel disease (IBD)
Gut organisms transported to breast milk ?	Immunoregulation
• Environmental saprophyte	
Mycobacterium vaccae	Allergy (mouse, dog)
• Ectoparasites	
Various	Response to TLR agonists in vitro

For details of these studies, please refer to Rook, 2011[17].

INDEX

acute lymphoblastic leukaemia. *See* ALL/ leukaemia
airway hyper-responsiveness, 24, 41
ALL, 14, 16
allergic disorders, 5, 9, 10, 11, 14, 15, 18, 27, 29, 30, 34, 35, 40, 42, 46, 52
ammonia-oxidising bacteria, 32, 34
anaphylaxis, 13
animal studies, 17, 23, 35
antibiotics, 7, 18, 25, 32, 33, 34, 46, 51, 67, 70
APC (antigen presenting cells), 65
asthma, 11, 12, 13, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 37, 39, 41, 42, 43, 46, 49, 52, 53, 54, 55, 59, 60, 65, 66, 67, 68
atopic dermatitis, 13, 17, 19, 20, 22, 26, 29, 40, 42, 52, 66, 67, 68
atopy, 5, 6, 10, 11, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 25, 26, 27, 28, 30, 33, 34, 35, 37, 41, 44, 46, 47, 49, 54, 55, 62, 67
autoimmune diseases. *See* CIDs
B. fragilis. *See* Bacteroides
Bacteroides, 25, 29, 34, 39, 40
BCG, 27, 30, 42, 70
Bifidobacteria, 25, 29, 34, 40
biodiversity, 5, 6, 7, 8, 10, 21, 28, 38, 57, 59, 64
biodiversity hypothesis, 6, 21, 38, 59
body mass index, 54
breastfeeding, 14, 16, 17, 26, 34
bronchiolitis, 27, 68
Caesarean section, 29, 34, 40
Campylobacter, 45, 62
carrier states, 33, 34
chemical products, 53
chemicals, 53, 61
chicken pox, 19, 20, 28, 35, 67, 68, 69
childhood infection, 16, 20, 21, 67
chlorination, 45
Cholera, 35
chronic inflammatory diseases. *See* CIDs
CIDs, 5, 6, 7, 8, 9, 11, 12, 13, 15, 16, 22, 30, 31, 33, 34, 35, 36, 39, 40, 41, 43, 44, 45, 46, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 70
city, 19, 25, 46, 52, 69
cleaning products, 44, 47
cleanliness, 5, 8, 9, 19, 44, 47, 48, 62, 63, 64
climate change, 55
common cold. *See* Rhinovirus
confounders (in epidemiological studies), 25, 26, 55, 56, 70
Coxsackie virus, 39, 40, 51, 53
Crohn's disease, 11, 12, 15, 26, 30, 41, 66, 71
cytokines, 9, 11, 26, 28, 66
day care, 6, 16, 20, 67
depression, 9, 11, 43
detergents, 32, 34, 47, 49
diabetes, 5, 9, 11, 15, 19, 23, 29, 53, 54, 65, 66, 69, 71
diarrhoea, 20, 21, 31, 49, 67
Dietzia, 35
diphtheria, 23, 35, 36, 46, 49, 50
dirt, 6, 7, 9, 61, 62, 64
diversity, 6, 8, 21, 25, 28, 29, 34, 38, 46, 48, 59, 64, 70
dust mites, 24, 28, 47, 62
E.coli, 25, 34, 47
ear infection, 20, 31, 67
eczema, 11, 12, 13, 14, 17, 19, 20, 22, 25, 26, 27, 29, 38, 40, 41, 42, 46, 49, 60, 62, 68
endemic diseases, 35
endotoxins,, 28
Enterobacter, 47
Enterococcus, 40, 47
environment of evolutionary adaptedness (EEA), 31
environmental organisms, 34, 42
environmental saprophytes, 32, 35, 43, 61
epidemics, 32, 36, 63
epidemiological transition, 31, 33, 35, 44
epigenetic effects, 35
Escherichia coli. *See* E.coli
family size, 6, 9, 14, 15, 20, 21, 31, 35, 54, 55, 58, 62, 69, 70
family structure, 14
farm effect, 6, 18, 24, 29, 34, 35, 39, 55
farming environment, 6, 17, 41
fertile field hypothesis, 39, 69
fetal programming (or fetal origins) hypothesis, 39
filth, 46

food allergy, 13
food poisoning, 36, 50, 51
food-borne pathogens, 36, 50, 51
gastroenteritis, 21, 36, 50
Gene-environment interactions, 11
Genetic susceptibility, 11, 55
gut commensal hypothesis, 41
gut commensals, 34, 40, 41
gut microbiota, 25, 29, 30, 32, 34, 37, 38, 41, 43, 46, 52, 54, 62, 69, 70
H.pylori, 16, 22, 24, 32, 33, 38, 41, 67
HAV, 21, 22, 23, 32, 33, 35, 36, 37, 38, 67, 70
hayfever, 9, 11, 12, 13, 14, 20, 21, 23, 24, 26, 30, 60, 67, 68, 69
HBV, 22, 23, 33
heirloom species, 32, 33, 34
Helicobacter pylori. *See* H.pylori
helminth, 6, 7, 21, 22, 26, 32, 33, 35, 36, 37, 39, 41, 43, 45, 51, 60, 70
high turnover and diversity hypothesis, 38
house dust mites, 28
human adaptation, 31
hypertension, 39
IBD, 9, 11, 13, 15, 26, 41, 42, 43, 45, 52, 66, 71
IgE, 11, 13, 18, 20, 22, 24, 26, 40, 66
immune dysregulation, 19, 25, 38, 42, 60
immune priming, 6, 21, 30, 36, 37, 38, 44, 59, 60
immune system, 5, 6, 9, 10, 11, 14, 19, 22, 23, 25, 26, 28, 29, 30, 31, 33, 34, 35, 37, 38, 39, 40, 43, 44, 46, 48, 52, 53, 55, 58, 59, 60, 65, 66, 69, 70
immunisation, 23, 30, 46, 68
immunocompromised, 35
Immunoglobulin E. *See* IgE
immunoregulation, 7, 8, 10, 19, 21, 22, 26, 28, 37, 38, 41, 43, 52, 60, 61, 63, 66
immunoregulators, 34
immunoregulatory disorders, 28
incidence, 11
inflammation, 11, 21, 23, 28, 39, 43, 54
inflammatory bowel disease. *See* IBD
Influenza, 27, 32, 33, 36, 63
inner cities, 18, 19
insect-borne infection, 38
Inuits, 18
Klebsiella, 47
Lactobacilli, 28, 34, 40
leukaemia, 16, 55
lifestyle, 5, 9, 10, 12, 15, 16, 18, 29, 33, 41, 49, 56, 57, 63, 64, 69
lipid hypothesis, 54
measles, 19, 20, 28, 32, 33, 35, 36, 49, 51, 67, 68
Measles-Mumps-Rubella (MMR) vaccination. *See* MMR
miasma, 46, 47
microbial deprivation, 9, 19, 59, 60
microbial hypothesis, 5, 9
microbial stimuli, 31, 66
microbiota, 6, 7, 17, 19, 24, 25, 27, 28, 29, 32, 33, 34, 37, 38, 43, 54, 63, 69, 70, 71
microflora hypothesis, 34, 69
migrant studies, 18, 19, 39, 69
milk, 13, 17, 18, 25, 31, 34, 38, 69, 71
MMR, 20, 21, 30, 68
morbidity, 36, 50
mortality, 13, 36, 49, 50, 55
MS, 11, 12, 13, 15, 16, 18, 26, 39, 41, 42, 43, 52, 54, 60, 61, 66, 71
multiple sclerosis, 5, 7, 9, 11, 19, 43, 65, 66
Mycobacteria, 27, 32, 35, 37, 59, 70
Neolithic period, 31, 32, 34, 35
non-gastrointestinal worms, 37
noroviruses, 33, 36
obesity, 7, 18, 29, 39, 54, 56, 57, 61, 63
obesity hypothesis, 54
OF hypothesis, 7, 30, 31, 33, 34, 35, 36, 37, 38, 44, 51, 52, 56, 58, 59, 60, 70
Old Friends, 5, 6, 7, 10, 26, 30, 33, 34, 35, 36, 37, 38, 40, 42, 43, 45, 56, 58, 60, 62, 63, 64, 70, 71
oral hygiene, 62
orofaecal transmission, 32, 33, 35, 38, 40, 42, 43, 51, 60, 61
osteoarthritis, 11
Paleolithic period, 6, 31, 32, 34, 51, 59, 63
pertussis, 30, 68
pets, 17, 28, 32, 47
pinworms, 32
pollution, 52, 56
poverty, 7, 18, 46, 55
prebiotics, 40
pregnancy, 7, 18, 19, 23, 30, 39, 49, 52, 54, 65, 67

prenatal exposure, 9, 17, 20, 39, 58, 67
prevalence, 11
probiotics, 7, 34, 40, 41, 70
Rapid Turnover hypothesis, 70
redundancy, 38, 39, 57
respiratory infection, 17, 20, 21, 25, 27, 31, 67
respiratory microbiota, 29, 62
Respiratory syncytial virus (RSV). *See* RSV
rhinoconjunctivitis, 12, 14, 20, 23, 41
rhinovirus, 17, 42
rodents, 18, 32, 46
RSV, 17, 27, 42, 70
rubella, 19, 20, 35, 67, 68
rural areas, 18, 45
rural environment, 16, 18, 25, 32, 33, 52
Salmonella, 21, 23, 32, 33, 36, 37, 50, 62, 71
sanitary revolution, 36, 40, 51, 52, 58, 60
sanitation, 5, 7, 8, 33, 35, 44, 45, 46, 51, 58, 61, 62, 64
saprophytic organisms, 35, 37, 42, 45, 58, 59, 70
sensitisation, 17, 23, 24, 27, 28, 41, 54
sewage, 45, 46
sewer, 45
sibling effect, 15
skin microbiota, 28, 29, 34, 51
soap, 26, 47, 49, 52
socioeconomic factors, 7, 12, 15, 18, 19, 23, 50, 55, 56, 63
soil, 27, 29, 31, 32, 34, 35, 37
Staphylococci, 28, 29, 32, 34, 36, 62, 70
Streptococci, 27, 28, 29
T1D, 9, 11, 12, 13, 15, 16, 19, 22, 23, 39, 43, 52, 61, 71
targeted hygiene, 8, 61, 64
TB, 35, 36, 42
Th1 immune responses, 27
Th1 lymphocytes, 42
Th2 lymphocytes, 42
thrifty phenotype hypothesis, 54
toilets, 45, 48
Toxoplasma, 21, 23, 32, 37
Toxoplasma gondii, 23, 33, 37
Treg, 38, 43, 65, 66, 71
trends in infectious disease, 7, 35
Trichuris suis, 41, 71
twin studies, 15, 27
type 2 diabetes. *See* diabetes and T1D
type1 diabetes. *See* T1D and diabetes
typhoid fever, 36, 45, 46, 50, 51
UC, 12, 15
ulcerative colitis, 11, 15, 26, 30, 41, 66, *See* UC
urban, 5, 6, 8, 10, 16, 18, 19, 21, 29, 31, 43, 52, 59, 61, 62, 63, See
urticaria, 13
vaccine, 20, 21, 30, 42, 68
Varicella zoster, 20
Vitamin D, 30, 41, 43, 52, 53, 54, 55, 56, 60, 70
washing, 5, 29, 48, 49, 52
water, 5, 7, 8, 21, 26, 31, 32, 35, 36, 37, 42, 44, 45, 46, 47, 48, 50, 51, 58, 61, 62, 64
whooping cough, 36, 49, 51, 68

REFERENCES

1 Stanwell- Smith R, Bloomfield SF. The Hygiene Hypothesis and the implications for hygiene. International Scientific Forum on Home Hygiene Available from: http://www.ifh-homehygiene.org/IntegratedCRD.nsf/111e68ea0824afe1802575070003f039/ce9bc2e0228ad9d480257522005b4748?OpenDocument

2 Bloomfield SF, Stanwell-Smith R, Crevel RWR, Pickup J. Too clean or not too clean: the Hygiene Hypothesis and home hygiene. Clin Exp Allergy 2006; 36(4):402-425.

3 Gerrard J, Geddes C, Reggin P. Serum IGE levels in white and metis communities in Saskatchewan. Ann Allergy 1976; 37:91-100.

4 Golding J, Peters TJ. Eczema and hay fever. In: Butler NR, Golding J, eds. From birth to five: a study of the health and behaviour of Britain's 5-year-olds. Oxford, UK: Pergamon Press, 1986; 171–186.

5 Strachan DP. Hay fever, hygiene and household size. Br Med J 1989; 299:1259-60.

6 Björkstén B. The hygiene hypothesis: do we still believe in it? In: Branzaeg P, Isolauri E, Prescott SL (eds): Microbial-Host Interaction: Tolerance versus Allergy. Nestlé Nutr Inst Workshop Ser Pediatr Program; vol 64: 11-22, Nestec Ltd, Vevey/S Karger AG, Basel: 2009

7 Rook GAW. 99th Dahlem Conference on Infection, inflammation and chronic inflammatory disorders: Darwinian medicine and the 'old friends' hypothesis. Clin Exper Immunol 2010; 160:70-79.

8 von Hertzen L, Hanski I, Haahtela T. Natural immunity: biodiversity loss and inflammatory diseases are two global megatrends that might be related. EMBO Reports 2011; 12(11):1089-93

9 Larson E. The 'hygiene hypothesis': how clean should we be? Am J Nursing 2002; 102(1): 81-89

10 Bloomfield S, Exner M, Fara GM, Scott EA. Prevention of the spread of infection – the need for a family-centred approach to hygiene promotion. Eurosurveillance 2008; 13(22):pii=18889. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18889

11 Salo PM. Exposure to multiple indoor allergens in U.S. homes and its relationship to asthma. J Allergy Clin Immunol 2008; 121:678-684.e2.

12 Heap GA, van Heel DA. The genetics of chronic inflammatory diseases. Hum Mol Genet 2009;18(R1): R101-R106 doi:10.1093/hmg/ddp001

13 Tan YD, Fornage M, George V, Xu H. Parent-child pair design for detecting gene-environment interactions in complex diseases. Hum Genet 2007; 121(6):745-57.

14 Weinberg CR. Less is more, except when less is less: Studying joint effects. Genomics 2009; 93(1):10-12.

15 AARDA (American Autoimmune Related Disease Association) and NCAPG (National Coalition of Autoimmune patient groups). The cost burden of autoimmune disease: the latest front in the

war on healthcare spending, AARDA, 2011. Report published online: http://www.aarda.org/pdf/cbad.pdf

16 Rook GA, Dalgleish A. Infection, immunoregulation and cancer. Immunol Rev 2011 Mar;240(1):141-59. doi: 10.1111/j.1600-065X.2010.00987.x.

17 Rook GA. Hygiene hypothesis and autoimmune diseases. Clin Rev Allergy Immunol 2011 Feb;42(1):5-15. doi: 10.1007/s12016-011-8285-8.

18 WGO (World Gastroenterology Organisation) Guidelines: Inflammatory bowel disease: a global perspective. June 2009. Available at: http://www.worldgastroenterology.org/inflammatory-bowel-disease.html (accessed July 2012)

19 Elliott DE, Summers RW, Weinstock JV. Helminths and the modulation of mucosal inflammation. Curr Op Gastroenterol 2005 21:51-8.

20 Ricklin-Gutzwiller ME, Reist M, Peel JE, Seewald W, et al. Intradermal injection of heat-killed Mycobacterium vaccae in dogs with atopic dermatitis: a multicentre pilot study. Vet Dermatol 2007; 18:87-93

21 Koch-Henriksen N, Sorensen PS. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol 2010; 9:520-32.

22 Wjst M. Introduction of oral vitamin D supplementation and the rise of the allergy pandemic. Allergy, Asthma & Clinical Immunology 2009; 5:8 doi:10.1186/1710-1492-5-8

23 Schadewaldt H. Geschichte der Allergie in vier Bänden. Dustri-Verlag 1980. [cited by Wjst 2009 op cit]

24 Jackson M. Allergy: The History of a Modern Malady. Reaktion Books: 2007.

25 Law M, Morris JK, Wald N, Luczynska C, Burney P. Changes in atopy over a quarter of a century, based on cross sectional data at three time periods. BMJ 2005; 21; 330(7501):1187–1188. doi: 10.1136/bmj.38435.582975.AE

26 Sibbald B, Rink E. Epidemiology of seasonal and perennial rhinitis: clinical presentation and medical history. Thorax 1991; 46(112):895-901

27 Janson C. The European Community Respiratory Health Survey: what are the main results so far? Eur Resp J 2001; 18:598–611.

28 Joint Task Force on Practice Parameters . The Diagnosis and Management of Rhinitis: An Updated Practice Parameter. J Allergy Clin Immunol 2008; 122:S1-S84.

29 McFadden ER. A century of asthma. doi: 10.1164/rccm.200402-185OE. Am J Respir Crit Care Med 2004;170(3):215-221.

30 Johansson SGO, Lundahl J. Asthma, atopy and IgE: what is the link? Curr Allergy Asthma Repts 2001; 1(2):89-90. doi: 10.1007/s11882-001-0071-x

31 Douwes J, Pearce N. Commentary: the end of the hygiene hypothesis? Int J Epidem 2008; 37:570-572. doi: 10.1093/ije/dyn077

32 Moncayo AL, Vaca M, Oviedo G, Erazo S et al. Asthma Risk factors for atopic and non-atopic asthma in a rural area of Ecuador. Thorax 2010;65:409-416 doi:10.1136/thx.2009.126490

33 Cserháti E. The history of bronchial asthma from the Renaissance till the beginning of the twentieth century. Acta Physiologica Hungarica 2005; 92(2):181-192. doi: 10.1556/APhysiol.92.2005.2.9

34 Centers for Disease Control and Prevention. National Surveillance for Asthma – United States, 1980-2004. Morbidity and Mortality Weekly Report. October 19, 2007; 56(SS08):1-14, 18-54.

35 Woodruff PG, Dolganov GM, Ferrando RE, Donnelly S, et al. Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression. Am J Respir Crit Care Med 2004;169:1001-6.

36 Wilson DH, Adams RJ, Tucker G, Appleton S, et al. Trends in asthma prevalence and population changes in South Australia, 1990–2003. MJA 2006; 184 (5):226-229

37 Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? Thorax 1999;54(3):268-72.

38 Asher MI, Montefort S, Björkstén B, Lai CK et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet 2006;368(9537):733-43.

39 Beasley R, Keil U, von Mutius E, Pearce N, for International Study of Asthma and Allergies in Childhood Steering Committee(ISAAC). Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 1988; 351:1225-32.

40 Pearce N, Aït-Khaled N, Beasley R, Mallol J, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). Thorax 2007;62(9):758-66.

41 Holt PG, Sly PD. Non-atopic intrinsic asthma and the 'family tree' of chronic respiratory disease syndromes. Clin Exp Allergy 2009; 39(6):807-11

42 Fleming DM, Sunderland R, Cross KW, Ross AM. Declining incidence of episodes of asthma: a study of trends in new episodes presenting to general practitioners in the period 1989-98. Thorax 2000; 55:657-61.

43 Gupta R, Sheikh A, Strachan DP, Anderson HR. Time trends in allergic disorders in the UK. Thorax 2007; 62(1):91-96

44 Akinbami LJ, Schoendorf KC. Trends in childhood asthma: prevalence, health care utilization, and mortality. Pediatrics 2002 Aug;110(2 Pt 1):315-22.

45 Anderson HR, Gupta R, Strachan DP, Limb ES. Review. 50 years of asthma: UK trends from 1955 to 2004. Thorax 2007;62:85-90 doi:10.1136/thx.2006.066407.

46 Ponsonby A-L, Glasgow N, Pezic A, Dwyer T, et al. A temporal decline in asthma but not eczema prevalence from 2000 to 2005 at school entry in the Australian Capital Territory with further consideration of country of birth. Int J Epidemiol 2008; 37(3):559-569.doi: 10.1093/ije/dyn029.

47 Wong GW, Leung TF, Ko FW, Lee KK, et al. Declining asthma prevalence in Hong Kong Chinese schoolchildren. Clin Exper Allergy 2004; 34(10):1550-5

48 American Lung Association Epidemiology and Statistics Unit. Research and Program Services Division. Trends in Asthma Morbidity and Mortality. July 2011. Available on line at: http://www.lungusa.org/finding-cures/our-research/trend-reports/asthma-trend-report.pdf

49 Lung and Asthma Information Agency. Trends in asthma mortality in Great Britain 97/3. http://www.laia.ac.uk/factsheets/973.pdf

50 Anandan C, Nurmatov U, van Schayck, OCP, Sheikh A. Is the prevalence of asthma declining? Systematic review of epidemiological studies. Allergy 2010; 65 (2):152-167

51 Leung DYM, NIcklas RA, Li JT, Bernstein L et al. Disease management of atopic dermatitis: An updated practice parameter. Ann Allergy Asthma Immunol 2004; 93:S1-S21.

52 Branum AM, Lukacs SL. Food allergy among U.S. children: Trends in prevalence and hospitalizations. NCHS data brief, no 10. Hyattsville, MD: National Center for Health Statistics. 2008.

53 Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: A 5-year follow-up study. J Allergy Clin Immunol 2003;112:1203–1207.

54 Arbes SJ. Prevalences of positive skin test responses to 10 common allergens in the U.S. population: Results from the Third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 2005; 116:377-383.

55 Gupta RS, Springston EE, Smith B, Warrier MR et al. Geographic variability of childhood food allergy in the United States. Clin Pediatr 2012, published online 17 May 2012. doi: 10.1177/0009922812448526. Online version at: http://cpj.sagepub.com/content/early/2012/05/14/0009922812448526

56 Decker W, Campbell, R, Manivannan, V, Luke A et al. The etiology and incidence of anaphylaxis in Rochester, Minnesota: A report from the Rochester Epidemiology Project. J Allergy Clin Immunol 2008;122:1161-5.

57 Ross MP, Ferguson M, Street D, Klontz K, et al. Analysis of food-allergic and anaphylactic events in the National Electronic Injury Surveillance System. J Allergy Clin Immunol 2008; 121(1):166-171.

58 Haby MM, Peat JK, Marks GB, Woolcock AJ, Leeder SR. Asthma in preschool children: prevalence and risk factors. Thorax 2001; 56(8):589-95.

59 Dik N, Tate RB, Manfreda J. Risk of physician-diagnosed asthma in the first 6 years of life. Chest 2004; 124:1147-1153.

60 McKeever TM, Lewis SA, Smith C. Siblings, multiple births and the incidence of allergic disease: a birth cohort study using the West Midlands general practice research database. Thorax 2001; 56:758-762

61 Goldberg S, Israeli E, Schwartz S, Shocat T. Asthma prevalence, family size and birth order. Chest 2007; 131(6):1747-1752

62 Altieri A, Castro F, Bermejo JL, Hemminki K. Number of Siblings and the Risk of Lymphoma, Leukemia, and Myeloma by Histopathology doi: 10.1158/1055-9965.EPI-06-0087 Cancer Epidemiol Biomarkers Prev July 2006 15(7); 1281-6.

63 Jarvis D, Chinn S, Luczynska C, Burney P. The association of family size with atopy and atopic disease. Clin Exp Allergy 1997; 27:240-45.

64 Bernsen RM, de Jongste JC, van der Wouden JC. Birth order and sibship size as independent risk factors for asthma, allergy and eczema. Pediatr Allergy Immunol 2003; 14:464-469

65 Karmaus W, Arshad H, Mattes J. Does the sibling effect has its origin in utero? Investigating birth order, cord blood immunoglobulin E concentration, and allergic sensitization at age 4 years. Am J Epidemiol 2001; 154:909-915

66 Devereux G, Barker RN, Seaton A. Antenatal determinants of neonatal immune response to allergens. Clin Exp Allergy 2002; 32(1):43-50

67 van Gool CJ, Thijs C, Dagnelie PC, Henquet CJM, et al. Determinants of neonatal IgE level: parity, maternal age, birth season and perinatal essential fatty acid status in infants of atopic mothers. Allergy 2004; 59:961-968.

68 Bodner C, Godden D, Seaton A. Family size, childhood infections and atopic disease. Thorax 1998; 53:28-32

69 Miyake Y, Tanaka K, Arakawa M. Sibling number and prevalence of allergic disorders in pregnant Japanese women: based on the Kyushu Okinawa Maternal and Child Health Study. BMC Public Health 2011; 11:561. doi:10.1186/1471-2458-11-561

70 Kinra S, Davey Smith G, Jeffreys M, Gunnell D, et al. Association between sibship size and allergic diseases in the Glasgow Alumni Study. Thorax 2006; 61(1):48-53.

71 Bernsen RM, van der Wouden JC. Association between sibship size and allergic diseases in the Glasgow Alumni Study (comment on Kinja 2006). Thorax 2006; 61(7):642.

72 Upchurch S, Harris JM, Cullinan P. Temporal changes in UK birth order and the prevalence of atopy. Allergy 2010; 65(8):1039-41

73 James WH. Multiple sclerosis and birth order. J Epidem Commun Health 1984; 38:21-22

74 Ajdacic-Gross V, Schmid M, Tschopp A, Gutzwiller F. Birth cohort effects in neurological disease: amyotrophic lateral sclerosis, Parkinson's disease and multiple sclerosis. Neuroepidemiology 2012; 38(1):56-63

75 Conradi S, Maizahn U, Schröter F, Friedemann P, et al. Environmental factors in early childhood are associated with multiple sclerosis: a case control study. BMC Neurology 2011; 11:123. doi:10.1186/1471-2377-11-123.

76 Lammi N, Moltchanova E, Blomstedt P, Eriksson P. The effect of birth order and parental age on the risk of type 1 and type 2 diabetes. Diabetologia 2007; 50(12):2433-8.

77 Sumnik Z, Drevinek P, Lanska V, Malcova H. Higher maternal age at delivery, and lower birth orders are associated with childhood type 1 diabetes mellitus. Exp Clin Endocrinol Diabetes 2004; 112(6):294-7.

78 Hugot JP, Cézard JP, Colombel FF, Belaicche J, et al. Clustering of Crohn's disease within affected sibships. European Journal of Human Genetics 2003; 11:179–184. doi:10.1038/sj.ejhg.5200932

79 Han DY, Fraser AG, Dryland P, Ferguson LR. Environmental factors in the development of chronic inflammation: a case control study on risk factors for Crohn's disease within New Zealand. Mutat Res 2010; 690(1-2):116-22.

80 Bager P, Nielsen NM, Frisch M, Wohifart J. Sibship characteristics and risk of multiple sclerosis: a nationwide cohort in Denmark. Am J Epidemiol 2006; 163(12):1112-7.

81 Sadovnick AD, Yee IM, Ebers GC; Canadian Collaborative Study Group. Multiple sclerosis and birth order: a longitudinal cohort study. Lancet Neurol 2005; 4(10):611-7

82 Whincup PH, Kaye SJ, Owen CG, Huxley R. Birth weight and risk of type 2 diabetes: a systematic review. JAMA 2008; 300(24):2886-97

83 Spehlmann ME, Begun AZ, Burghardt J, Lepage P. Epidemiology of inflammatory bowel disease in a German twin cohort: results of a nationwide study. Inflamm Bowel Dis 2008; 14(7):968-76

84 Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature 2011; 474(7351):307-317. doi: 10.1038/nature10209.

85 Karmaus W, Botezan C. Does a higher number of siblings protect against the development of allergy and asthma? A review. J Epidem Commun Health 2002; 56(3):209-217.

86 Louhiala PJ, Jaakkola N, Ruotsalainen R, Jaakkola JJ. Form of day care and respiratory infections among Finnish children. Am J Public Health 1995; 85(8 pt 1):1109-1112

87 von Mutius E, Fritzsch C, Weiland SK, Roll G, Magnussen H. Prevalence of asthma and allergic disorders among children in the united Germany: a descriptive comparison. BMJ 1992; 305:1395-9.

88 Krämer U, Heinrich J, Wjist M, Wichmann HE. Age of entry to day nursery and allergy in later childhood. Lancet 1999; 353:450-4.

89 de Meer G, Janssen NAH, Brunekreef B. Early childhood environment related to microbial exposure and the occurrence of atopic disease at school age Allergy 2005; 60:619-625.

90 Backman A, Björkstén F, Ilmonen S, Juntunen K, Suonemi I. Do infections in infancy affect sensitisation to airborne allergens and development of atopic disease? Allergy 1984; 39:309-15.

91 Infante-Rivard C, Amre D, Gautrin D, Malo JL. Family size, day-care attendance and breastfeeding in relation to the risk of childhood asthma. Am J Epidemiol 2001; 153(7):653-8.

92 Hagerhed-Engman L, Bornehag CG, Sundell J, Aberg N. Day-care attendance and increased risk for respiratory and allergic symptoms in preschool age. Allergy 2006; 61(4):447-53.

93 Caudri D, Wijga A, Scholtens S, Kerkhof M, et al. Early daycare is associated with an increase in airway symptoms in early childhood but is no protection against asthma or atopy at 8 years. Am J Resp Crit Care Med, 2009; 180:491-8. doi: 10.1164/rccm.20093-03270C

94 Wiemels J. Perspectives on the causes of childhood leukemia. Chem Biol Interact 2012; 196(3):59-67.[Epub Feb 2012 ahead of print]

95 Greaves M, Buffler PA. Infections in early life and risk of childhood ALL (Letter). Br J Cancer 2009; 100(5):863.

96 Gilham C, Peto J, Simpson J, Roman E, Eden TOB, et al. Day care in infancy and risk of childhood acute lymphoblastic leukaemia: findings from UK case-control study. BMJ 2005; 330(7503):1294-7. doi: 10.1136/bmj.38428.521042.8F

97 Ma X, Buffler PA, Selvin S, Matthay KK et al. Daycare attendance and risk of childhood acute lymphoblastic leukaemia. Br J Cancer 2002; 86(9):1419-24.

98 Li S, Jin X, Yan C, Wu S. Bed- and room- sharing in Chinese school-aged children: prevalence and association with sleep behaviours. Sleep Med 2008; 9(5):555-63

99 Li F, Zhou Y, Li S, Jiang F. Prevalence and risk factors of childhood allergic diseases in eight metropolitan cities in China: a multicenter study. BMC Public Health 2011; 11:437. doi:10.1186/1471-2458-11-437

100 Farrell S, Doherty GM, Milliken I, Shield MD, McCallion WA. Risk factors for Helicobacter pylori infection in children: an examination of the role played by intrafamilial bed sharing. Pediatr Infect Dis J 2005; 24(2):149-52.

101 Schwarz S, Morelli G, Kusecek B, Manica A, et al. Horizontal versus familial transmission of Helicobacter pylori. PLoS Pathog 2008; 4(10):e1000180.

102 Okuda M, Myashiro E, Koike M Okuda S, et al. Breast-feeding prevents Helicobacter pylori infection in early childhood. Pediatr Int 2001; 43(6):714-5.

103 Mahmud MA, Chappell CL, Hossain MM, et al. Impact of breast-feeding on Giardia lamblia infections in Bilbeis, Egypt. Am J Trop Hyg 2001; 65(3):57-60.

104 Ehlayel MS, Bener A, Abdulrahman HM. Protective effect of breastfeeding on diarrhea among children in a rapidly growing newly developed society. Turk J Pediatr 2009; 51(6):527-33.

105 Bulkow LR, Singleton RJ, Karron RA, Harrison LH; Alaska RSV Study Group. Risk factors for severe respiratory syncytial virus infection among Alaska native children. Pediatrics 2002; 109(2):210-6

106 Arifeen S, Black RE, Antelman G, Baqui A, et al. Exclusive breastfeeding reduces acute respiratory infection and diarrhea deaths among infants in Dhaka slums. Pediatrics 2001; 108(4):E67

107 Ip S, Chung M, Raman G, Chew P, et al. Breastfeeding and maternal and infant health outcomes in developed countries. Evid Rep Technol Assess 2007; 153:1-186.

108 Rönmark E, Jönsson E, Platts-Mills T, Lundbäck B. Different pattern of risk factors for atopic and non-atopic asthma among children – report from the Obstructive Lung Disease in Northern Sweden Study. Allergy 1999; 54:926-935.

109 Oddy WH. Breast feeding and childhood asthma. (Editorial). Thorax 2009;64:558-559. doi:10.1136/thx.2008.105130

110 Tanaka K, Miyake Y, Sasaki S. Association between breastfeeding and allergic disorders in Japanese children. Int J Tuberc Lung Dis 2010; 14(4):513-8.

111 Bener A, Ehlayel MS, Alsowaidi S, Sabbah A. Role of breast feeding in primary prevention of asthma and allergic diseases in a traditional society. Eur Ann Allergy Clin Immunol 2007; 39(10):337-43.

112 Ehlayel MS, Bener A. Duration of breast-feeding and the risk of childhood allergic diseases in a developing country. Allergy Asthma Proc 2008; 29(4):386-91

113 Sonnenschein-van der Voort AM, Jaddoe VW, Van der Valk RJ, Willemsen SP, et al. Duration and exclusiveness of breastfeeding and childhood asthma-related symptoms. Eur Respir J 2012 Jan;39(1):81-9. Epub 2011 Jul 20.

114 Kemp A, Kakakios A. Asthma prevention: breast is best? J Pediatr Child Health 2004; 40(7):337-9

115. Yang YW, Tsai CL, Lu CY. Exclusive breastfeeding and incident atopic dermatitis in childhood: a systematic review and meta-analysis of prospective cohort studies. Br J Dermatol 2009 Aug;161(2):373-83.

116 Stabell Benn C, Wohlfahrt J, Aaby P, Westergaard T, et al (i). Breastfeeding and risk of atopic dermatitis, by parental history of allergy, during the first 18 months of life. Am J Epidemiol 2004; 160(3):217-223

117 Flohr C, Nagel G, Weinmayr G, Kleiner A et al. Lack of evidence for a protective effect of prolonged breastfeeding on childhood eczema: lessons from the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Two. Br J Dermatol 2011; 165(6):1280-89. doi: 10.1111/j.1365-2133.2011.10588.x

118 Duncan JM, Sears MR. Breastfeeding and allergies: time for a change in paradigm? Curr Opin Allergy Clin Immunol 2008; 8(5):398-405

119 Jones CA, Holloway JA, Popplewell EJ, et al. Reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the subsequent development of atopy, eczema, or both. J Allergy Clin Immunol 2002; 109:858–866.

120 Jones CA. Maternal transmission of infectious pathogens in breast milk. J Paediatr Child Health 2001; 37(6):576-82.

121 Kawada M, Okuzumi K, Hitomi S, Sugishita C. Transmission of Staphylococcus aureus between health, lactating mothers and their infants by breastfeeding. Hum Lact 2003; 19(4):411-7

122 Chatzakis E, Scoulica E, Papageorgiou N, Maraki S. Infant colonization by Staphylococcus aureus: role of maternal carriage. Eur J Clin Microbiol Infect Dis 2011; 9(11):111-7

123 Michie C, Lockie F, Lynn W. The challenge of mastitis. Arch Dis Child 2003; 88(9):818-21.

124 Perez PF, Doré J, Leclerc M, Levenez F, et al. Bacterial imprinting of the neonatal immune system: lessons from maternal cells? Pediatrics 2007; 119: e724-32.

125 Von Mutius E. 99th Dahlem Conference on Infection, inflammation and chronic inflammatory disorders: Farm lifestyles and the hygiene hypothesis. Clinical and Experimental Immunology, 2010; 160:130-135

126 Braun-Fährlander C, Gassner M, Grize L, Neu U, et al. Prevalence of hay fever and allergic sensitisation in farmers' children and their peers living in the same rural community. Clin Exp Allergy 1999; 29:28-34.

127 Gassner-Bachman M, Wuthrich B. Farmers' children suffer less from hay fever and asthma. [in German, English abstract]. Dtsch Med Wochenschr 2000; 125:924-31.

128 von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, et al. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy 2000; 30(2):187-93.

129 Riedler J, Braun-Fährlander, Eder W, Shreuer M, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet 2001; 358:1129-33.

130 Radon K, Danuser B, Inversen M, Jörres R, et al. Respiratory symptoms in European animal farm workers. Eur Respir J 2001; 17:747-54.

131 Varraso R, Oryszczyn MP, Mathieu N, Le Moual N et al. Farming in childhood, diet in adulthood and asthma history. Eur Respir J 2012 39:67-75; published ahead of print 2011, doi:10.1183/09031936.00115010

132 Kramer MS, Matush L, Bogdanovich N, Dahhou M, et al. The low prevalence of allergic disease in Eastern Europe: are risk factors consistent with the hygiene hypothesis? Clin Exp Allergy 2009 May;39(5):708-16.

133 Remes ST, Iivanninen K, Koskela W, Pekkanen J. Which factors explain the lower prevalence of atopy amongst farmers' children? Clin Exp Allergy 2003; 33:427-34

134 Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? J Allergy Clin Immunol 2006; 117:1-374.

135 Zekveld C, Bibakis I, Biabaki-Liakou V, Pedioti A. The effects of farming and birth order on asthma and allergies. Eur Respir J 2006; 28(1):82-8

136 Downs SH, Marks GB, Mitakakis TZ, Lëuppi JD, et al. Having lived on a farm and protection against allergic disease in Australia. Clin Exp Allergy 2001; 331:570-5.

137 Wickens K, Lane JM, Fitzharris P, Siebers R, et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. Allergy 2002; 57:1171-9

138 Smit LA, Zuurbier M, Doekes G, Wouters IM, et al. Hay fever and asthma symptoms in conventional and organic farmers in the Netherlands. Occup Environ Med 2007; 117:1-374.

139 Monsó E, Schenker M, Radon K, Riu E, et al. Region-related risk factors for respiratory symptoms in European and Californian farmers. Eur Respir J 2003; 21:323-31.

140 Monsó E, Magarolas R, Radon K, Danuser B, et al. Respiratory symptoms of obstructive lung disease in European crop farmers. Am J Respir Crit Care Med 2000; 162:1246-50.

141 Stabell Benn C, Melbye M, Wolfahrt J, Björkstén B, Aaby P (ii). Cohort study of sibling effect, infectious disease and risk of atopic dermatitis during the first 18 months of life. BMJ 2004; 328:1223-26.

142 Aichbhaumik N, Zoratti EM, Strickler R, Wegienka G, et al. Prenatal exposure to household pets influences fetal immunoglobulin E production. Clin Exp Allergy 2008; 38:1787-94.

143 Simpson A. Effect of household pet ownership on infant immune response and subsequent sensitization. J Asthma Allergy 2010; 3:131–137. doi: 10.2147/JAA.S6958

144 Bergroth E, Remes S, Pekkanen J, Kauppila T, et al. Respiratory Tract Illnesses During the First Year of Life: Effect of Dog and Cat Contacts. Pediatrics 2012 Aug;. 130(2):211-20. Published online July 9 2012. doi: 10.1542/peds.2011-2825

145 Lauener RP, Birchler T, Adamski J, Braun-Fahrländer C, et al. Expression of CD 14 and Toll-like receptor 2 in farmers' and non-farmers' children. Lancet 2002; 360:465-6.

146 Ege MJ, Frei R, Bieli C, Schram-Bijerk D, et al. Not all farming environments protect against the development of asthma and wheeze in children. J Allergy Clin Immunol 2007; 119:1140-7

147 Ege MJ, Bieli C, Frei R, van Strien RT, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J Allergy Clin Immunol 2006; 117:817-23.

148 Schaub B, Liu J, Hoppler S, Schleich I, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J Allergy Clin Immunol. 2009; 123(4):774-82.e5.

149 Genuneit J. Exposure to farming environments in childhood and asthma and wheeze in rural populations: a systematic review with meta-analysis. Pediatr Allergy Immunol 2012 May 25. doi: 10.1111/j.1399-3038.2012.01312.x. [Epub ahead of print].

150 Matricardi PM. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: Controversial aspects of the 'hygiene hypothesis'. Clin Exp Immunol 2010; 160:98-105.

151 Cullinan P, Harris JM, Newman T, Taylor AJ, et al. Can early infection explain the sibling effect in adult atopy? Eur Respir J 2003; 22:956-61.

152 Krause T, Koch A, Friborg J, Poulsen LK, et al. Frequency of atopy in the Arctic in 1987 and 1998. Lancet 2002;360(9334):691-2

153 Andersen S, Mulvad G, Pedersen HS, Laurberg P. Gender diversity in developing overweight over 35 years of Westernization in an Inuit hunter cohort and ethno-specific body mass index for evaluation of body-weight abnormalities. Eur J Endocrinol 2004;151:735–740.

154 Fromont A, Binquet C, Sauleau EA, Fournel I, et al. Geographic variations of multiple sclerosis in

France. Brain 2010; 133:1889-1899.

155 Söderström U, Aman J, Hjern A. Being born in Sweden increases the risk for type 1 diabetes – a study of migration of children to Sweden as a natural experiment. Acta Paediatrica 2012; 101(1):73-77. Published online: 11 Aug 2011. doi: 10.1111/j.1651-2227.2011.02410.x

156 Okada H, Kuhn C, Feillet H, Bach J-F. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clin Exp Immunol 2010; 160(1):1-9.

157 Platts-Mills TAE, Woodfolk JA, Sporik RB. The increase in asthma cannot be ascribed to cleanliness. Am J Respir Crit Care Med 2001; 164:1107-8.

158 Platts-Mills TAE, Erwin E, Heymann P, Woodfolk J. Is the hygiene hypothesis still a viable explanation for the increased prevalence of asthma? Chest. 2005;127:1232-1241.

159 Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 2002; 347:911–20.

160 Werner S, Buser K, Kapp A, Werfel T. The incidence of atopic dermatitis in school entrants is associated with individual life-style factors but not with local environmental factors in Hannover, Germany. Br J Dermatol 2002; 147:95–104.

161 Patterson CC, Carson DJ, Hadden DR. Epidemiology of childhood IDDM in Northern Ireland 1989–1994: low incidence in areas with highest population density and most household crowding. Northern Ireland Diabetes Study Group. Diabetologia 1996; 39:1063–9.

162 Asher MI. Urbanisation, asthma and allergies. Thorax 2011; 66(12):1025-1026. doi: 10.1136/thoraxjnl-2011-201019

163 Weinmayr G, Forastiere F, Weiland SK, Rzehak P, et al. International variation in prevalence of rhinitis and its relationship with sensitisation to perennial and seasonal allergens. Eur Respir J 2008; 32(5):1250-61.

164 Flohr C, Weiland SK, Weinmayr G, Björkstén B, et al. The role of atopic sensitization in flexural eczema: findings from the International Study of Asthma and Allergies in Childhood Phase Two. J Allergy Clin Immunol 2008; 121(1):141-147.

165 Sevin CM, Peebles RS. Infections and asthma: new insights into old ideas. Clin Exper Allergy 2010; 40:1142-1154.

166 Nafstad P, Brunekreef B, Skrondal A, Nystad W. Early respiratory infections, asthma, and allergy: 10-year follow-up of the Oslo Birth Cohort. Pediatrics 2005; 116:e255–e262

167 Benn CS, Melbye M, Wohlfahrt J, Björkstén B, Aaby P. Cohort study of sibling effect, infectious diseases, and risk of atopic dermatitis during first 18 months of life. BMJ 2004; 328(7450):1223.

168 McKeever TM, Lewis SA, Smith C, Hubbard R. The importance of prenatal exposure on the development of allergic disease. A Birth cohort study using the West Midlands General Practice Database. Am J Resp and Crit Car Med 2002; 166(6):827-832 doi: 10:1164/rccm.200202-158OC.

169 Bremner SA, Carey IM, DeWilde S, Richards N. Infections presenting for clinical care in early life and later risk of hay fever in two UK birth cohorts. Allergy 2008; 63:274-283.

170 Sun Y, Sundell J. Early daycare attendance increases the risk for respiratory infections and asthma of children. Journal of Asthma 2011; 48(8):790-796. doi:10.3109/02770903.2011.604884

171 Dunder T, Tapianen T, Pokka T, Uhari M. Infections in child day care centres and later development of asthma, allergic rhinitis and atopic dermatitis: prospective follow-up survey 12 years after controlled randomized hygiene intervention. Arch Pediatr Adolesc Med 2007; 161(10):972-977.

172 Kim JS. Prediction, prevention and the hygiene hypothesis. Infections in child day care centres and later development of asthma, allergic rhinitis and atopic dermatitis: prospective follow-up survey 12 years after controlled randomized hygiene intervention. Pediatrics 2008; 122(8) Supp 4: S179-180

173 Silverberg JI, Kleiman E, Silverberg NB, Durkin HG, et al. Chickenpox in childhood is associated with decreased atopic disorders, IgE, allergic sensitization, and leukocyte subsets. Pediatr Allergy Immunol 2012 Feb;23(1):50-8. doi: 10.1111/j.1399-3038.2011.01224.x. Epub 2011 Oct 21.

174 Gibbs S, Surridge H, Adamson R, Cohen B, et al. Atopic dermatitis and the hygiene hypothesis: a case-control study. Int. J. Epidemiol. 2004; 33 (1):199-207. doi: 10.1093/ije/dyg267

175 Bager P, Westergaard T, Rostgaard K, Hjalgrim H, Melbye M. Age at childhood infections and risk of atopy. Thorax 2002;57:379-382 doi:10.1136/thorax.57.5.379

176 Shaheen SO, Aaby P, Hall AJ, Barker DJ, et al. Measles and atopy in Guinea-Bissau. Lancet 1996; 347:1792-6

177 Paunio M, Heinonen OP, Virtanen M, Leinikki P, et al. Measles history and atopic diseases: a population based cross-sectional study. JAMA 2000; 283(3):343-346.

178 Flölstrup H, Swartz J, Bergström A, Alm JS, et al. Allergic disease and sensitization in Steiner school children. J Allergy Clin Immunol 2006; 117(1):59-66

179 Hvlid A, Melbye M. Measles-Mumps-Rubella Vaccination and asthma-like disease in early childhood. Am J Epidemiol 2008; 168(11):1277-1283.

180 McDade TW, Rutherford J, Adair L, Kuzawa CW. Early origins of inflammation: microbial exposures in infancy predict lower levels of C-reactive protein in adulthood. Proc Bio Sci 2010; 277(1684):1129-37. doi: 10.1098/rspb.2009.1795

181 McDade T, Tallman PS, Madimenos FC, Liebert MA, et al. Analysis of variability of high sensitivity C-Reactive Protein in Lowland Ecuador reveals no evidence of chronic low-grade inflammation. Am J Hum Biol 2012; May (Epub ahead of print) doi 10.1002/ajhb.22296

182 Gurven M, Kaplan H, Crimmins E, Finch C, Winking J. Lifetime inflammation in two epidemiological worlds. J Gerontol Biol Sci 2008; 63A(2):196-9.

183 Hanski I, von Hertzen L, Fyhrquist N, Koskinen K, et al. Environmental biodiversity, human microbiota and allergy are interrelated. PNAS 2012; 109(21):8334-8339. www.pnas.org/cgi/doi/10.1073/pnas.1205624109

184 Ege KJ, Mayer M, Normand AC, Genueit J, et al. Exposure to Environmental Microorganisms and Childhood Asthma. N Engl J Med 2011; 364:701-709.

185 Sordillo JE, Hoffman EB, Celedón JC, Litonjua AA, et al. Multiple microbial exposures in the home may protect against asthma. Clin Exp Allergy 2010; 40(6):902-10.

186 Gern JE. Barnyard microbes and childhood asthma (Editorial) New Eng J Med 2011; 364(8):769-770.

187 Harris PR, Wright SW, Serrano C, Riera F, et al. Helicobacter pylori gastritis in children is associated with a regulatory T-cell response. Gastroenterology 2008; 134(2):491-9.

188 Greaves MW. Pathophysiology of chronic urticaria. Int Arch Allergy Immunol 2002;127:3–9

189 Hernando-Harder AC, Booken N, Goerdet S, Singer MV, Harder H. *Helicobacter pylori* infection and dermatologic diseases. Eur J Dermatol 2009;19:431–444.

190 Jarvis D, Lucynska C, Chinn S, Burney P. The association of HAV and Helicobacter pylori with sensitization to common allergens, asthma and hay fever in a population of young British adults. Allergy 2004; 59:1063-7.

191 Kosunen TU, Hook-Nikanne J, Salomaa A, Sarna S, et al. Increase in allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to Helicobacter pylori infections. Clin Exp Allergy 2002; 32:373-8.

192 Matricardi PM, Rosmini F, Riondino S, Fortini M, et al. Exposure to foodborne and orofaecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. BMJ 2000; 320:412-17.

193 Chen Y, Blaser MJ. Inverse associations of Helicobacter pylori with asthma and allergy. Arch Intern Med 2007; 167(8):821-7.

194 Chen Y, Blaser MJ. Helicobacter pylori colonization is inversely associated with childhood asthma. J Infect Dis 2008; 198(4):553-60.

195 Shiotani A, Miyanishi T, Kamada T, Haruma K. Helicobacter pylori infection and allergic diseases: epidemiological study in Japanese university students. J Gastroenterol 2008; 23:e29-e33.

196 Konturek PC, Rienecker H, Hahn EG, Raithel M. Helicobacter pylori as a protective factor against food allergy. Med Sci Monit 2008; 14:452-CR458

197 Zuel-Fakkar NM, Girgis SA. Study of Helicobacter pylori in children with atopic dermatitis. J Egyptian Women's Dermatologic Soc 2011; 8(1):17-20.

198 Amberbir A, Medhin G, Erku, W, Alem A, et al. Effects of *Helicobacter pylori*, geohelminth infection and selected commensal bacteria on the risk of allergic disease and sensitization in 3-

year-old Ethiopian children. Clin Exper Allergy 2011; 41:1422–1430. doi: 10.1111/j.1365-2222.2011.03831.x

199 Fullerton D, Briton JR, Lewis SA, Pavord ID, et al. *Helicobacter pylori* and lung function, asthma, atopy and allergic disease – a population-based cross-sectional study in adults. Int J Epidemiol 2009; 38:419-26.

200 Baccioglu A, Kalpaklioglu F, Guliter S, Yakarylimaz F. *Helicobacter pylori* in allergic inflammation – fact or fiction? Allergol Immunopathol (Madr) 2008; 36:85-9.

201 Tsang KW, Lam WK, Chan KN, Hu W, et al. Helicobacter pylori sero-prevalence in asthma. Respir Med 2000; 94:756-9.

202 Jun ZJ, Lei Y, Shimizu Y, Dobashi K, Mori M. *Helicobacter pylori* seroprevalence in patients with mild asthma. Tohoku J Exp Med 2005; 207:287-91.

203 Corrado G, Luzzi I, Pacchiarotti C, Lucarelli S, et al. *Helicobacter Pylori* seropositivity in children with atopic dermatitis as sole manifestation of food allergy. Pediatr Allergy Immunol 2000; 11:101–105.

204 Blaser MJ, Atherton JC. Helicobacter pylori persistence: biology and disease. J Clin Invest 2004; 113(3):321-333.

205 Matricardi PM, Rosmini F, Panella V, Ferrigno L, Bonini S. Hay fever and asthma in relation to markers of infection in the United States. J Allergy Clin Immunol 2002; 110:381-7.

206 Matricardi PM, Rosmini F, Rerrigno L, Nisini R, et al. Cross-sectional retrospective study of prevalence of atopy among Italian military students with antibodies against the HAV virus. Br Med J 1997; 314:999-1003.

207 Linneberg A, Østergaard C, Tvede M, Andersen LP, et al. IgG antibodies against microorganisms and atopic disease in Danish adults: the Copenhagen Allergy Study. J Allergy Clin Immunol 2003; 111:847-53.

208 Bodner C, Anderson WJ, Reid TS, Godden DJ. Childhood exposure to infection and the risk of adult onset wheeze and atopy. Thorax 2000; 55:383-7.

209 Umetsu D, McIntire JJ, DeKruyff RH. TIM-1, Hepatitis A Virus and the Hygiene Theory of Atopy: Association of TIM-1 with Atopy. J Pediatr Gastroent & Nutr 2005; 40:S43

210 Umetsu DT, DeKruyff RH. 99th Dahlem Conference on Infection, Inflammation and Chronic Inflammatory Disorders: Microbes, apoptosis and TIM-1 in the development of asthma. Clin Exp Immunol 2010; 160(1):125-129. doi: 10.1111/j.1365-2249.2010.04136.x

211 Cakir M, Akcay S, Karakas T, Gedik Y, et al. Prevalence of atopy in children with type 1 diabetes mellitus, hepatitis B virus carriers, and healthy children: role of T helper 1 (TH1)-type immune response. Allergy Asthma Proc 2008; 29(2):166-70.

212 Kocobas E, Yapicioglu H, Dincer Y, Kendirli S, et al. The prevalence of atopy in children with antibodies against hepatitis A virus and hepatitis B virus. Turk J Pediatr 2006; 48:189-196.

213 Friedrich N, Kramer A, Mentel R, Gürtler U, et al. No influence of atopic diseases on antibody titres following tetanus, diphtheria and hepatitis B immunisation among adults. Eur J Clin Microbiol Infect Dis 2007; 26:887-894.

214 Kocobas CN. Do hepatitis B virus carriers develop atopic diseases? Allergy 2001; 56(11):1100-1101.

215 Seiskari T, Kondrashova A, Viskari H, Kaila M, et al. Allergic sensitization and microbial load--a comparison between Finland and Russian Karelia. Clin Exp Immunol 2007;148(1):47-52.

216 Tracy S, Drescher KM, Jackson JD, Kim K, Kono K. Enteroviruses, type 1 diabetes and hygiene: a complex relationship. Rev Med Virol 2010; 20(2):106-16.

217 Oikarinen S, Martiskainen M, Tauriainen S, Huhtala H. Enterovirus RNA in blood is linked to the development of type 1 diabetes. Diabetes 2011; 60(1):276-9.

218 Stene LC, Oikarinen S, Hyöty H, Barriga KJ, et al. Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the Diabetes and Autoimmunity Study in the Young (DAISY). Diabetes 2010; 59(12):3174-80.

219 Graham GL, Sanders N, Tan Y, Allison J, et al. Rotavirus Infection Accelerates Type 1 Diabetes in Mice with Established Insulitis. J Virology 2008; 82(13):6139–6149.

220 Filippi CM, von Herrath MG. Viral trigger for type 1 diabetes: pros and cons. Diabetes 2008; 57:2863-2871.

221 Harrison LC, Honeyman MC, Morahan G, Wentworth JM, et al. Type 1 diabetes: lessons for other autoimmune diseases? J Autoimmun 2008 Nov;31(3):306-10.

222 Pelosi U, Porcedda G, Tiddia F. The inverse association of salmonellosis in infancy with allergic rhinoconjuntivitis and asthma at school-age: a longitudinal study. Allergy 2005; 60:626-30.

223 Janson C, Asbjornsdottir H, Birgisdottir A, Sigurjonsdottir RB, et al. The effect of infectious burden on the prevalence of atopy and respiratory allergies in Iceland, Estonia, and Sweden. J Allergy Clin Immunol 2007 Sep;120(3):673-9.

224 Michos A, Terzidis A, Kanarious M, Kalampoki V, et al. Association of allergic sensitisation with infectious disease burden in Roma and non-Roma children. Pediatr Allergy Immunol 2011; 22(2):243-248 doi: 10.1111/j.1399-3038.2010.01086.x

225 Alcantara-Neves J, Veiga RV, Dattoli VCC, Flaccone R, et al. The effect of single and multiple infections on atopy and wheezing in children. J Allerg Clin Immunol 2012;129(2):359-67. http://dx.doi.org/10.1016/j.jaci.2011.09.015 Epub 2011 Oct 27.

226 Martinez FD. CD14, endotoxin, and asthma risk: actions and interactions. Proc Am Thorac Soc 2007; 4(3):221-5

227 Delfino RJ, Staimer N, Tjoa T. Personal endotoxin exposure in a panel study of school children with asthma. Environ Health 2011; 10:69.

228 Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 2002; 347:869-77.

229 Douwes J, van Strien R, Doekes G, Smit J, et al. Does early indoor microbial exposure reduce the risk of asthma? The Prevention and Incidence of Asthma and Mite Allergy birth cohort study. J Allergy Clin Immunol 2006 May;117(5):1067-73.

230 Schram-Bijerk D, Doekes G, Boeve M, Riedler J, et al. Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children – the PARSIFAL study. Allergy 2005; 60:611-8.

231 van Strien RT, Englel R, Holst O, Bufe A, et al. Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health. J Allergy Clin Immunol 2004; 113:860-7.

232 Smit LA, Heederik D, Doekes G, Blom C, et al. Exposure-response analysis of allergy and respiratory symptoms in endotoxin-exposed adults. Eur Respir J 2008; 31:1241-8.

233 Portengen L, Preller L, Tielen M, Doekes G, Heederik D. Endotoxin exposure and atopic sensitization in adult pig farmers. J Allergy Clin Immunol 2005; 5:797-802.

234 Basinas J, Schlünssen V, Heederik D, Sigsgaard T, et al. Sensitisation to common allergens and respiratory symptoms in endotoxin exposed workers: a pooled analysis. Occup Environ Med 2012;69:99-106. doi:10.1136/oem.2011.065169

235 Korecka A, Arulampalam V. The gut microbiome: scourge, sentinel or spectator? J Oral Microbiol 2012, 4:9367, doi: 10:3402/jom.v4i0.9367

236 Berg RD. The indigenous gastrointestinal microflora. Trends Microbiol 1996; 4:430-435.

237 Martin R, Jimenez E, Heilig H, Fernandez L, et al. Isolation of Bifidobacteria from Breast Milk and Assessment of the Bifidobacterial Population by PCR-Denaturing Gradient Gel Electrophoresis and Quantitative Real-Time PCR. Appl Environ Microbiol 2009; 7(4):965-969

238 Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, et al. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 2000; 30:61-67.

239 Brandtzaeg P. Development of the mucosal immune system in humans. In: Bindels JG, Goedhart AC, Visser HKA, eds. Recent developments in infant nutrition. UK: Kluwer Academic Publishers; 1996:349-376.

240 Sudo N, Sawamura S, Tanaka K, Aiba Y, et al. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol 1997; 159:1739-1745.

241 Björkstén B. Evidence of probiotics in prevention of allergy and asthma. Curr Drug Targets Inflamm Allergy 2005; 4(5):599-604

242 Ackerman J. The ultimate social network: researchers who study the friendly bacteria that live inside all of us are starting to sort out who is in charge – microbes or people? Scientific American 2012; 306(6):37-43.

243 Chung H, Pamp SJ, Hill JA, Surana NK, et al. Gut immune maturation depends on colonization with a host-specific microbiota. Cell 2012, June 22; 149(7):1578-93. doi 10.1016/j.cell.2012.04.037

244 De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA 2010 107:14691-6.

245 Wang M, Karlsson C, Olsson C, Adlerberth I, et al. Reduced diversity in the early faecal microbiota of infants with atopic eczema. J Allergy Clin Immunol 2008; 121:129–34.

246 Bisgaard H, Li N, Bonnelykke K, Chawes BL, et al. Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. J Allergy Clin Immunol 2011; 128(3):646-72 doi:10.1016/j.jaci.2011.04.060

247 Forno E, Onderdonk AB, McCracken J, Litonjua AA, et al. Diversity of the gut microbiota and eczema in early life. Clin Molecular Allergy 2008; 6:11. doi:10.1186/1476-7961-6-11

248 Adlerberth I, Strachan DP, Matricardi PM, Ahrne S, et al. Gut microbiota and development of atopic eczema in 3 European birth cohorts. J Allergy Clin Immunol 2007; 120:343-50.

249 Matricon J. Immunopathogenesis of inflammatory bowel disease [article in French]. Med Sci (Paris) 2010; 26(4):405-10.

250 Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007; 448(7152):427-34.

251 Björkstén B, Naaber P, Sepp E, Mikelsaar M The intestinal microflora in allergic Estonian and Swedish 2-year -old children. Clin Exp Allergy 1999; 29:342-46.

252 Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005; 122:107-18

253 Risnes C R, Belanger K, Murk W, Bracken MB. Antibiotic exposure by 6 months and asthma and allergy at 6 years. Am J Epidemiol 2011; 173(3):310-318

254 Kozyrskyj AL, Ernst P, Becker AB. Increased risk of childhood asthma from antibiotic use in early life. Chest 2007; 131:1753-9

255 Celedon AC, Fuhlbrigge A, Rifas-shima S, Weiss ST, Finkelstein A. Antibiotic use in the first year of life and asthma in early childhood. Clin Exp Allergy 2004; 34:1011-6.

256 McDonnell Norms Group. Antibiotic overuse: the influence of social norms. J Am Coll Surg 2008; 207(2):265-75. doi:10:1016/h.jamcollsurg.2008.02.035. Available on line at: http://www.jsmf.org/about/s/smf-norms.pdf (accessed June 2012).

257 Paul IM, Maselli JH, Hersh AL, Boushey HA, et al. Antibiotic Prescribing During Pediatric Ambulatory Care Visits for Asthma. Pediatrics 2011; 127(6):1014-1021.

258 Penders J, Kummeling I, Thijs C. Infant antibiotic use and wheeze and asthma risk: a systematic review and meta-analysis. Eur Respir J 2011; 38(2):295-302.

259 Savage JH, Matsui EC, Wood RA, Keet CA. Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. J Allergy Clin Immunol 2012 Aug; 130(2):453-460. June epub ahead of print. doi: 10.1016/j.jaci.2012.05.006

260 Kummeling I, Stelma FF, Dagnelie PC, Snijders DEP, et al. Early life exposure to antibiotics and the subsequent development of eczema, wheeze and allergic sensitization in the first 2 years of life: the KOALA Birth Cohort Study. Pediatrics 2007; 119(1):225-231. doi: 10.1542/peds.2006-0896

261 Hill DA, Siracusa MC, Abt MC, Kim BS, et al. Commensal bacteria – derived signals regulate basophil hematopoiesis and allergic inflammation. Nature Medicine 2012; 18:538–546 doi:10.1038/nm.2657

262 Weinstock JV, Elliott DE. Helminths and the IBD Hygiene Hypothesis. Inflamm Bowel Dis 2009; 15(1):128-133.

263 Gale EA. A missing link in the hygiene hypothesis? Diabetologia. 2002; 45(4):588-94.

264 Rook GAW. Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. Immunology 2009; 126(1):3-11. doi:10.1111/j.1365-2567.2008.03007.x

265 Yazdanbakhsh M, Matricardi PM. Parasites and the hygiene hypothesis: regulating the immune system? Clin Rev Allergy Immunol 2004; 26:15-24.

266 Taylor ML, Le Goff A, Harris E, Malone JE, et al. Removal of regulatory T cell activity reverses hyporesponsiveness and leads to filarial parasite clearance in vivo. J Immunol 2005; 174:4924-4933.

267 Taylor MD, Harris A, Babayan A, Bain O, et al. CTLA-4^{+} and CD4^{+}CD25^{+} regulatory T cells inhibit protective immunity to filarial parasites in vivo. J Immunol 2007; 179:4626-4634.

268 Taylor MD, van der Werf N, Harris, A, Graham AL, et al. Early recruitment of natural CD4^{+}Foxp3^{+} regulatory T cells by infective larvae determines the outcome of filarial infection. Eur J Immunol 2009; 39:192-206.

269 Dittrich AM, Erbacher A, Specht S, Diesner F, et al. Helminth infection with *Litomosoides sigmodontis* induces regulatory T cells and inhibits allergic sensitization, airway inflammation, and hyperreactivity in a murine asthma model. J Immunol 2008;180:1792-1799.

270 Flohr C, Yeo L. Atopic dermatitis and the hygiene hypothesis revisited. Curr Probl Dermatol 2011; 41:1-34.

271 Mpairwe H, Webb EL, Muhangi L, Ndibazza J, et al. Antihelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. Pediatr Allergy Immunol 2011; 22:305-312.

272 Hilty M, Burke C, Pedro H, Cardenas P. Disordered microbial communities in asthmatic airways. PLoS One 2010; 5:e8578

273 Keski-Nisula I, Katila ML, Remes S, Heinonen S, Pekkanen J. Intrauterine bacterial growth at birth and risk of asthma and allergic sensitization among offspring at the age of 15-17 years. J Allergy Clin Immunol 2009; 123:1305-11.

274 Bisgaard H, Hermansen MN, Buchvald F, Loland L et al. Childhood asthma after bacterial colonisation of the airway in neonates. N Engl J Med 2007; 357:1487-95.

275 Eder W, Ege MJ, von Mutius E. The asthma epidemic. N Engl J Med 2006; 355(21):2226-35.

276 Hopkin JM. Atopy, asthma and the mycobacteria (Editorial). Thorax 2000; 55:443-445.

277 Obihara CC, Bollen CW, Beyers N, Kimpen JL. Mycobacterial infection and atopy in childhood: a systematic review. Pediatri Allergy Immunol 2007; 18(7):551-9.

278 Miyake Y, Arakawa M, Tanaka K, Sasaki S, Ohya Y. Tuberculin reactivity and allergic disorders in schoolchildren, Okinawa, Japan. Clin Exp Allergy 2008; 38(3):486-92.

279 Wu Q, Martin RJ, LaFasto S, Efaw BJ, et al. Toll-like receptor 2 down-regulation in established mouse allergic lungs contributes to decreased mycoplasma clearance. Am J Respir Crit Care Med 2008; 177:720-9.

280 Kraft M, Adler KB, Ingram JL, Crews AL, et al. *Mycoplasma pneumoniae* induces airway epithelial cell expression of MUC5AC in asthma. Eur Respir J 2008; 31:43-46.

281 Jackson DJ, Gangnon RE, Evans MD, Roberg KA, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med 2008; 178:667-72.

282 Miller EK, Edwards KM, Weinberg GA, Iwane MK, et al. A novel group of rhinoviruses is associated with asthma hospitalization. J Allergy Clin Immunol 2009; 123:98-104.

283 Lee KK, Hegele RG, Manfreda J, Wooldrage K, et al. Relationship of early childhood viral exposures to respiratory symptoms, onset of possible asthma and atopy in high risk children: the Canadian asthma primary prevention study. Pediatr Pulmonol 2007; 42:290-7.

284 Riese RJ, Finn PW, Shapiro SD. Influenza and asthma: adding to the respiratory burden. Nat Immunol 2004; 5:243-244. doi:10.1038/ni0304-243.

285 Bordon Y. Asthma and allergy: influenza virus and an innate form of asthma. Nat Rev Immunol 2011; 11:443/ doi:10.1038/nri3013

286 Wu P, Dupont WD, Griffin MR, Carroll KN, et al. Evidence of a causal role of winter virus infection during infancy in early childhood asthma. Am J Respir Crit Care Med 2008; 178:1123-9.

287 Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J Respir Crit Care Med 2000; 161:1501-7

288 Thomsen SF, van der Sluis S, Stensballe LG, Posthuma D et al. Exploring the association between severe respiratory syncytial virus infection and asthma: a registry-based twin study. Am J Respir Crit Care Med 2009; 179:1091-7.

289 Kuehni CE, Spycher BD, Silverman M. Causal links between RSV infection and asthma: no clear answers to an old question. Am J Resp Crit Care Med 2009; 179:1079-80.

290 Koponen P, Helminen M, Paassilta M, Luukkaala T, Korppi M. Preschool asthma after bronchiolitis in infancy. Eur Respir J 2012 39:76-80; published ahead of print 2011, doi:10.1183/09031936.00040211

291 Brussee JE, Smit HA, van Strien RT, Corver K, et al. Allergen exposure in infancy and the development of sensitization, wheeze, and asthma at 4 years. J Allergy Clin Immunol 2005; 115(5):946-52.

292 Simpson A. Effect of household pet ownership on infant immune response and subsequent sensitization. J Asthma Allergy 2010; 3:131-137. doi: 10.2147/JAA.S6958

293 Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. Eur Respir J 2006; 27:615-26.

294 von Hertzen LC, Savolainen J, Hannuksela M, Klaukka T, et al. Scientific rationale for the Finnish Allergy Programme 2008–2018: emphasis on prevention and endorsing tolerance. Allergy 2009; 64:678–701. doi: 10.1111/j.1398-9995.2009.02024

295 Grice E, Kong HH, Conlan S, Deming CB, et al. Topographical and temporal diversity of the human skin microbiome. Science 2009; 324:1190-92.

296 Lai Y, Di Nardo A, Nakatsuji T, Leichtie A, et al. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. Nat Med 2009 Dec;15(12):1377-82.

297 Capone KA, Dowd SE, Stamatas GN, Nikolovski J. Diversity of the human skin microbiome early in life. J Invest Dermatol 2011; 131(10):2026–2032. doi: 10.1038/jid.2011.168

298 Dotterud LK, Wilsgaard T, Vorland LH, Falk ES. The effect of UVB radiation on skin microbiota in patients with atopic dermatitis and healthy controls. Int J Circumpolar Health 67:2-3 2008

299 Dahl MV. Staphylococcus aureus and atopic dermatitis. Arch Dermatol 1983;119:840-846.

300 Mancini AJ, Kaulback K, Chamlin SL. The socioeconomic impact of atopic dermatitis in the United States: a systematic review. Pediatr Dermatol 2008; 25:1–6.

301 Callard RE, Harper JI. The skin barrier, atopic dermatitis and allergy: a role for Langerhans cells? Trends Immunol 2007; 28(7):294-8.

302 Spergel JM, Paller AS. Atopic dermatitis and the atopic march. J Allergy Clin Immunol 2003; 112(6 Suppl):S118-27.

303 Benn CS, Thorsen P, Jensen JS, Kjaer BB, et al. Maternal vaginal microflora during pregnancy and the risk of asthma hospitalization and use of antiasthma medication in early childhood. J Allergy Clin Immunol 2002; 110(1):72-7.

304 Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol 2011; 9:244-53.

305 Huffnagle GB. The Microbiota and Allergies/Asthma. PLoS Pathog 2010; 6(5): e1000549. doi:10.1371/journal.ppat.1000549

306 Noverr, MC, Noggle RM, Toews GB, Huffnagle GB. Role of Antibiotics and Fungal Microbiota in Driving Pulmonary Allergic Responses. Infection and Immunity 2004; 72(9):4996–5003 doi: 10.1128/IAI.72.9.4996–5003.2004

307 Blaser MJ. Who are we? Indigenous microbes and the ecology of human diseases. EMBO Rep 2006; 7(10):956–960. doi: 10.1038/sj.embor.7400812

308 Decker E, Hornef M, Stockinger S. Caesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. Gut Microbes 2011; 2(2):91-98. Accessed June 2012 at: http://www.landesbioscience.com/journals/gutmicrobes/DeckerGMIC2-2.pdf

309 Ly, NP. Conference presentation on Caesarean section and allergy. Am Thoracic Society 23 May 2008.

310 Ly NP, Ruiz-Pórez B, Onderdonk AB, Tzianabos AO, et al. Mode of delivery and cord blood cytokines: a birth cohort study. Clin Mol Allergy 2006;4:13. doi: 10.1186/1476-7961-4-13

311 Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? Diabetes Care 2010; 33(10):2277-2284. Accessed 25 June 2012 at: http://care.diabetesjournals.org/content/33/10/2277.full

312 Pistiner M, Gold DR, Abdulkerim H, Hoffman E, Celedón JC. Birth by cesarean section, allergic rhinitis and allergic sensitization among children with a parental history of atopy. J Allergy Clin Immunol 2008; 122(2):274-9.

313 Algert CS, Bowen JR, Lain SL, Allen HD, et al. Pregnancy exposures and risk of childhood asthma admission in a population birth cohort. Pediatr Allergy Immunol 2011; 22(8):836-42. doi: 10.1111/j.1399-3038.2011.01206.x

314 Koppen S, de Groot R, Neijens HJ, Nagelkerke N,et al. No epidemiologic evidence for infant vaccinations to cause allergic disease. Vaccine 2004; 22(25-26):3375-3385.

315 Sanchez-Solis M, Garcia-Marcos L. Do vaccines modify the prevalence of asthma and allergies? Expert Rev Vaccines 2006; 5(5):631-640.

316 Rottem M. Asthma prevalence and exacerbations in children: is there an association with childhood vaccination? Expert Rev Clin Immunol 2008;4(6):687-694. doi:10.1586/1744666X.4.6.687.

317 Cates CJ, Jefferson TO, Rowe BH. Vaccines for preventing influenza in people with asthma. Cochrane Database Syst Rev 2008; (2):CD000364.

318 Minne A, Jaworska J, Gerhold K, Ahrens B, et al. Intranasal delivery of whole influenza vaccine prevents subsequent allergen-induced sensitization and airway hyper-reactivity in mice. Clin Exp Allergy 2007; 37(8):1250–1258 .

319 Bernsen RM, van der Wouden JC. Measles, mumps and rubella infections and atopic disorders in MMR-unvaccinated and MMR-vaccinated children. Pediatr Allergy Immunol 2008; 19(6):544-51.

320 Bernsen RM, Nagelkerke NJ, Thijs C, van der Wouden JC. Reported pertussis infection and risk of atopy in 8- to 12-yr-old vaccinated and non-vaccinated children. Pediatr Allergy Immunol 2008;19(1):46-52.

321 Balicer RD, Grotto I, Mimouni M, Mimouni D. Is childhood vaccination associated with asthma? A meta-analysis of observational studies. Pediatrics 2007; 120(5): e1269-e1277. doi: 10.1542/peds.2006-3569.

322 Björkstén B. Diverse microbial exposure – Consequences for vaccine development. Vaccine 2012 Jun 19; 30(29):4336-40. Epub Nov 2011. http://dx.doi.org/10.1016/j.vaccine.2011.10.074

323 Rook GA, Adams V, Hunt J, Palmer R, et al. Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. Springer Semin Immunopathol 2004;25(3-4):237-55.

324 Caldwell JC. Population health in transition. Bull World Health Organ 2001; 79:1159-60.

325 Armelagos GJ, Brown PJ, Turner B. Evolutionary, historical and political economic perspectives on health and disease. Soc Sci Med 2005; 61:755-65

326 Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? Nat Rev Microbiol 2009; 7:887-894

327 Shreiner A, Huffnagle GB, Noverr MC. The 'Microflora Hypothesis' of allergic disease. Adv Exp Med Biol 2008; 635:113-134, doi: 10.1007/978-0-387-09550-9_10

328 Whitlock DR, Feelisch M. Soil bacteria, nitrite and the skin. In: Rook GAW ed. The Hygiene Hypothesis and Darwinian Medicine. Series: Progress in Inflammation Research. Birkhäuser: Basel 2009, XII, 308, pp103-116. ISBN: 978-3-7643-8902-4

329 Rook GAW, Martinelli R, Brunet LR. Innate immune responses to mycobacteria and the downregulation of atopic responses. Curr Opin Allergy Clin Immunol 2003; 3:337-42.

330 Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature 1996; 383:787-93.

331 Herz U, Gerhold K, Gruber C. BCG infection suppresses allergic sensitization and development of increased airway reactivity in an animal model. J Allergy Clin Immunol 1998; 102:867-74

332 Zuany-Amorim C, Sawicka E, Manlius C, Le Moine A, et al. Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-Cells. Nat Med 2002; 8:625-9.

333 Gagneux S, Small PM. Global phylogeography of *Mycobacterium tuberculosis* and implications for tuberculosis product development. Lancet Infect Dis 2007; 7:328–37.

334 von Mutius E, Pearce N, Beasley R, Cheng S, et al. International patterns of tuberculosis and the prevalence of symptoms of asthma rhinitis, and eczema. Thorax 2000; 55:449-53.

335 Aiello A E, Larson EL, Sedlak R (i). The health revolution: medical and socioeconomic advances Am J Infect Control 2008; 36:16-27. doi:10.1016/j.ajic.2008.09.007

336 Velimirovic B. Infectious diseases in Europe- a fresh look. Copenhagen: WHO, 1984.

337 Monto AS, Sullivan KM. Acute respiratory illness in the community: frequency of illness and the agents involved. Epidemiol Infect 1993;110:145-60.

338 Parliamentary Office of Science and Technology, UK. Food poisoning. The Parliamentary Office of Science and Technology, London: 2003, no:13. http://www.parliament.uk/business/publications/research/briefing-papers/POST-PN-193

339 Tam CC, Rodrigues LC, Viviani L, Dodds JP, et al. IID2 Study Executive Committee. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut 2012;61(1):69-77. Epub 2011 Jun 27.

340 CDC Norovirus: surveillance, disease burden and disease reduction activities. http://www.cdc.gov/norovirus/php/reporting.html

341 Lindesmith L, Moe C, Marionneau S, Ruvoen N, et al. Human susceptibility and resistance to Norwalk virus infection. Nat Med 2003; 9 (5):548–53. doi:10.1038/nm860

342 Soriano-Gabarró M, Mrukowicz J, Vesikari T, Verstraeten T. Burden of rotavirus disease in European Union countries. Pediatr Infect Dis J. 2006; 25:S7-S11.

343 Hayward A, Knott F, Petersen I, Livermore DM, et al. Increasing hospitalizations and general practice prescriptions for community-onset staphylococcal disease, England. Emerging Infectious Diseases [serial on internet]. 2008 May [*cited June 2012*]. Available from http://wwwnc.cdc.gov/eid/article/14/5/07-0153.htm

344 Von Hertzen LC. Puzzling associations between childhood infections and the later occurrence of asthma and atopy. Ann Med 2000; 32:397-400.

345 Pelosi U, Porcedda G, Tiddia F, Tripodi S, et al. The inverse association of salmonellosis in infancy with allergic rhinoconjunctivitis and asthma at school-age: a longitudinal study. Allergy 2005; 60:626–30.

346 Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunuol 2009; 9:313-324.

347 Armelagos GJ. The paleolithic disease-scape, the hygiene hypothesis and the second epidemiological transition. In: GAW Rook (Ed): The Hygiene hypothesis and Darwinian Medicine. Series: Progress in Inflammation Research. Birkhäuser, Basel 2009, XII, 308. ISBN: 978-3-7643-8902-4

348 Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? Allergy 1998; 53:20-5.

349 Wang M, Karlsson C, Olsson C, Adlerberth I, et al. Reduced diversity in the early faecal microbiota of infants with atopic eczema. J Allergy Clin Immunol 2008; 121:129–34.

350 Björkstén B, Naaber P, Sepp E, Mikelsaar M, et al. The intestinal microflora in allergic Estonian and Swedish 2-year -old children. Clin Exp Allergy 1999; 29:342-46.

351 Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 2008; 453:620–5.

352 Olszak T, An D, Zeissing S, Pinilla Vera M, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science 2012; 336(6080):489-93. http://www.sciencemag.org/content/early/recent 22 March 2012 / Page 1/ 10.1126/science.1219328

353 Vassallo M, Walker W. Neonatal Microbial Flora and Disease Outcome. In: Barker, D.J.P. (Southampton) (eds): The Window of Opportunity: Pre-Pregnancy to 24 Months of Age. Basel, Karger, 2008, vol 61, pp 211-224 (doi: 10.1159/000113496)

354 Coppieters KT, Wiberg A, Tracy SM, von Herrath MG, et al. The Role of Viruses in Type 1 Diabetes: A Difficult Dilemma. Clin Exper Immunol 2012; 168(1):5-11. doi: 10.1111/j.1365-2249.2011.04554.x

355 Barker DJ. The developmental origins of chronic adult disease. Acta Paediatr 2004; Suppl 93:26–33

356 Brand S, Teich R, Dicke T, Harb H, et al. Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. J Allergy Clin Immunol 2011; 128 (3):618-625.e7.

357 Kaplan JL, Shi HN, Walker WA. The Role of Microbes in Developmental Immunologic Programming. Pediatric Research 2011; 69:465–472; doi:10.1203/PDR.0b013e318217638a.

358 Holt PG, Strickland DH. Soothing signals: transplacental transmission of resistance to asthma and allergy. J Exp Med 2009; 206(13):2861-2864. www.jem.org/cgi/doi/10.1084/jem.20092469

359 Forrester T. Historic and early life origins of hypertension in Africans. J Nutr 2004; 134:211–216.

360 Eifan AO, Akkoc T, Yildiz A, Keles S, et al. Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial. Clin Exp Allergy 2010; 40:922–932.

361 Sewell WA, Thomas WR. Immunotherapy of allergic diseases by bacterial products. Immunol Cell Biol 2011; 89:749-750; doi: 10.1038/icb.2011.24.

362 Prescott SL, Björkstén B. Probiotics for the prevention or treatment of allergic diseases. J Allergy Clin Immunol 2007; 120:255-62.

363 Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. PNAS 2010; 107(27):12204-12209

364 Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. J Allergy Clin Immunol 2009; 123:335-41.

365 Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, et al. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. J Allergy Clin Immunol 2007; 119:192-8.

366 Taylor A, Dunstan J, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitisation in high risk children: a randomised controlled trial. J Allergy Clin Immunol 2007; 119:184-91.

367 Thomas DW, Greer FR. Probiotics and prebiotics in pediatrics. Pediatrics 2010; 126(6):1217-31.

368 Noguerira JCR, Goncalves M da CR. Probiotics in allergic rhinitis. Braz J Otorhinolarynogol 2011; 77(1):129-34.

369 Ly NP, Litonjua A, Gold DR, Celedón JC. Gut microbiota, probiotics and vitamin D: interrelated exposures influencing allergy, asthma and obesity? J Allergy Clin Immunol 2011; 127(5):1087-1094.

370 Mortimer K, Brown A, Feary J. Dose-ranging study for trials of therapeutic infection with *Necator americanus* in humans. Am J Trop med Hyg 2006; 75:914-20.

371 Blount D, Hooi D, Feary J, Venn A. Immunologic profiles of persons recruited for a randomized, placebo-controlled clinical trial of hookworm therapy. Am J Trop Med Hyg 2009; 81(5):911-916.

372 Summers RW, Elliott DE, Urban JF, Jr., Thompson R, Weinstock JV (i). Trichuris suis therapy in **Crohn's disease**. Gut 2005 54:87-90.

373 Summers RW, Elliott DE, Urban JF, Jr., Thompson RA, Weinstock JV (ii). Trichuris suis therapy for active **ulcerative colitis**: a randomized controlled trial. Gastroenterology 2005; 128:825-32.

374 Fleming J, Isaak A, Lee J, Luzzio C, et al. Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. Mult Scler 2011 17:743-54.

375 Correale J, Farez M. Association between parasite infection and immune responses in multiple sclerosis. Ann Neurol 2007 61:97-108.

376 Arnold IC, Dehzad N, Reuter S, Martin H, et al. Helicobacter pylori infection prevents allergic asthma in mouse models through the induction of regulatory T cells. J Clin Invest 2011;121(8):3088–3093. doi:10.1172/JCI45041.

377 Smit JJ, Folkerts G, Nijkamp FP. Mycobacteria, genes and the 'hygiene hypothesis'. Curr Opin Allergy Clin Immunol 2004; 4:57-62

378 Berth-Jones J, Arkwright PD, Marasovic D, Savani N, et al. Mycobacterium vaccae suspension in children with moderate to severe atopic dermatitis: a randomized, double-blind, placebo-controlled trial. Clin Exp Allergy 2006; 36:115-1121

379 Marro A, Pirles M, Schiaffino L, Bin L, et al. Successful immunotherapy of canine flea allergy with injected Actinomycetales preparations. Immunotherapy 2011;3(8):971-978. doi:10.2217/imt.11.93

380 Erb KJ, Holloway JW, Sobeck A, Moll H, Le Gros G. Infection of mice with Mycobacterium bovis Bacillus Calmette-Guérin (BCG) suppresses allergen-induced airway eosinophilia. J Ex Med 1998; 187:561-569.

381 Arkwright PD, David TJ. Intradermal administration of a killed *Mycobacterium vaccae* suspension (SRL 172) is associated with improvement in atopic dermatitis in children with moderate-to-severe disease. J Allergy Clin Immunol 2001; 107:531-4.

382 Camporota L, Corkhill A, Long H, Lordan J, et al. The effects of *Mycobacterium vaccae* on allergen-induced airway responses in atopic asthma. Eur Respir J 2003; 21(2):287-93.

383 Barlan IB, Bahceciler N, Akdis M, Akdis CA. Role of bacillus Calmette-Guérin as an immunomodulator for the prevention and treatment of allergy and asthma. Curr Opin Allergy Clin Immunol 2005;5(6):552-7.

384 Alm JS, Lilja G, Pershagen G, Scheynius A. Early BCG vaccination and development of atopy. Lancet 1997; 350:400-3.

385 Aaby P, Shaheen SO, Heyes CB, Goudiaby A et al. Early BCG vaccination and reduction in atopy in Guinea-Bissau. Clin Exp Allergy 2000; 30(5):644-50.

386 Omenaas E, Jentoft HF, Vollmer WM, Buist AS, Gulsvik A. Absence of relationship between tuberculin reactivity and atopy in BCG vaccinated adults. Thorax 2000; 55:454-8.

387 Krishna MT, Salvi SS. Could administration of bacille Calmette-Guérin vaccination at birth protect from the development of asthma and allergic diseases in the western world? Has this question been adequately investigated? Pediatr Allergy Immunol 2002;13(3):172-6.

388 Cohon A, Arruda LK, Martins MA, Guilherme L, Kalil J. Evaluation of BCG administration as an adjuvant to specific immunotherapy in asthmatic children with mite allergy. J Allergy Clin Immunol 2007; 120:210–213.

389 Steenhuis TJ, van Aalderen WM, Bloksma N, Nijkamp FP, et al. Bacille-Calmette-Guerin vaccination and the development of allergic disease in children: a randomized, prospective, single-blind study. Clin Exp Allergy 2008 Jan;38(1):79-85.

390 El-Zein M, Parent ME, Benedetti A, Rousseau MC. Does BCG vaccination protect against the development of childhood asthma? A systematic review and meta-analysis of epidemiological studies. Int J Epidemiol 2010; 39:469–486.

391 Click RE. Successful treatment of asymptomatic or clinically terminal bovine *Mycobacterium avium* subspecies *paratuberculosis* infection (Johne disease) with the bacterium Dietzia used as a probiotic alone or in combination with dexamethasone: Adaption to chronic human diarrheal diseases. Virulence Mar/Apr 2011; 2(2):131-143.

392 Yoo J, Tcheurekdjian H, Lynch SV, Cabana M, Boushey HA. Microbial manipulation of immune function for asthma prevention: inferences from clinical trials. Proc Am Thorac Soc. 2007; 4:277-82.

393 Umetsu DT, DeKruyff RH. Microbes, apoptosis and TIM-1 in the development of Asthma. Clin Exp Immunol 2010; 160:125-9.

394 van den Biggelaar AH, Rodrigues LC, van Ree R, van der Zee JS, et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 2004; 189:892-900.

395 Stene LC, Nafstad P. Relation between occurrence of type 1 diabetes and asthma. Lancet 2001 357:607

396 Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 2002; 347:911-20.

397 Rook GAW, Stanford JL. Give us this day our daily germs. Immunol Today 1998; 19:113-6.

398 Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease; revisiting the hygiene hypothesis. Nature Reviews 2001; 1:69-75.

399 Grainger JR, Smith KA, Hewitson JP, McSorley HJ, et al. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-beta pathway. J Exp Med 2010; 207:2331-41.

400 Round JL, Lee SM, Li J, Tran G, et al. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 2011; 332:974-7.

401 Smits HH, Engering A, van der Kleij D, de Jong EC, et al. Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. J Allergy Clin Immunol 2005; 115:1260-7.

402 Le Bert N, Chain BM, Rook G, Noursadeghi M. DC Priming by *M. vaccae* inhibits Th2 responses in contrast to specific TLR2 priming and is associated with selective activation of the CREB Pathway. PLoS One. 2011 6(4):e18346. doi:10.1371/journal.pone.0018346.

403 Akdis M, Verhagen J, Taylor A, Karamloo F, et al. Immune Responses in Healthy and Allergic Individuals Are Characterized by a Fine Balance between Allergen-specific T Regulatory 1 and T Helper 2 Cells. J Exp Med 2004; 199:1567-75.

404 Perez-Machado MA, Ashwood P, Thomson MA, Latcham F, et al. Reduced transforming growth factor-beta1-producing T cells in the duodenal mucosa of children with food allergy. Eur J Immunol 2003; 33:2307-15.

405 Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. J Exp Med 2004; 199:971-9.

406 Kriegel MA, Lohmann T, Gabler C, Blank N et al. Defective suppressor function of human CD4+ CD25+ regulatory T cells in autoimmune polyglandular syndrome type II. J Exp Med 2004 199:1285-91.

407 Ferraro A, Socci C, Stabilini A, Valle A, et al.. Expansion of Th17 cells and functional defects in T regulatory cells are key features of the pancreatic lymph nodes in patients with type 1 diabetes. Diabetes 2011 60:2903-13.

408 Makita S, Kanai T, Oshima S, Uraushihara K, et al. CD4+CD25bright T cells in human intestinal lamina propria as regulatory cells. J Immunol 2004; 173:3119-30.

409 Veltkamp C, Anstaett M, Wahl K, Moller S, et al. Apoptosis of regulatory T lymphocytes is increased in chronic inflammatory bowel disease and reversed by anti-TNFalpha treatment. Gut 2011 60:1345-53.

410 Osada Y, Kanazawa T. Parasitic helminths: new weapons against immunological disorders. J Biomed Biotechnol 2010; 2010:743758. doi: 10.1155/2010/743758

411 Correale J, Farez MF. The impact of parasite infections on the course of multiple sclerosis. J Neuroimmunol 2011; 11(51):233-6.

412 Raison CL, Lowry CA, Rook GAW. Inflammation, sanitation and consternation: Loss of contact with co-evolved, tolerogenic micro-organisms and the pathophysiology and treatment of major depression. Arch Gen Psychiat 2010; 67:1211-24.

413 Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med 2001; 344:961-6.

414 MP Ravenel, ed., A Half Century of Public Health: Jubilee Historical Volume of the American Public Health Association (New York American Public Health Association, 1921).

415 Aiello AE, Larson EL, Sedlak R (ii). Hidden heroes of the health revolution: Sanitation and personal hygiene. Am J Infect Control 2008;36:S128-51. doi:10.1016/j.ajic.2008.09.008

416 WHO/ UNICEF. Drinking water: equity, safety and sustainability. 2011.WHO/UUNICER Joint Monitoring Programme for Water Supply and Sanitation. http://www.wssinfo.org/fileadmin/user_upload/resources/report_wash_low.pdf

417 European Food Standards Agency. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2008. Available at: http://www.efsa.europa.eu/en/scdocs/doc/1496.pdf.

418 Molodecky NA, Kaplan GG. Environmental risk factors for inflammatory bowel disease. Gastroenterol Hepatol (NY) 2010 May; 6(5):339–346

419 Baron S, Turck D, Leplat C, Merle V, et al. Environmental risk factors in paediatric inflammatory bowel diseases: a population based case control study. Gut 2005; 54:357-363. doi:10.1136/gut.2004.054353

420 Aiello AE, Larson EL, Sedlak R (iii). The good old days? Disease, despair and dying young. Am J Infec Control 2008; 36(10 Suppl):S111-5.

421 Working Party of the British Society for Antimicrobial Chemotherapy. Working Party Report: Hospital antibiotic control measures in the UK. J Antimicrob Chemotherapy 1994; 34:21-42. http://jac.oxfordjournals.org/content/34/1/21.full.pdf+html

422 Hamad B. The antibiotics market. Nat Rev Drug Discov 2010; 9:675-676 doi:10.1038/nrd3267

423 Spyridis N, Sharland M. The European Union Antibiotic Awareness Day: the paediatric perspective. Arch Dis Child 2008;93:909-910 doi:10.1136/adc.2008.149625

424 Foliaki S, Nielsen SK, Björkstén B, Von Mutius E, et al. ISAAC Phase I Study Group. Antibiotic sales and the prevalence of symptoms of asthma, rhinitis, and eczema: The International Study of Asthma and Allergies in Childhood (ISAAC). Int J Epidemiol 2004; 33(3):558-63.

425 Hartley D. Water in England. Publisher: MacDonald and Jane's; First Thus edition (1978) ISBN-10: 0354040480/ ISBN-13: 978-0354040488

426 Fleck A. Technology and Its Social Consequences, in A History of Technology, Volume 5: The Late 19th Century. London: Oxford University Press; 1958.

427 Greene VW. Cleanliness and the health revolution. New York: The Soap and Detergent Association, 1984.

428 Bloomfield SF. 2012. The chain of infection transmission in the home and everyday life settings, and the role of hygiene in reducing the risk of infection. http://www.ifh-homehygiene.org/IntegratedCRD.nsf/111e68ea0824afe1802575070003f039/9df1597d90588986802572970061709 3?OpenDocument

429 Josephson KL, Rubino JR, Pepper IL. Characterization and quantification of bacterial pathogens and indicator organisms in household kitchens with and without the use of a disinfectant cleaner. J Appl Microbiol 1997; 83:737-50

430 Ojima M, Toshima Y, Koya E, Ara K, et al. Hygiene measures and microorganisms in Japanese households. J Appl Microbiol 2002; 93:800–809.

431 Scott, EA., Bloomfield, SF, Barlow, CG. An investigation of microbial contamination in the domestic environment. J Hygiene 1982; 89:279-293.

432 Judah G, Donachie P, Cobb E, Schmidt W. Dirty hands: bacteria of faecal origin on commuters' hands. Epidemiol Infect 2010; 138:409-414.

433 Brasche S, Bischof W. Daily time spent indoors in German homes – baseline data for the assessment of indoor exposure of German occupants. Environ Health 2005; 208(4):247-53.

434 Scott EA, Bloomfield SF, Barlow CG. Evaluation of disinfectants in the domestic environment under 'in use' conditions. J Hygiene 1984;92:193-203.

435 Rusin P, Orosz-Coughlin P, Gerba C. Reduction of faecal coliform, coliform and heterotrophic plate count bacteria in the household kitchen and bathroom by disinfection with hypochlorite cleaners. J Appl Microbiol 1998; 85:819-828.

436 Cogan TA, Bloomfield SF, Humphrey TJ. The effectiveness of hygiene procedures for the prevention of cross contamination from chicken carcasses in the domestic kitchen. Lett Appl Microbiol 1999; 29:354-358.

437 Cogan TA, Slader J, Bloomfield SF, Humphrey TJ. Achieving hygiene in the domestic kitchen: the effectiveness of commonly used cleaning procedures. J Appl Microbiol 2002; 92:885-892.

438 Barker J, Vipond IB, Bloomfield SF. Effects of cleaning and disinfection in reducing the spread of Norovirus contamination via environmental surfaces. J Hosp Infec 2004; 58:42–49

439 Kembel SW, Jones E, Kline J, Northcutt D, Stenson J et al. Architectural design influences the diversity and structure of the built environment microbiome. The ISME Journal 2012:1–11

440 Flores GE, Bates ST, Knights D, Lauber CL, et al. Microbial Biogeography of Public Restroom Surfaces. PLoS ONE 2011; 6(11): e28132. doi:10.1371/journal.pone.0028132

441 Sherriff A, Golding J and the ALSPAC study team (i). Factors associated with different hygiene practices in the homes of 15 month old infants. Arch Dis Child 2002; 86:30-35.

442 Sherriff A, Golding J and the ALSPAC study team (ii). Hygiene levels in a contemporary population cohort are associated with wheezing and atopic eczema in preschool children. Arch Dis Child 2002; 87(1):26-9

443 Font-Ribera L, Villanueva CM, Nieuwenhuijsen MJ, Zock JP, et al. Swimming Pool Attendance, Asthma, Allergies and Lung Function in the ALSPAC Child Cohort. Am J Respir Crit Care Med 2011; 183:582-588

444 Miyake Y, Ohya Y, Tanaka K, Yokoyama T, et al. Home environment and suspected atopic eczema in Japanese infants: the Osaka Maternal and Child Health Study. Pediatr Allergy Immunol 2007; 18(5):425-32.

445 De Benedetto A, Kubo A, Beck LA. Skin barrier disruption: a requirement for allergen sensitization? J Invest Derm 2012; 132:949-963. doi:10.1038/jid.2011.435; epub 5 Jan 2012

446 Stelmach I, Smejda K, Jerzynska J, et al. Decreased markers of atopy in children with presumed early exposure to allergens, unhygienic conditions, and infections. Ann Allergy Asthma Immuol 2007; 99(2):170-177.

447 Hicks J, Allen A. A Century of Change: Trends in UK statistics since 1900 House of Commons Library research paper. http://www.parliament.uk/documents/commons/lib/research/rp99/rp99-111.pdf

448 Schluger NW. The Acute Respiratory Infections (ARIA) Atlas, 1st edition 2010. World Lung Foundation, New York. ISBN: 978-1-4507-3262-8 Available online at: http://www.ariatlas.org/tools/downloads/files/ARIA.pdf

449 Monto AS. Viral respiratory infections in the community: epidemiology, agents and interventions. Am J Med 1995; 99(6B):24S.

450 Grüber C, Kell T, Kulig M, Roll S. History of respiratory infections in the first 12 yr among children from a birth cohort. Pediatr Allergy Immunol 2008; 19(6):505-12.

451 Fendrick AM, Monto AS, Nightingale B, Sarnes M. The economic burden of non-influenza related viral respiratory tract infection in the United States. Arch Intern Med 2003; 163:487-494.

452 Edelsberg J, Taneja C, Zervos M, Haque N, et al. Trends in US hospital admissions for skin and soft tissue infections. Emerg Infect Dis [serial on the Internet]. 2009 Sep; 15(9) (cited June 2012). Available from http://wwwnc.cdc.gov/eid/article/15/9/08-1228.htm doi: 10.3201/eid1509.081228

453 Afsar FS. Skin infections in developing countries. Curr Opin Pediatr 2010; 22(4):459-66.

454 Fairweather D, Rose NR. Women and autoimmune diseases. Emerg Infect Dis [serial on the Internet]. 2004 November [accessed June 2012]. Available from: http://wwwnc.cdc.gov/eid/article/10/11/04-0367.htm

455 Bach J-F. Infections and autoimmune diseases. J Autoimmunity 2005;25:74-80.

456 Paccagnini D, Sieswerda L, Rosu V, Masala S, et al. (2009) Linking Chronic Infection and Autoimmune Diseases: *Mycobacterium avium* Subspecies *paratuberculosis*, *SLC11A1* Polymorphisms and Type-1 Diabetes Mellitus. PLoS ONE 4(9): e7109. doi:10.1371/journal.pone.0007109

457 D'Amato G, Liccardi G, D'Amato M. Environmental risk factors (outdoor air pollution and climatic changes) and increased trend of respiratory allergy. J Investig Allergol Clin Immunol 2000; 10(3):123-128.

458 Rios JLM, Boechat JL, Sant'Anna CC, França AT. Atmospheric pollution and the prevalence of asthma: study among schoolchildren of 2 areas in Rio de Janeiro, Brazil. Ann Allergy Asthma Immunol 2004; 92:629-634.

459 Lødrup Carlsen KC. The Environment and Childhood Asthma (ECA) study in Oslo: ECA-1 and ECA-2. Pediatr Allergy Immunol 2002;13(suppl):29-31.

460 Charpin D, Pascal L, Birnbaum J, Armengaud A, et al. Gaseous air pollution and atopy. Clin Exp Allergy 1999; 29:1474-1480; doi: 10.1046/j.1365-2222.1999.00685.x.

461 Strachan DP. (b) The role of environmental factors in asthma. Br Med Bull 2000; 56:865-882.

462 Pandya RJ, Solomon G, Kinner A, Balmes JR. Diesel exhaust and asthma: hypotheses and molecular mechanisms of action. Environ Health Perspect 2002 Feb;110 Suppl 1:103-12.

463 Gilmour MI. Influence of air pollutants on allergic sensitization: the paradox of increased allergies and decreased resistance to infection. Toxicol Pathol Jan 2012; 40(2):312-4.

464 Baïz B, Slama R, Bene MC, Charles MA, et al. Maternal exposure to air pollution before and during pregnancy related to changes in newborns cord blood lymphocyte subpopulations. The EDEN study cohort. BMC Pregnancy Childbirth 2011;11(1):87.

465 Bernstein DL. Traffic-related pollutants and wheezing in children. J Asthma 2012 Feb;49(1):5-7. Epub 2012 Jan 3.

466 Jeong SH, Kim JH, Son BK, Hong SC, et al. Comparison of air pollution and the prevalence of allergy-related diseases in Incheon and Jeju City. Korean J Pediatr 2011 Dec;54(12):501-6.

467 DeWitt JC, Peden-Adams MM, Keller JM, Germolec DR. Immunotoxicity of perfluorinated compounds: recent developments. Toxicol Pathol 2012; 40(2):300-311.

468 Putman E, van Loveren H, Bode G, Dean J, et al. Assessment of the immunotoxic potential of human pharmaceuticals: a workshop report. Drug Inf J 2002; 36:417-427.

469 Galloway T, Handy R. Immunotoxicity of organophosphorous pesticides. Ecotoxicology 2003; 12(1-4):345-63.

470 Seo M, Kobayashi R, Nagase H. Immunotoxic effects of trichloroethylene and tetrachloroethylene. J Health Sci 2011; 57(6):488-496.

471 Carpenter DO. Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. Rev Environ Health 2006 Jan-Mar;21(1):1-23.

472 Abedi-Valugerdi M, Nilsson C, Zargari A, Gharibdoost F et al. Bacterial lipopolysaccharide both renders resistant mice susceptible to mercury-induced autoimmunity and exacerbates such autoimmunity in susceptible mice. Clin Exp Immunol 2005;141(2):238-47.

473 Vehik K, Hamman RF, Lezotte D, Norris JM et al. Trends in high-risk HLA susceptibility genes among Colorado youth with type 1 diabetes. Diabetes Care 2008 Jul;31(7):1392-6.

474 Funseth E, Wicklund-Glynn A, Friman G, Ilbäck N. Redistribution of accumulated 2,3,7,8-tetrachlorodibenzo-p-dioxin during coxsackievirus B3 infection in the mouse. Toxicol Lett 2000 Jul 27;116(1-2):131-41.

475 Feingold BJ, Vegosen L, Davis M, Leibler J, et al. A niche for infectious disease in environmental health: rethinking the toxicological paradigm. Environ Health Perspect 2010; 118(8):1165-72.

476 Sherriff A, Farrow A, Golding J, the ALSPAC Study Team, Henderson J. Frequent use of chemical household products is associated with persistent wheezing in pre-school age children. Thorax 2005; 60:45-49. Doi: 10.1136/thx.2004.021154.

477 Henderson J, Sherriff A, Farrow A, Ayres JG. Household chemicals, persistent wheezing and lung function: effect modification by atopy? Eur Respir J 2008; 31(3):547-54

478 Franklin P. Household chemicals: good housekeeping or occupational hazard? Eur Resp J 2008; 31(3):489-91

479 Kim J-H, Ellwood PE, Asher MI. Diet and asthma: looking back, moving forward. Resp Research 2009; 10:49 doi: 10.1186/1465-9921-10-49

480 Gao J, Gao X, Li W, Zhu Y, Thompson PJ. Observational studies on the effect of dietary antioxidants on asthma: a meta-analysis. Respirology 2008; 13(4):528-536.

481 Devereux G. Early life events in asthma – diet. Pediatr Pulmonol 2007; 42(8):663-673.

482 Rathi N, Rathi A. Vitamin D and child health in the 21st century. (Review). Indian Pediatr 2011 Aug;48(8):619-25.

483 van der Mei IA (i), Ponsonby AL, Dwyer T, Blizzard L. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. J Neurol 2007; 254(5):581-90.

484 van der Mei IA (ii), Ponsonby AL, Englesen O, Pasco JA. The high prevalence of vitamin D insufficiency across Australian populations is only partly explained by season and latitude. Environ Health Perspect 2007; 115(8):1132-9.

485 Bozzetto S, Carraro S, Giordano G, Boner A, Baraldi E. Asthma, allergy, and respiratory infections: the vitamin D hypothesis. Allergy 2012; 67(1):10-17. doi: 10.1111/j.1398-9995.2011.02711.x.

[486] Mason RS, Sequeira VB, Gordon-Thomson C. Vitamin D: the light side of sunshine. Review. Eur J Clin Nutr 2011 Sep;65(9):986-93. doi: 10.1038/ejcn.2011.105.

[487] De Vries A, Howie SE. Diet and asthma – can you change what you or your children are by changing what you eat? Pharmacol Ther 2009; 122(1):78-82.

[488] Castro-Rodriguez JA, Garcia-Marcos L, Alfonseda Rojas JD. Mediterranean diet as a protective factor for wheezing in preschool children. J Pediatr 2008; 152(6):823-828.

[489] Patel BD, Welch AA, Bingham SA, Luben RN. Dietary antioxidants and asthma in adults. Thorax 2006; 61(5):388-393.

[490] Wickens K, Barry D, Friezema A, Rhodius R. Fast foods – are they a risk factor for asthma? Allergy 2005; 61(12):1048-1053.

[491] Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull 2001; 60:5-20.

[492] Beuther DA, Weiss ST, Sutherland ER. Obesity and asthma. Am J Respir Crit Care Med 2006;174:112–119.

[493] Ford ES. The epidemiology of obesity and asthma. J Allergy Clin Immunol 2005;115:897–909.

[494] Shore SA, Johnston RA. Obesity and asthma. Pharmacol Ther 2006;110:83–102

[495] Luder E, Ehrlich RI, Lou WY, Melnik TA, Kattan M. Body mass index and the risk of asthma in adults. Respir Med 2004; 98:29-37.

[496] Guerra S, Wright AL, Morgan WJ, Sherill DL, et al. Persistence of asthma symptoms during adolescence: role of obesity and age at the onset of puberty. Am J Respir Crit Care Med 2004; 170:78-85

[497] Musso G, Gambino R, Cassader M. Obesity, diabetes and gut microbiota: the hygiene hypothesis expanded? doi: 10.2337/dc10-0556 Diabetes Care 2010; 33(10):2277-2284

[498] Ajslev TA, Andersen CS, Gamborg M, Sørensen TIA, Jess T. Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. Int J Obesity 2011; 35:522-529 doi:10.1038/ijo.2011.27

[499] Tuohy KM, Costabile A, Fava F. The gut microbiota in obesity and metabolic disease – a novel therapeutic target Nutritional Therapy & Metabolism 2009; 27(3):113-133

[500] Serino M, Luche E, Gres S, Baylac A, et al. Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. Gut 2012; 61(4):543-53. doi:10.1136/gutjnl-2011-301012

[501] Tilg H, Moschen AR, Kaser A. Obesity and the Microbiota (Review). Gastroenterology 2009; 136:1476–1483/ http://dx.doi.org/10.1053/j.gastro.2009.03.030

[502] Hersoug L-G, Linneberg A. The link between the epidemics of obesity and allergic diseases: does obesity induce decreased immune tolerance? Allergy 2007; 62(10):11205-1213.

[503] Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects:

associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19:972–978.

504 Bastard JP, Jardel C, Bruckert E, Blondy P, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 2000;85:3338–3342.

505 Festa AD, Agostino R Jr, Williams K, Karter AJ, et al. The relation of body fat mass and distribution to markers of chronic inflammation. Int J Obes Relat Metab Disord 2001;25:1407–1415.

506 Engstrom G, Hedblad B, Stavenow L, Lind P, et al. Inflammation-sensitive plasma proteins are associated with future weight gain. Diabetes 2003;52:2097–2101.

507 Esposito K, Pontillo A, Ciotola M, Di Palo C, et al. Weight loss reduces interleukin-18 levels in obese women. J Clin Endocrinol Metab 2002;87:3864–3866.

508 Chiellini C, Santini F, Marsili A, Berti P, et al. Serum haptoglobin: a novel marker of adiposity in humans. J Clin Endocrinol Metab 2004;89:2678–2683.

509 Thornton CA, Jones RH, Doekhie AH, Bryant AH, et al. Inflammation, obesity, and neuromodulation in pregnancy and fetal development. Adv Neuroimmune Biol 2011:193-203. doi:10.3233/NIB-2011-015.

510 Platts-Mills TAE. Asthma severity and prevalence: an ongoing interaction between exposure, hygiene and lifestyle. PloS Med 2005; 2(2):e34. doi: 10.1371/journal.pmed.0020034.

511 Sears ME, Genius SJ. Environmental determinants of chronic disease and medical approaches: recognition, avoidance, supportive therapy, and detoxification. J Environ Public Health 2012; 2012:356798. Epub 2012 Jan 19.

512 Tsai FC, Macher JM. Concentrations of airborne culturable bacteria in 100 large US office buildings from the BASE study. Indoor Air 2005; 15: 71–81.

513 Rogers CA**.** A breath of "fresh air". Editorial. Aerobiologica 2005; 21:151-153 doi:10.1007/s10453-005-9016-5

514 Weinmayr G, Weiland SK, Björkstén B, Brunekreef B, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children (ISAAC Phase Two Study Group. Am J Respir Crit Care Med. 2007 Sep 15;176(6):565-74.

515 Shapira Y, Agmon-Levin N, Shoenfeld Y. Geoepidemiology of autoimmune rheumatic diseases. Nat Rev Rheumatol. 2010 Aug;6(8):468-76.

516 Greenwood HL, Edwards N, Hoogbruin A, Kahwa EK. A review of equity issues in quantitative studies on health inequalities: the case of asthma in adults. BMC Med Res Methodol 2011; 11:104. doi: 10.1186/1471-2288-11-104

517 Fleming DM, Sunderland R, Cross KW, Ross AM. Declining incidence of episodes of asthma: a study of trends in new episodes presenting to general practitioners in the period 1989-98. Thorax 2000; 55:657-661; doi: 10.1136/thorax.55.8.657.

518 D'Amato G, Liccardi G, D'Amato M, Cazzola M. The role of outdoor air pollution and climatic changes on the rising trends in respiratory allergy. Respir Med 2001;95:606-611; doi: 10.1053/rmed.2001.1112.

519 Ziska LH, Caulfield FA. Rising CO_2 and pollen production of common ragweed (*Ambrosia artemisiifolia*), a known allergy-inducing species: implications for public health. Aust J Plant Physiol 2000; 27:893-898; doi: 10.1071/PP00032.

520 Ziska LH, Gebhard DE, Frenz DA, Faulkner S et al. Cities as harbingers of climate change: common ragweed, urbanization, and public health. J Allergy Clin Immunol 2003; 111:290-295; doi: 10.1067/mai.2003.53.

521 Beggs PJ. Impacts of climate change on aeroallergens: past and future. Clin Exp Allergy 2004;34: 1507-1513; doi: 10.1111/j.1365-2222.2004.02061.x.

522 Fitter AH, Fitter RSR. Rapid changes in flowering time in British plants. Science 2002; 296:1689-1691.

523 Chapman SJ Hill AVS. Human genetic susceptibility to infectious disease. Nat Rev Genet 2012/03//print 13(3)(175-188). http://dx.doi.org/10.1038/nrg3114 M3 - 10.1038/nrg3114 N1 - 10.1038/nrg3114

524 Hill AVS. Aspects of Genetic Susceptibility to Human Infectious Diseases. Ann Rev Genetics 2006; 40:469-486 doi: 10.1146/annurev.genet.40.110405.090546

525 Hill AVS. Evolution, revolution and the genetics of infectious disease susceptibility. Phil Trans R. Soc B 2012:367, doi: 10.1098/rstb.2011.0275

526 Rodenhiser D, Mann M. Epigenetics and human disease: translating basic biology into clinical applications (Review). CMAJ 2006; 174: 3. doi: 10.1503/cmaj.050774.

527 Gilbert SF, McDonald E, Boyle N, Buttino N. Symbiosis as a source of selectable epigenetic variation: taking the heat for the big guy. Phil. Trans. R. Soc. B 2010 365:671-8. doi: 10.1098/rstb.2009.0245.

528 Kemeny ME, Schedlowski M. Understanding the interaction between psychosocial stress and immune-related diseases: a stepwise progression [Review]. Brain, Behavior, and Immunity 2007; 21: 1009–18.

529 Stojanovich L. Stress and autoimmunity. Autoimmun Rev 2010 Mar;9(5):A271-6.

530 Marshall GD. The adverse effects of psychological stress on immunoregulatory balance: applications to human inflammatory diseases. Immunol Allergy Clin North Am 2011 Feb; 31(1):133-40.

531 Moran N. Symbiosis as an adaptive process and source of phenotypic complexity. PNAS 2007;104 Suppl 1:8627-60.

532 Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science 2010; 330(6012):1768-73.

533 Patz JA, Confalonieri UEC, Amerashinghe FP, Chua KB. Human health: ecosystem regulation of infectious diseases. Millennium Ecosystem Assessment, 2005 Chapter 14, pp391-415. Accessed June 2012 from http://www.millenniumassessment.org/documents/document.283.aspx.pdf

534 United Nations. World Urbanization Prospects: the 2007 revision. United Nations, New York.

535 IFH. Guidelines for prevention of infection and cross infection in the domestic environment. International Scientific Forum on Home Hygiene. Milan: Intramed Communications 1998.

536 IFH. Recommendations for selection of suitable hygiene procedures for use in the domestic environment. International Scientific Forum on Home Hygiene. Milan: Intramed Communications 2001.

537 IFH. Home hygiene - prevention of infection at home: a training resource for carers and their trainers. (2003) International Scientific Forum on Home Hygiene. Available from: http://www.ifh-homehygiene.org/IntegratedCRD.nsf/571fd4bd2ff8f2118025750700031676/9aaaeb306bb3c50c80257522004b4fdc?OpenDocument

538 IFH. Home hygiene in developing countries: prevention of infection in the home and peri-domestic settings (2006) International Scientific Forum on Home Hygiene. http://www.ifh-homehygiene.org/IntegratedCRD.nsf/571fd4bd2ff8f2118025750700031676/19155ab46073e67f8025752200546d83?OpenDocument

539 Larson E, Duarte CG. Home hygiene practices and infectious disease symptoms among household members. Public Health Nurs 2001; 18(2):116-7.

540 Koren O, Spor A, Felin J, Fåk F et al. Human oral, gut and plaque microbiota in patients with atherosclerosis. PNAS March, 2011; 108(Suppl 1):4592-4598. doi: 10.1073/pnas.1011383107

541 Meurman JH. Oral microbiota and cancer [review]. J Oral Microbiol 2010; 2:10:3402/jom.v210.5195

542 Bloomfield SF, Exner M, Fara GM, Nath KJ, et al. The global burden of hygiene-related diseases in relation to the home and community. International Scientific Forum on Home Hygiene, 2009 Available from: http://www.ifh-homehygiene.org/IntegratedCRD.nsf/111e68ea0824afe1802575070003f039/29858aa006faaa22802572970064b6e8?OpenDocument

543 Scallan E, Hoekstar RM, Angulo FJ, Tauxe RV, et al (i). Foodborne Illness Acquired in the United States-Major Pathogens. Emerg Infect Dis 2011; 17:7-15

544 Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM (ii). Foodborne Illness Acquired in the United States-Unspecified Agents. Emerg Infect Dis 2011; 17:16-22

545 European Food Standards Agency. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2008. Available at: http://www.efsa.europa.eu/en/scdocs/doc/1496.pdf. Accessed 10/11/2011

546 Rocourt J, Moy G, Vierk, R, Schlundt J. The present state of foodborne disease in OECD countries. Food Safety Department, World Health Organization Geneva, Switzerland. 2003. Available at:

http://www.who.int/foodsafety/publications/foodborne_disease/en/OECD%20Final%20for%20WEB.pdf. Accessed 10/11/2011

547 Widdowson MA, Monroe SS, Glass RI. Are noroviruses emerging? Emerg Infect Dis 2005; 11:735-7.

548 Goldmann DA. Transmission of viral respiratory infections in the home. Pediatr Infect Dis J 2000; 19:S97-102

549 Jefferson T, Del Mar C, Dooley L, Ferroni E. Physical interventions to interrupt or reduce the spread of respiratory viruses: systematic review. BMJ 2009; 339:doi:10.1136/bmj.b3675

550 Bridges CB, Kuehnert MJ, Hall CB . Transmission of influenza: implications for control in healthcare settings. Clin Infect Dis 2003; 37:1094-101

551 Farmer P. Perspectives: Social Inequalities and Emerging Infectious Disease. Emerg Infect Dis 1996; 2:259-269

552 Semenza JC. Strategies to intervene on social determinants of infectious diseases. Euro Surveill 2010; 15: pii: 19611.

553 Anon. Foodborne Disease Strategy: The FSA strategy for reduction of foodborne illness in the UK. 2010-15. 2010; Food Standards Agency, London, UK. FSA Document number: 10/05/0. Accessed on 10/11/2011

554 Scharff RL. Health-related costs from foodborne illness in the United States, published by the Produce Safety Project 2010; Available at: http://www.producesafetyproject.org/admin/assets/files/Health-Related-Foodborne-Illness-Costs-Report.pdf-1.pdf

555 Anon. European Centre for Disease Control and Prevention. Disease Programmes. TATFAR. Terms of Reference. 2009. Available at: http://www.ecdc.europa.eu/en/activities/diseaseprogrammes/TATFAR. Accessed 10/11/2011

556 TATFAR. Recommendations for future collaboration between the US and EU. Transatlantic Taskforce on Antimicrobial Resistance, 2011. Available at: http://ecdc.europa.eu/en/activities/diseaseprogrammes/TATFAR/Documents/210911_TATFAR_Report.pdf

557 Vidal-Navarro L, Pfeiffer C, Bouziges N, Sotto A, Lavigne JP. Faecal carriage of multidrug-resistant Gram-negative bacilli during a non-outbreak situation in a French university hospital. J Antimicrob Chemother 2010; 65:2455–2458

558 Poirel L, Herve V, Houmbrouck-Alert C, Nordmann P. Long-term carriage of NDM-1-producing Escherichia coli. J Antimicrob Chemother 2011; 66(9):2185-6. doi: 10.1093/jac/dkr236.

559 Klein E, Smith DL, Laxminarayan R. Community-associated Methicillin- Resistant Staphylococcus aureus in Outpatients, United States, 1999–2006. Emerg Infect Dis 2009; 15:1925-30.

560 Köck R, Becker K, Cookson B, van Gemert-Pijnen JE, et al. Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe. Euro Surveill 2010; 15: pii: 19688.

561 Abbas AK, Lichtman AH. Basic Immunology. Functions and disorders of the immune system, 3rd Edition. Saunders/ Elsevier, 2010. ISBN: 141605569X, 9781416055693

562 NIAID (National Institute of Allergy and Infectious Diseases). Understanding the immune system: how it works. US Dept of Health and Human Services, 2007. http://www.niaid.nih.gov/topics/immunesystem/documents/theimmunesystem.pdf

563 Schmidt-Weber CB, Akdis M, Akdis CA. Th-17 cells in the big picture of immunology. J Allergy & Clin Immunol 2007;120(2):247-54. doi:10.1016/j.jaci.2007.06.039

564 Mesquita D Jr, Cruvinel WM, Câmara NOS, Kállas EG, Andrade LEC. Autoimmune diseases in the TH17 era. Braz J Med Biol Res 2009; 42:476-486.

565 NIAID (National Institute of Allergy and Infectious Diseases). Understanding the immune system: how it works. US Dept of Health and Human Services, 2007. http://www.niaid.nih.gov/topics/immunesystem/documents/theimmunesystem.pdf