

# Microbial 'old friends', immunoregulation and socioeconomic status

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## Introduction

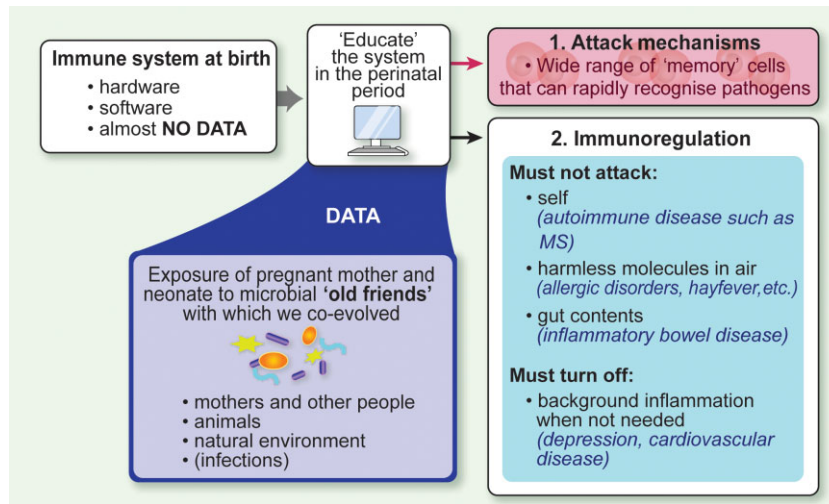
The expression 'hygiene hypothesis' was invented in 1989, and since then has had wide, often misleading, media appeal [1]. The problem has been that although based on a crucial underlying insight (that microbial experience modulates our immune systems), it was initially interpreted narrowly in the context of allergic disorders, and there was a tendency to assume that the relevant microbes were the common infections of childhood. However, the concept has broadened so that it is now a fundamental component of Darwinian or 'evolutionary' medicine, with implications for essentially all aspects of human health. The allergic disorders are a tiny part of the story, and neither hygiene nor the

## Summary

The immune system evolved to require input from at least three sources that we collectively term the 'old friends': (i) the commensal microbiotas transmitted by mothers and other family members; (ii) organisms from the natural environment that modulate and diversify the commensal microbiotas; and (iii) the 'old' infections that could persist in small isolated hunter-gatherer groups as relatively harmless subclinical infections or carrier states. These categories of organism had to be tolerated and co-evolved roles in the development and regulation of the immune system. By contrast, the 'crowd infections' (such as childhood virus infections) evolved later, when urbanization led to large communities. They did not evolve immunoregulatory roles because they either killed the host or induced solid immunity, and could not persist in hunter-gatherer groups. Because the western lifestyle and medical practice deplete the 'old' infections (for example helminths), immunoregulatory disorders have increased, and the immune system has become more dependent upon microbiotas and the natural environment. However, urbanization maintains exposure to the crowd infections that lack immunoregulatory roles, while accelerating loss of exposure to the natural environment. This effect is most pronounced in individuals of low socioeconomic status (SES) who lack rural second homes and rural holidays. Interestingly, large epidemiological studies indicate that the health benefits of living close to green spaces are most pronounced for individuals of low SES. Here we discuss the immunoregulatory role of the natural environment, and how this may interact with, and modulate, the proinflammatory effects of psychosocial stressors in low SES individuals.

**Keywords:** host–pathogen interactions, inflammation, regulatory T cells

common childhood infections play an important role. For this reason we prefer terms such as the biodiversity hypothesis or the 'old friends' mechanism [2]. The 'old friends' mechanism should now be seen as one component of the broad spectrum of interactions between mammals and their microbial environment [3]. At one end of the spectrum are the endosymbiotic events that led to the organelles in mammalian eukaryotic cells. There are also endogenous viruses and retrotransposons that lurk in our tissues and form major components of our DNA [4]. Next, in terms of their interdependency with human physiology, and particularly important in the context of this paper, are the various microbiotas (gut, skin, airway, oropharyngeal, genitourinary). These microbiotas perform significant parts of



**Fig. 1.** The immune system requires 'educational' input. The microbiota of others, organisms from the natural environment and other tolerated organisms (such as helminths) with which we co-evolved are required to expand the effector and regulatory branches of the immune system. During subsequent encounters with pathogens, danger signals generated by tissue damage enhance effector mechanisms and attenuate regulatory pathways to permit an appropriate immune response. Adequate background levels of regulatory T cells and dendritic cells and other regulatory mechanisms are required to maintain suppression of responses to 'forbidden targets' and to switch off inflammation completely when the danger is eliminated, so that proinflammatory mediators do not continue to circulate.

mammalian metabolism and contain about 100-fold more genes than does the human genome [5]. Metabolomic analyses show that much of 'our' metabolism is microbial [6]. The commensal microbiotas are also involved in development of mammalian organ systems, including gut, immune system, bone and brain (reviewed in [3]). The brain provides an example relevant to this paper. The brain of germ-free mice has altered chemistry and gene expression, and the animals behave abnormally [7]. The hypothalamic–pituitary–adrenal (HPA) axis of germ-free animals is also abnormal, manifested as altered central nervous system (CNS) gene expression and abnormal responses to stress [8,9]. To correct these abnormalities it is necessary to reconstitute the gut microbiota with appropriate organisms within the first 6 weeks of life [7,8]. Moving along the spectrum of human–microbe interdependency, we next have to consider our encounters with harmless organisms from the natural environment, present in large quantities in air, soil and water [10]. Finally, we come to the infections, the type of microbial interaction initially implicated by the hygiene hypothesis.

This paper is focused upon four questions: (i) why do some microbial exposures regulate the immune system (in addition to triggering its development in the neonate); (ii) which categories of organism have this immunoregulatory property; (iii) what relevance does this immunoregulation have to depression and stress resilience; and (iv) how do these mechanisms interact with the clear influence of socio-economic status (SES) on many aspects of health? We emphasize that immunoregulation by microbial exposures is no longer a 'hypothesis'. We will not discuss the existence

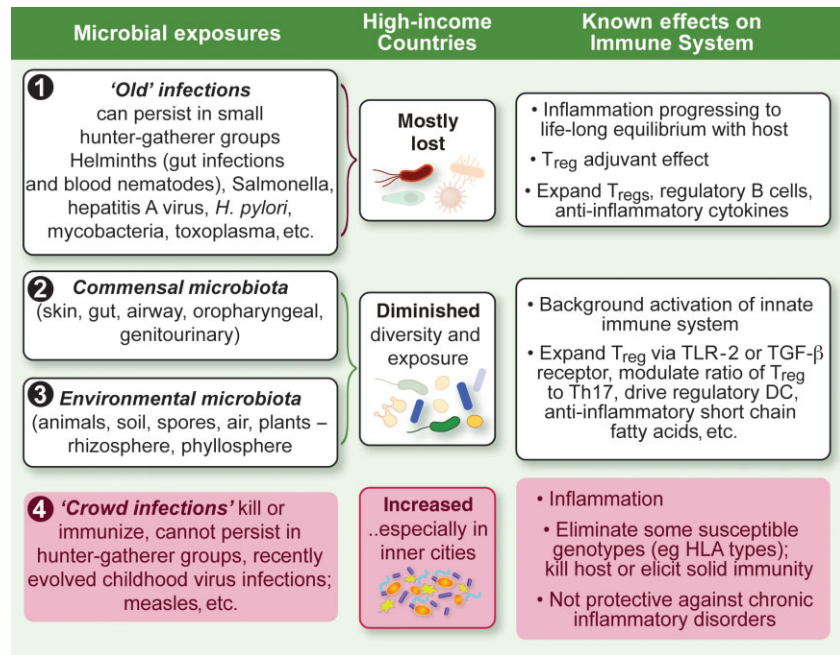
of these effects, but rather their relative importance, because they certainly do not act alone.

### Which microbial exposures regulate the immune system, and why do they do it?

At birth the immune system has genetically inherited mechanisms, but it lacks data. It has some knowledge of self, acquired as lymphocytes mature in the thymus, and minimal knowledge of the outside world, transferred from the mother across the placenta. After birth it needs microbial exposures for at least three reasons. The first two are somewhat obvious and are part of the microbe-dependent signals for the development of the immune system mentioned in the previous paragraph. Exposure to a broad biodiversity of organisms builds up a memory of diverse molecular structures that accelerates subsequent rapid recognition of novel dangerous organisms [11,12]: this works because all life forms share fundamental molecular building blocks [3]. Secondly, microbial components such as peptidoglycans and lipopolysaccharide (LPS) taken in from the gut maintain background activation of the innate immune system [13].

It is less obvious that microbial exposures also play a role in setting up the control mechanisms that stop the immune system from causing inappropriate inflammatory responses. Thus, the system needs to develop a network of regulatory pathways and regulatory T cells ( $T_{reg}$ ) that stop inappropriate immune attacks on self, harmless allergens and gut contents, including harmless microorganisms (Fig. 1). These are the pathological targets involved in autoimmune

**Fig. 2.** A simplified scheme that divides organisms into categories based on whether or not they co-evolved with humans and needed to be tolerated, and so developed immunoregulatory roles within the immune system. The 'old' infections were able to persist in isolated hunter-gatherer groups as carrier states and latent infections, and so evolved the ability to modulate the immune system. The microbiotas also needed to be tolerated, and an unknown subset of organisms within the commensal microbiotas is derived from the environment, including animal sources. The crowd infections evolved relatively recently and either kill the host or immunize, and constitute the only category that is increased rather than decreased in high-income settings. Epidemiological studies show that the crowd infections are not immunoregulatory.



diseases [14,15], allergic disorders [16] and inflammatory bowel diseases (IBD) [17], respectively, all of which are increasing in prevalence in high-income countries. These down-regulatory mechanisms must also be able to shut off inflammatory responses that are no longer needed, because chronically raised background inflammation, easily documented as raised C-reactive protein (CRP) or interleukin (IL)-6, correlates with a multitude of health problems, including cardiovascular disease, depression and reduced stress resilience [18,19], all of which are increasing problems in modern urbanized high-income countries. In high-income communities there are often persistent high background levels of CRP in the absence of any demonstrable reason for ongoing inflammation. In sharp contrast, a longitudinal study in a low-income country revealed that large peaks of CRP accompanied episodes of infection (therefore, cross-sectional studies suggest higher CRP levels in low- than in high-income settings), but after resolution of the infection CRP levels fell to values close to zero [20]. Thus, in the under-developed low-income setting, inflammation appears when needed but is completely shut off when not needed, whereas in high-income urban communities there can be a failure to shut off unwanted inflammation.

### Which categories of organism have an immunoregulatory role?

Which categories of organism have an immunoregulatory role? The short answer is that the immunoregulatory organisms are those with which we co-evolved, and that had to be tolerated. These include the microbiotas (gut, airway, skin,

genitourinary, oropharyngeal) which, as commensals performing crucial physiological functions, clearly must not be attacked [12,21–23]. We also evolved to tolerate harmless organisms (bacteria, archaea, fungi, viruses, protozoa, etc.) from the natural environment in water, air and soil because these were inevitably taken into the body daily in large quantities (some of these might, in fact, become incorporated into the microbiota, as discussed later). Finally, we evolved to tolerate a specific subset of infections from our evolutionary past, sometimes referred to as the 'old infections' [24,25]. The crucial feature of the 'old infections' was their ability to persist in small isolated human hunter-gatherer groups, because they caused persistent non-fatal carrier states or subclinical disease (Fig. 2). These 'old infections' include 'paleolithic' strains of *Mycobacterium tuberculosis* that were less pathogenic than modern ones [25], helminths including gut parasites, but also blood nematodes that never enter the gut [26] and *Helicobacter pylori* (reviewed in [2]). Infections such as blood nematodes are not always harmless but once established, attempts by the immune system to eliminate them merely cause pointless immunopathology, leading to complications such as elephantiasis [26].

### Mechanisms of immunoregulation

We are only just beginning to understand some of the mechanisms involved in this microbe-driven immunoregulation. Some 'old friends' (including members of the human gut microbiota such as *Bacteroides fragilis*), or molecules that they secrete, are known to specifically expand T<sub>reg</sub> populations [22,27–29], or to cause dendritic cells (DC)

to switch to regulatory DC that preferentially drive immunoregulation [30,31]. The latter mechanism implies a 'T<sub>reg</sub> adjuvant' effect. For example, when patients suffering from early relapsing multiple sclerosis (MS) become infected with helminths the disease stops progressing, and circulating myelin-recognizing T<sub>reg</sub> appear in the peripheral blood [32,33], an exciting observation that has led to formal clinical trials [34]. Release of immunoregulatory molecules is not confined to organisms in the gut, given that such activities have also been isolated from blood nematodes [35,36]. Another recent suggestion is that a certain level of the proinflammatory cytokine tumour necrosis factor (TNF)- $\alpha$  is required to drive apoptosis of potentially autoreactive T cells, and that in the absence of sufficient TNF-releasing infections such T cells can persist to cause type 1 diabetes (T1D) or multiple sclerosis [37]. Numerous other regulatory mechanisms are discussed in recent publications [22]. The relevance of these regulatory mechanisms for chronic inflammatory disorders is now well supported by data from animal models. Given their regulatory effects, it is not surprising that 'old friends' can be shown to drive immunoregulation, and to block or treat pathophysiology in models of allergies, autoimmune disease and IBD [29,38,39].

#### Non-immunoregulatory crowd infections

These old infections are very different from the 'crowd infections' that started to infect man after the First Epidemiological Transition, when the Neolithic revolution led to agriculture and large settlements and eventually to urbanization (category 4 in Fig. 2). The 'crowd infections' are mainly viruses such as measles that could not persist in sparsely distributed hunter-gatherer bands, because they either killed the host or induced solid immunity [24]. Crowd infections need large populations and networks of social contacts, so that the infection can return to cause an epidemic when herd immunity declines [24]. Such populations did not exist until well after 10 000 BCE, when agriculture and permanent settlements initiated the transition to larger population densities. Thus humans did not co-evolve with the crowd diseases, and the crowd diseases did not need to be tolerated (they killed the host or generated solid immunity) so, as anticipated, they play little role in setting up immunoregulatory pathways. Epidemiological studies have confirmed that the crowd infections do not protect children from allergic disorders [40–42] and often, in fact, trigger them [43]. Crowd infections also fail to protect from autoimmunity and IBD [44–47]. Thus the crowd infections do not explain the original ground-breaking observations of Strachan that led to the coining of the misleading term 'hygiene hypothesis' [1]. The crowd infections can, of course, impact upon human evolution. For instance, they may eliminate certain susceptible genotypes or major histocompatibility complex (MHC) phenotypes. However, spo-

radic dangerous infections do not co-evolve essential ongoing roles in mammalian physiology; but the 'old friends' (the old infections, and the commensal and environmental microbiotas) that accompanied human evolution do have crucial immunoregulatory roles that are disturbed by the modern urban lifestyle. What aspects of that lifestyle are important?

#### Loss of contact with 'old friends' in high-income countries

It is of interest to summarize the lifestyle changes that reduce contact with the 'old friends' in high-income settings where chronic inflammatory disorders are increasing [48], because it casts light upon the relative immunoregulatory importance of the different groups of organisms. First, as reviewed in detail elsewhere [19], the chronic inflammatory disorders tend to increase when populations immigrate from low-income to developed high-income countries, and within any given country they tend to be more common in urban than in rural communities. This is equally true for psychiatric disorders, including autism, schizophrenia and depression (reviewed in [19]). It is obvious that these lifestyle changes will deplete the old infections, listed as category 1 in Fig. 1. For example, helminths are almost eliminated from high-income cities.

Perhaps of greater interest, and less fully understood, are the lifestyle changes that reduce contact with the commensal microbiota of others (category 2 in Fig. 2) and the microbiota of the natural environment (category 3 in Fig. 2).

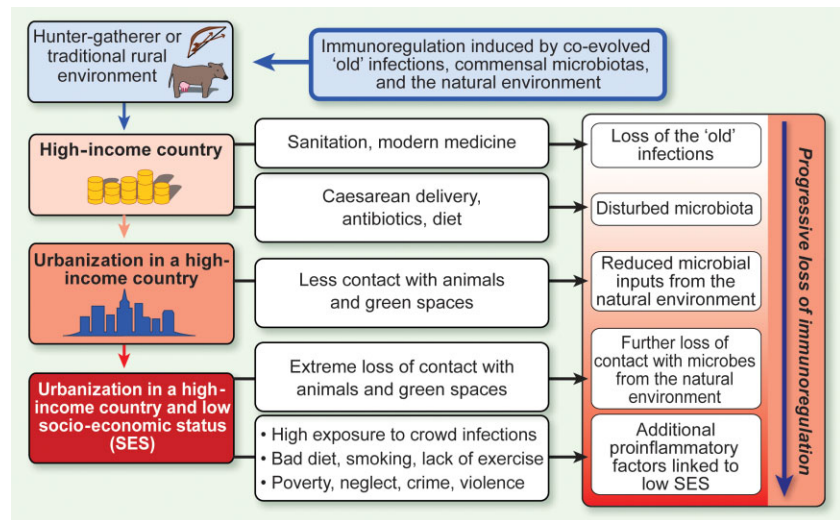
#### Caesarean section, birth order, antibiotics and diet

Birth by caesarean section causes development of the gut microbiota to be delayed and to take an unusual course [49] (Fig. 3). This has been linked to increased T1D [50,51] and asthma [52,53], while increased contact with maternal microbiota may be protective [54]. Caesarean birth also increases the risk of coeliac disease [55], but there is little or no effect on the risk of IBD [55–57]. Birth order also modulates the gut microbiota [58], and this probably accounts for the protection against allergic disorders attributable to having older siblings [58] reported originally by Strachan [1].

Antibiotics, particularly excessive antibiotic use during pregnancy or the neonatal period, induces changes in the microbiota that have been implicated in asthma [59,60], cow's milk allergy [61], irritable bowel syndrome [62], IBD [63] and obesity [64].

Typical modern western diets have also been shown to exert profound effects on the microbiota [65]. For example, the gut microbiota of Italians is very different from that of people living in traditional villages in Burkina Faso [66], and the authors attributed this difference to diet because

**Fig. 3.** Progressive loss, in high-income settings, of contact with the immunoregulatory organisms with which humans co-evolved. In communities with low socioeconomic status there are numerous additional factors that also tend to increase background inflammation, including psychosocial stressors, bad diet, obesity and continued exposure to the crowd infections.



the African microbiomes contained genes for the hydrolysis of cellulose and xylan, abundant in African but not in Italian diets [66]. Similarly, in faeces from 98 Americans, enterotypes (bacterial ecosystems in the gut microbiota) correlated with long-term dietary habits, particularly the consumption of animal fat *versus* carbohydrates [67].

### The natural environment and animals

In addition to factors discussed thus far, the composition of the commensal microbiota also depends upon input from the natural environment (reviewed in [10]). The skin microbiota from individuals living close to agricultural land in Finland was more diverse than that from individuals living close to urban centres, and was associated with reduced atopic sensitization [21]. Moreover, the ability of exposure to the farming environment early in life to protect from asthma seems to correlate closely with airborne fungal and bacterial biodiversity in children's bedrooms [68]. Similarly, the biodiversity of the gut microbiota was greater in Amazonian Amerindians than in Malawians, while the biodiversity of gut microbiota from the United States was lower than that of either of these low-income rural groups [69]. Clearly, diet could play a role, but recent experiments with piglets suggest the importance of environmental inputs. The gut microbiota of piglets that were housed in a natural outdoor environment was rich in Firmicutes, particularly *Lactobacillus* strains, whereas the hygienic indoor piglets had reduced *Lactobacilli* and more potentially pathogenic phylotypes [70]. Moreover, biopsies of the gut mucosa revealed that these 'indoor' piglets had increased type 1 interferon activity, increased MHC class 1 and up-regulation of many chemokines [70], implying a more inflammatory state in the guts of animals whose microbiota had not been modified and diversified by exposure to the natural environment. In mice [71] and humans, a correlation between reduced gut microbial biodiversity and poor

control of inflammation is a common finding [72–74]. It therefore seems likely that microbial inputs are required to maintain diversity of the gut microbiota, and that such diversity plays a role in the regulation of inflammation, although at present the relationship between environmental strains and colonizing strains is not documented (discussed in [10]). Data on gut bacteria are accumulating rapidly, so for these organisms the issue may soon be addressed, but data for bacteriophages and other viruses or for archaea and fungi are only just beginning to appear [23,75].

### Animals, faeces and spores

Contact with animals might provide part of the explanation for this environmental input. The protective effect of the farming environment was noted in the 19th century [76], and has been associated with cowsheds [77]. Contact with dogs, with which humans have co-evolved for many millennia [78], also protects from allergic disorders [79,80], and people seem to share their microbiota via dogs [81], which greatly increase the microbial biodiversity of the home [82,83]. In a developing country the presence of animal faeces in the home correlated with better ability to control background inflammation (CRP levels) in adulthood [20], and in Russian Karelia (where the prevalence of childhood atopy is four times lower and T1D is six times lower than in Finnish Karelia), house dust contained a sevenfold higher number of clones of animal-associated species than was present in Finnish Karelian house dust [84].

It is interesting to speculate here that these effects have something to do with faeces and spores. Approximately one-third of the bacteria in the gut microbiota are spore-forming, and spores are readily demonstrable in human faeces [85]. Spores are remarkably resistant and can remain viable for thousands, possibly millions of years (reviewed in [86]). It has been calculated that many billions of tonnes of animal and human faeces are generated every year, so

faeces-derived spores accumulate in the natural environment. Human faeces average up to  $10^4$  spores/g, while soil contains approximately  $10^6$  spores/g [87]. However, spores in soil have tended to be studied by environmental microbiologists and ecologists, and the soil has been regarded as the natural habitat of spore-forming organisms such as *Bacillus* spp., despite awareness that many of them can germinate and replicate in the intestinal tracts of insects and other animals [86] [some of these are toxic to the insect, and so are used as biological insecticides (reviewed in [86])]. Recently it has been reported that spores of *Bacillus subtilis* can germinate in the small bowels of mice and rabbits [88–90]. Moreover, after germination they replicated in the small bowel and then resporulated as they entered the colon. The same occurs in humans. *B. subtilis* strains were obtained from biopsies of human ileum and from faecal samples [85]. Most of these strains are able to form biofilms, sporulate anaerobically and secrete antimicrobials, properties that could facilitate survival in the gut [85]. There is therefore a growing view that *B. subtilis* and other environmental spore-forming species are gut commensals rather than soil microorganisms [87]. This might be very relevant to the ‘old friends’ mechanism, particularly to the clear importance of exposure to animals, agricultural land and green spaces. For example, *B. subtilis* is an important stimulus for development of the gut-associated lymphoid tissue (GALT) in rabbits, and sporulation of live bacilli within the GALT is considered critical to this process [90]. Interestingly, *B. subtilis* is capable of driving GALT in synergy with *B. fragilis*, an organism that also drives formation of murine GALT, and secretes a polysaccharide antigen that drives proliferation of  $T_{reg}$  [22].

### The natural environment and human wellbeing

In the previous section we emphasized the relevance of natural and agricultural environments, including the animals they contain, because several remarkable epidemiological studies have found that living in proximity to green spaces reduces overall mortality, cardiovascular disease and depressive symptoms, and increases subjective feelings of wellbeing [91–94]. This beneficial effect has been attributed to multiple factors, including exercise, exposure to sunlight and an evolved psychological reward from contemplating the ideal hunter-gatherer habitat, perhaps similar to ‘habitat selection’ in other species [94,95]. A detailed review and critique of these explanations has been published elsewhere [10].

In view of the large literature on urban–rural differences in chronic inflammatory and psychiatric disorders (reviewed in [19]), and on the protective effects of close exposure [77,96] or even mere proximity [21] to the farming environment or to airborne microbial biodiversity [68], it is strange that the likely connection between the ‘green space’ effect and the ‘hygiene hypothesis’ or ‘old

friends’ mechanism has only recently been made. Urban populations are heavily exposed to the crowd infections, but as pointed out above, we did not co-evolve with the crowd infections, they did not co-evolve roles in setting up our immunoregulatory mechanisms and exposure to them does not protect against the chronic inflammatory disorders [40–47]. Conversely, urban populations are deprived of exposure to the natural and agricultural environment. This is particularly true of low SES individuals who perhaps do not have rural secondary homes or holiday travel to rural settings. This point, which needs verification by epidemiologists, could explain why the beneficial effect of proximity to green space is strikingly more marked at the lower end of the socioeconomic scale [91–93].

### Inflammation and SES

Of course, this does not mean that we can attribute all urban health deficits to lack of immunoregulatory microbial exposures, so to what extent can we do so? Many factors are linked to increased background levels of inflammation (measured as CRP or IL-6) in people of low SES. These include unhealthy behaviours such as smoking, drugs, poor diet and obesity, as well as psychosocial stressors such as violence, distrust, neglect, poverty and crime [97–99]. For example, CRP levels of 3 mg/l or more are found in increased percentages of children living in areas with high levels of poverty or crime [100].

At least some of this predisposition to inflammation is developmental, and is established during the perinatal period. Perinatal stress results in adults who themselves show exaggerated inflammatory responses to stress [101–104]. For example, maltreated children develop higher levels of IL-6 in response to a standardized social stressor (the Trier Social Stress Test; TSST) when tested as adults in comparison to a non-maltreated control group [101,105], and maltreated children tend to have higher levels of CRP 20 years later [103].

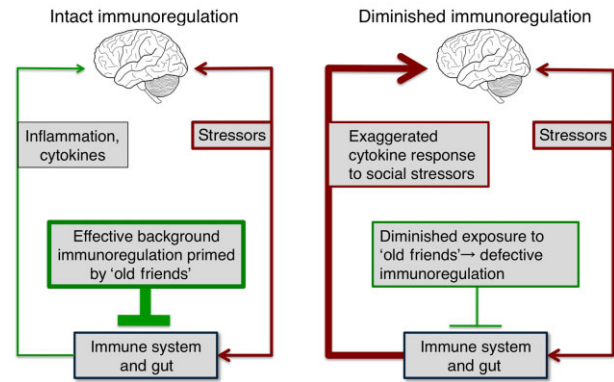
Interestingly, negative life events during the first years of life, whether they affect the child directly or indirectly via traumatic experiences of the mother, also predispose to some of the chronic inflammatory disorders that are increasing in high-income countries and that are associated more traditionally with the ‘hygiene hypothesis’. For example, the autoimmune disease T1D is increased in low SES children ([106,107], reviewed in [108]). Low SES children also tend to have more severe asthma, accompanied by increased expression of proinflammatory pathways [109]. In some settings IBD is also more common in low SES children [110]. Similarly SES-associated inflammation partly explains the SES-related incidence of type 2 diabetes [111]. These observations imply a broad-based immunoregulatory problem in low SES communities.

There are several explanations for this effect. First, prenatal stress causes long-term alterations in the HPA axis func-

tion, both in animal models [112,113] and in humans exposed to prenatal [114] or early childhood stress [115], or to a childhood background of low SES [97]. Secondly, prenatal stress has effects on the microbiota of rats and rhesus monkeys that persist into adulthood [116,117], and it is likely that the same is true in humans, although this has not, to our knowledge, been documented. However, we do know that in humans fluctuations in the microbiota early after surgery may lead to an increased risk of immunoregulatory failure, manifested in this clinical group as graft-versus-host disease [118], and changes in the microbiota of severely stressed critically ill humans are rapid and prolonged [119]. The nature of the microbiota, as discussed earlier, modulates development of the brain and HPA axis [7–9] and the microbiota plays a major role in the inflammatory response to stress [9]. Much emphasis has been placed upon the role of poor diet and obesity in the health deficits associated with low SES settings, and indeed obesity has proinflammatory effects, but much of this is again mediated via the microbiota [120]. These, and no doubt other mechanisms that are beyond the scope of this review, cause low SES urban children to have poor regulation of inflammation that predisposes them to cardiovascular disease, depression and metabolic syndrome [97–99,111] and other chronic inflammatory disorders [108–110].

### The ‘old friends’ mechanism and inflammation in low SES settings

Does the ‘old friends’ mechanism have anything to do with the increased inflammation observed in low SES settings? There are a number of ways in which these consequences of low SES might overlap with, and be modified or exaggerated by, the ‘old friends’ mechanism. For example, the release of inflammatory mediators is subject to negative immunoregulatory feedback. Therefore, poor immunoregulation will lead to exaggerated inflammatory responses. Because low SES inner-city children lack exposure to all the categories of immunoregulatory organisms except the microbiota of others, but are bombarded by the non-immunoregulatory crowd infections, they are at risk for frequent inflammatory responses (Fig. 3). If these occur during pregnancy, the increased levels and persistence of inflammatory mediators may predispose to developmental abnormalities of the brain, such as those that underlie some cases of schizophrenia or autism [121] and other neurological disorders [122]. Essentially, all the common crowd infections are associated epidemiologically with these neurological/psychiatric disorders when the infection occurs during pregnancy [121–123], and abnormal brain development is seen when inflammation is provoked during pregnancy in monkeys [124] or rodents [125]. A similar mechanism might explain the associations of low SES with T1D ([106,107], reviewed in [108]), asthma [109] and IBD, as mentioned above [110].



**Fig. 4.** Exaggerated and prolonged cytokine release in response to a psychosocial stressor in individuals with diminished immunoregulation. Stress drives release of proinflammatory mediators via pathways that involve the immune system and the gut. The inflammatory response to a given level of a stressor is modulated and eventually terminated by immunoregulatory mechanisms. If immunoregulation is defective, as can occur when there has been inadequate exposure to immunoregulation-inducing ‘old friends’, then a given level of stressor will result in greater and more prolonged inflammatory response. Reprinted from Rook *et al.* (2013) *Evolution, Medicine and Public Health* (1): 46–64, doi: 10.1093/emph/eot004, by permission of Oxford University Press and the Foundation for Evolution, Medicine, and Public Health [19].

An immunoregulatory deficit is equally relevant to inflammation driven by psychosocial stressors. In the absence of effective priming of immunoregulation by ‘old friends’, a given level of psychosocial stress might be expected to cause a greater release of inflammatory mediators (Fig. 4), which predispose towards [18,126], and can drive, psychiatric symptoms [127,128], whereas exposure to ‘old friends’ might be expected to reduce cytokine levels and so increase stress resilience [19]. Recent studies in the Philippines have found that even a childhood trauma as severe as maternal deprivation can fail to result in a raised background CRP in adulthood in those individuals who were heavily exposed to a microbe-rich environment and animal faeces in childhood [129]. In US citizens such adverse childhood events tend to have serious consequences for later health, as outlined above [101–105]. Similarly, recent psychosocial stress did not cause detectable rise in CRP in these adults who had received heavy microbial exposures as infants [129]. In a fascinating further twist it was found that, in this community, depressive symptoms were not associated with raised biomarkers of inflammation [130], whereas depressive symptoms are often associated with raised biomarkers of inflammation in high-income countries [126,127,131]. In view of the growing awareness of the relevance of contact with animals and spores outlined earlier, this finding is intriguing and suggests that in low-income settings exposure to animal-derived microbes might

improve regulation of inflammation, and so increase stress-resilience, although this observation clearly needs to be confirmed in other populations.

## Conclusions and critique

Much confusion has arisen from the assumption that multiple older siblings provide protection from allergic disorders because they transmit the common childhood infections [1], whereas it now seems more likely that this protection is attributable to exchange of commensal microbiota [58]. This paper is an attempt to show that a broad evolutionary and biological approach can provide a framework that makes sense of several paradoxes, including the fact that inner-city children, in whom childhood infections are rife, tend to have more rather than fewer inflammatory disorders, including allergies. If we consider our microbiological encounters in terms of our evolutionary history, and the nature of our relationships to each category of organism, we can see that low SES urban children are at the extreme end of a spectrum of loss of the organisms with which we co-evolved (Fig. 3). A second objective of the paper is to point out that these effects have little or nothing to do with hygiene [132]. Indeed, increased hygiene in the low SES home might help to protect from the crowd infections, while also protecting from 'new enemies' such as gastroenteritis-inducing *Escherichia coli* strains and *Campylobacter*. Our third objective is to illustrate how the low SES lifestyle, including the psychosocial stressors that it entails, will synergize with the consequences of the 'old friends' mechanism to increase susceptibility to a broad range of inflammation-dependent health problems. We hope that these insights will help to focus attention on the need for increased hygiene, coupled with innovative design for homes and sustainable cities that promote appropriate microbial exposures. Meanwhile, there is enormous need for a greater understanding of the relationship between organisms in the natural environment and those that colonize us so that we can optimize the design of urban green spaces.

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## Disclosure

C. L. R. reports the following activities for the previous 2 years: advisory board participation and related travel funds for PamLab, Lilly and North American Center for Continuing Education; development and presentation of disease state slides for PamLab, Pfizer and Johnson & Johnson, as well as related travel funds for these activities; development of continuing medical education material for North American Center for Continuing Education and for CME Incite.

## References

- 1 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; **299**:1259–60.
- 2 Rook GAW. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: darwinian medicine and the 'hygiene' or 'old friends' hypothesis. *Clin Exp Immunol* 2010; **160**:70–9.
- 3 McFall-Ngai M, Hadfield MG, Bosch TC *et al.* Animals in a bacterial world, a new imperative for the life sciences. *Proc Natl Acad Sci USA* 2013; **110**:3229–36.
- 4 Holmes EC. The evolution of endogenous viral elements. *Cell Host Microbe* 2011; **10**:368–77.
- 5 O'Hara AM, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep* 2006; **7**:688–93.
- 6 Wikoff WR, Anfora AT, Liu J *et al.* Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci USA* 2009; **106**:3698–703.
- 7 Heijtz RD, Wang S, Anuar F *et al.* Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci USA* 2011; **108**:3047–52.
- 8 Sudo N, Chida Y, Aiba Y *et al.* Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *J Physiol* 2004; **558**:263–75.
- 9 Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav Immun* 2011; **25**:397–407.
- 10 Rook GAW. Regulation of the immune system by biodiversity from the natural environment: an ecosystem service essential to health. *Proc Natl Acad Sci USA* 2013; **110**:18360–7.
- 11 Su LF, Kidd BA, Han A, Kotzin JJ. Virus-specific DMM. CD4(+) memory-phenotype T cells are abundant in unexposed adults. *Immunity* 2013; **38**:373–83.
- 12 Naik S, Bouladoux N, Wilhelm C *et al.* Compartmentalized control of skin immunity by resident commensals. *Science* 2012; **337**:1115–9.
- 13 Clarke TB, Davis KM, Lysenko ES, Zhou AY, Yu Y, Weiser JN. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nat Med* 2010; **16**:228–31.
- 14 Fleming JO. Helminth therapy and multiple sclerosis. *Int J Parasitol* 2013; **43**:259–74.
- 15 Stene LC, Nafstad P. Relation between occurrence of type 1 diabetes and asthma. *Lancet* 2001; **357**:607.



- 16 Eder W, Ege MJ, von Mutius E. The asthma epidemic. *N Engl J Med* 2006; **355**:2226–35.
- 17 Elliott DE, Summers RW, Weinstock JV. Helminths and the modulation of mucosal inflammation. *Curr Opin Gastroenterol* 2005; **21**:51–8.
- 18 Gimeno D, Kivimaki M, Brunner EJ *et al.* Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychol Med* 2009; **39**:413–23.
- 19 Rook GAW, Lowry CA, Raison CL. Microbial Old Friends, immunoregulation and stress resilience. *Evol Med Public Health* 2013; **2013**(1):46–64.
- 20 McDade TW, Tallman PS, Madimenos FC *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; **24**:675–81.
- 21 Hanski I, von Hertzen L, Fyhrquist N *et al.* Environmental biodiversity, human microbiota, and allergy are interrelated. *Proc Natl Acad Sci USA* 2012; **109**:8334–9.
- 22 Round JL, Lee SM, Li J *et al.* The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 2011; **332**:974–7.
- 23 Hoffmann C, Dollive S, Grunberg S *et al.* Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. *PLOS ONE* 2013; **8**:e66019.
- 24 Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. *Nature* 2007; **447**:279–83.
- 25 Comas I, Coscolla M, Luo T *et al.* Out-of-Africa migration and Neolithic coexpansion of *Mycobacterium tuberculosis* with modern humans. *Nat Genet* 2013; **45**:1176–82.
- 26 Babu S, Blauvelt CP, Kumaraswami V, Nutman TB. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. *J Immunol* 2006; **176**:3248–56.
- 27 Grainger JR, Smith KA, Hewitson JP *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.
- 28 Atarashi K, Tanoue T, Shima T *et al.* Induction of colonic regulatory T cells by indigenous clostridium species. *Science* 2011; **331**:337–41.
- 29 Karimi K, Inman MD, Bienenstock J, Forsythe P. *Lactobacillus reuteri*-induced regulatory T cells protect against an allergic airway response in mice. *Am J Respir Crit Care Med* 2009; **179**:186–93.
- 30 Smits HH, Engering A, van der Kleij D *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.
- 31 Hart AL, Lammers K, Brigidi P *et al.* Modulation of human dendritic cell phenotype and function by probiotic bacteria. *Gut* 2004; **53**:1602–9.
- 32 Correale J, Farez M. Association between parasite infection and immune responses in multiple sclerosis. *Ann Neurol* 2007; **61**:97–108.
- 33 Correale J, Farez MF. The impact of parasite infections on the course of multiple sclerosis. *J Neuroimmunol* 2011; **233**:6–11.
- 34 Fleming J, Isaak A, Lee J *et al.* Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. *Mult Scler* 2011; **17**:743–54.
- 35 Harnett MM, Melendez AJ, Harnett W. The therapeutic potential of the filarial nematode-derived immunomodulator, ES-62 in inflammatory disease. *Clin Exp Immunol* 2010; **159**:256–67.
- 36 Kron MA, Metwali A, Vodanovic-Jankovic S, Elliott D. Nematode AsnRS resolves intestinal inflammation in murine T-cell transfer colitis. *Clin Vaccine Immunol* 2012; **20**:276–81.
- 37 Faustman DL, Wang L, Okubo Y *et al.* Proof-of-concept, randomized, controlled clinical trial of bacillus Calmette–Guerin for treatment of long-term type 1 diabetes. *PLOS ONE* 2012; **7**:e41756.
- 38 Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009; **9**:313–23.
- 39 Osada Y, Kanazawa T. Parasitic helminths: new weapons against immunological disorders. *J Biomed Biotechnol* 2010; **2010**:743–58.
- 40 Benn CS, Melbye M, Wohlfahrt J, Bjorksten B, Aaby P. Cohort study of sibling effect, infectious diseases, and risk of atopic dermatitis during first 18 months of life. *Br Med J* 2004; **328**:1223–8.
- 41 Dunder T, Tapiainen T, Pokka T, Uhari M. Infections in child day care centers 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**:972–7.
- 42 Bremner SA, Carey IM, DeWilde S *et al.* Infections presenting for clinical care in early life and later risk of hay fever in two UK birth cohorts. *Allergy* 2008; **63**:274–83.
- 43 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.
- 44 Cardwell CR, Carson DJ, Yarnell J, Shields MD, Patterson CC. Atopy, home environment and the risk of childhood-onset type 1 diabetes: a population-based case–control study. *Pediatr Diabetes* 2008; **9**:191–6.
- 45 Amre DK, Lambrette P, Law L *et al.* Investigating the hygiene hypothesis as a risk factor in pediatric onset Crohn's disease: a case–control study. *Am J Gastroenterol* 2006; **101**:1005–11.
- 46 Bernstein CN, Rawsthorne P, Cheang M, Blanchard JF. A population-based case–control study of potential risk factors for IBD. *Am J Gastroenterol* 2006; **101**:993–1002.
- 47 Koloski NA, Bret L, Radford-Smith G. Hygiene hypothesis in inflammatory bowel disease: a critical review of the literature. *World J Gastroenterol* 2008; **14**:165–73.
- 48 Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002; **347**:911–20.
- 49 Dominguez-Bello MG, Costello EK, Contreras M *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci USA* 2010; **107**:11971–5.
- 50 Bonifacio E, Warncke K, Winkler C, Wallner M, Ziegler AG. Cesarean section and interferon-induced helicase gene polymorphisms combine to increase childhood type 1 diabetes risk. *Diabetes* 2011; **60**:3300–6.
- 51 Cardwell CR, Stene LC, Joner G *et al.* Cesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. *Diabetologia* 2008; **51**:726–35.

- 52 Thavagnanam S, Fleming J, Bromley A, Shields MD, Cardwell CR. A meta-analysis of the association between Caesarean section and childhood asthma. *Clin Exp Allergy* 2008; **38**:629–33.
- 53 Guibas GV, Moschonis G, Xepapadaki P *et al.* Conception via *in vitro* fertilization and delivery by Caesarean section are associated with paediatric asthma incidence. *Clin Exp Allergy* 2013; **43**:1058–66.
- 54 Hesselmar B, Sjöberg F, Saalman R, Aberg N, Adlerberth I, Wold AE. Pacifier cleaning practices and risk of allergy development. *Pediatrics* 2013; **131**:e1829–37.
- 55 Decker E, Engelmann G, Findeisen A *et al.* Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. *Pediatrics* 2010; **125**:e1433–40.
- 56 Bager P, Simonsen J, Nielsen NM, Frisch M. Cesarean section and offspring's risk of inflammatory bowel disease: a national cohort study. *Inflamm Bowel Dis* 2012; **18**:857–62.
- 57 Malmborg P, Bahmanyar S, Grahnquist L, Hildebrand H, Montgomery S. Cesarean section and the risk of pediatric Crohn's disease. *Inflamm Bowel Dis* 2012; **18**:703–8.
- 58 Penders J, Gerhold K, Stobberingh EE *et al.* Establishment of the intestinal microbiota and its role for atopic dermatitis in early childhood. *J Allergy Clin Immunol* 2013; **132**:601–7.
- 59 Stensballe LG, Simonsen J, Jensen SM, Bonnelykke K, Bisgaard H. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. *J Pediatr* 2013; **162**:832–8 e3.
- 60 Russell SL, Gold MJ, Hartmann M *et al.* Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Rep* 2012; **13**:440–7.
- 61 Metsala J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM. Mother's and offspring's use of antibiotics and infant allergy to cow's milk. *Epidemiology* 2013; **24**:303–9.
- 62 Villarreal AA, Aberger FJ, Benrud R, Gundrum JD. Use of broad-spectrum antibiotics and the development of irritable bowel syndrome. *Wisconsin Med J* 2012; **111**:17–20.
- 63 Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. *Am J Gastroenterol* 2010; **105**:2687–92.
- 64 Trasande L, Blustein J, Liu M, Corwin E, Cox LM, Blaser MJ. Infant antibiotic exposures and early-life body mass. *Int J Obes (Lond)* 2013; **37**:16–23.
- 65 Maslowski KM, Mackay CR. Diet, gut microbiota and immune responses. *Nat Immunol* 2011; **12**:5–9.
- 66 De Filippo C, Cavalieri D, Di Paola 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.
- 67 Wu GD, Chen J, Hoffmann C *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; **334**:105–8.
- 68 Ege MJ, Mayer M, Normand AC *et al.* Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* 2011; **364**:701–9.
- 69 Yatsunenko T, Rey FE, Manary MJ *et al.* Human gut microbiome viewed across age and geography. *Nature* 2012; **486**:222–7.
- 70 Mulder IE, Schmidt B, Stokes CR *et al.* Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. *BMC Biol* 2009; **7**:79–99.
- 71 Hildebrand F, Nguyen TL, Brinkman B *et al.* Inflammation-associated enterotypes, host genotype, cage and inter-individual effects drive gut microbiota variation in common laboratory mice. *Genome Biol* 2013; **14**:R4.
- 72 Turnbaugh PJ, Hamady M, Yatsunenko T *et al.* A core gut microbiome in obese and lean twins. *Nature* 2009; **457**:480–4.
- 73 Rehman A, Lepage P, Nolte A, Hellmig S, Schreiber S, Ott SJ. Transcriptional activity of the dominant gut mucosal microbiota in chronic inflammatory bowel disease patients. *J Med Microbiol* 2010; **59**:1114–22.
- 74 Claesson MJ, Jeffery IB, Conde S *et al.* Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012; **488**:178–84.
- 75 Minot S, Bryson A, Chehoud C, Wu GD, Lewis JD, Bushman FD. Rapid evolution of the human gut virome. *Proc Natl Acad Sci USA* 2013; **110**:12450–5.
- 76 Blackley CH. Experimental researches on the causes and nature of catarrhus aestivus (hay-fever and hay-asthma). London: Baillière Tindall and Cox, 1873.
- 77 Riedler J, Braun-Fahrlander C, Eder W *et al.* Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; **358**:1129–33.
- 78 Axelsson E, Ratnakumar A, Arendt ML *et al.* The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* 2013; **495**:360–4.
- 79 Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002; **288**:963–72.
- 80 Aichbaumik N, Zoratti EM, Strickler R *et al.* Exposure to household pets influences fetal immunoglobulin E production. *Clin Exp Allergy* 2008; **38**:1787–94.
- 81 Song SJ, Lauber C, Costello EK *et al.* Cohabiting family members share microbiota with one another and with their dogs. *Elife* 2013; **2**:e00458.
- 82 Fujimura KE, Johnson CC, Ownby DR *et al.* Man's best friend? The effect of pet ownership on house dust microbial communities. *J Allergy Clin Immunol* 2010; **126**:410–2, 2 e1–3.
- 83 Dunn RR, Fierer N, Henley JB, Leff JW, Menninger HL. Home life: factors structuring the bacterial diversity found within and between homes. *PLOS ONE* 2013; **8**:e64133.
- 84 Pakarinen J, Hyvarinen A, Salkinoja-Salonen M *et al.* Predominance of Gram-positive bacteria in house dust in the low-allergy risk Russian Karelia. *Environ Microbiol* 2008; **10**:3317–25.
- 85 Hong HA, To E, Fakhry S, Baccigalupi L, Ricca E, Cutting SM. *Bacillus subtilis* isolated from the human gastrointestinal tract. *Res Microbiol* 2009; **160**:134–43.
- 86 Nicholson WL. Roles of *Bacillus* endospores in the environment. *Cell Mol Life Sci* 2002; **59**:410–6.
- 87 Hong HA, To E, Fakhry S, Baccigalupi L, Ricca E, Cutting SM. Defining the natural habitat of *Bacillus* spore-formers. *Res Microbiol* 2009; **160**:375–9.
- 88 Casula G, Cutting SM. *Bacillus* probiotics: spore germination in the gastrointestinal tract. *Appl Environ Microbiol* 2002; **68**:2344–52.
- 89 Tam NK, Uyen NQ, Hong HA *et al.* The intestinal life cycle of *Bacillus subtilis* and close relatives. *J Bacteriol* 2006; **188**:2692–700.
- 90 Rhee KJ, Sethupathi P, Driks A, Lanning DK, Knight KL. Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. *J Immunol* 2004; **172**:1118–24.
- 91 Maas J, Verheij RA, Groenewegen PP, de Vries S, Spreeuwenberg P. Green space, urbanity, and health: how strong is the relation? *J Epidemiol Community Health* 2006; **60**:587–92.

- 92 Mitchell R, Popham F. Effect of exposure to natural environment on health inequalities: an observational population study. *Lancet* 2008; **372**:1655–60.
- 93 Dadvand P, de Nazelle A, Figueras F *et al.* Green space, health inequality and pregnancy. *Environ Int* 2012; **40**:110–5.
- 94 Aspinall P, Mavros P, Coyne R, Roe J. The urban brain: analysing outdoor physical activity with mobile EEG. *Br J Sports Med* 2013; Mar 6. [Epub ahead of print].
- 95 Morris DW. Adaptation and habitat selection in the eco-evolutionary process. *Proc Biol Sci* 2011; **278**:2401–11.
- 96 Radon K, Windstetter D, Poluda AL, Mueller B, von Mutius E, Koletzko S. Contact with farm animals in early life and juvenile inflammatory bowel disease: a case–control study. *Pediatrics* 2007; **120**:354–61.
- 97 Miller GE, Chen E, Fok AK *et al.* Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proc Natl Acad Sci USA* 2009; **106**:14716–21.
- 98 Chen E, Miller GE. Socioeconomic status and health: mediating and moderating factors. *Annu Rev Clin Psychol* 2013; **9**:723–49.
- 99 Seeman T, Epel E, Gruenewald T, Karlamangla A, McEwen BS. Socio-economic differentials in peripheral biology: cumulative allostatic load. *Ann NY Acad Sci* 2010; **1186**:223–39.
- 100 Broyles ST, Staiano AE, Drazba KT, Gupta AK, Sothorn M, Katzmarzyk PT. Elevated C-reactive protein in children from risky neighborhoods: evidence for a stress pathway linking neighborhoods and inflammation in children. *PLOS ONE* 2012; **7**:e45419.
- 101 Carpenter LL, Gawuga CE, Tyrka AR, Lee JK, Anderson GM, Price LH. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology* 2010; **35**:2617–23.
- 102 Danese A, Moffitt TE, Pariante CM, Ambler A, Poulton R, Caspi A. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. *Arch Gen Psychiatry* 2008; **65**:409–15.
- 103 Danese A, Pariante CM, Caspi A, Taylor A, Poulton R. Childhood maltreatment predicts adult inflammation in a life-course study. *Proc Natl Acad Sci USA* 2007; **104**:1319–24.
- 104 Entringer S, Kumsta R, Nelson EL, Hellhammer DH, Wadhwa PD, Wust S. Influence of prenatal psychosocial stress on cytokine production in adult women. *Dev Psychobiol* 2008; **50**:579–87.
- 105 Pace TW, Mletzko TC, Alagbe O *et al.* Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *Am J Psychiatry* 2006; **163**:1630–3.
- 106 Sepa A, Frodi A, Ludvigsson J. Mothers' experiences of serious life events increase the risk of diabetes-related autoimmunity in their children. *Diabetes Care* 2005; **28**:2394–9.
- 107 Vlajinac H, Sipetic S, Marinkovic J, Bjekic M, Kocev N, Sajic S. The Belgrade childhood diabetes study – comparison of children with type 1 diabetes with their siblings. *Paediatr Perinat Epidemiol* 2006; **20**:238–43.
- 108 Peng H, Hagopian W. Environmental factors in the development of Type 1 diabetes. *Rev Endocr Metab Disord* 2006; **7**:149–62.
- 109 Chen E, Miller GE, Walker HA, Arevalo JM, Sung CY, Cole SW. Genome-wide transcriptional profiling linked to social class in asthma. *Thorax* 2009; **64**:38–43.
- 110 Ekblom A, Adami HO, Helmick CG, Jonzon A, Zack MM. Perinatal risk factors for inflammatory bowel disease: a case–control study. *Am J Epidemiol* 1990; **132**:1111–9.
- 111 Stringhini S, Batty GD, Bovet P *et al.* Association of lifecourse socioeconomic status with chronic inflammation and type 2 diabetes risk: the Whitehall II prospective cohort study. *PLoS Med* 2013; **10**:e1001479.
- 112 Kapoor A, Dunn E, Kostaki A, Andrews MH, Matthews SG. Fetal programming of hypothalamo–pituitary–adrenal function: prenatal stress and glucocorticoids. *J Physiol* 2006; **572**:31–44.
- 113 Weinstock M. The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain Behav Immun* 2005; **19**:296–308.
- 114 Entringer S, Kumsta R, Hellhammer DH, Wadhwa PD, Wust S. Prenatal exposure to maternal psychosocial stress and HPA axis regulation in young adults. *Horm Behav* 2009; **55**:292–8.
- 115 Heim C, Newport DJ, Heit S *et al.* Pituitary–adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA* 2000; **284**:592–7.
- 116 O'Mahony SM, Marchesi JR, Scully P *et al.* Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biol Psychiatry* 2009; **65**:263–7.
- 117 Bailey MT, Lubach GR, Coe CL. Prenatal stress alters bacterial colonization of the gut in infant monkeys. *J Pediatr Gastroenterol Nutr* 2004; **38**:414–21.
- 118 Jenq RR, Ubeda C, Taur Y *et al.* Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *J Exp Med* 2012; **209**:903–11.
- 119 Hayakawa M, Asahara T, Henzan N *et al.* Dramatic changes of the gut flora immediately after severe and sudden insults. *Dig Dis Sci* 2011; **56**:2361–5.
- 120 Karlsson F, Tremaroli V, Nielsen J, Backhed F. Assessing the human gut microbiota in metabolic diseases. *Diabetes* 2013; **62**:3341–9.
- 121 Meyer U, Feldon J, Dammann O. Schizophrenia and autism: both shared and disorder-specific pathogenesis via perinatal inflammation? *Pediatr Res* 2011; **69**:26R–33R.
- 122 Hagberg H, Gressens P, Mallard C. Inflammation during fetal and neonatal life: implications for neurologic and neuropsychiatric disease in children and adults. *Ann Neurol* 2012; **71**:444–57.
- 123 Brown AS, Derkits EJ. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *Am J Psychiatry* 2010; **167**:261–80.
- 124 Willette AA, Lubach GR, Knickmeyer RC *et al.* Brain enlargement and increased behavioral and cytokine reactivity in infant monkeys following acute prenatal endotoxemia. *Behav Brain Res* 2011; **219**:108–15.
- 125 Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci* 2007; **27**:10695–702.
- 126 Valkanova V, Ebmeier KP, Allan CL. CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. *J Affect Disord* 2013; **150**:736–44.
- 127 Raison CL, Lowry CA, Rook GAW. Inflammation, sanitation and consternation: loss of contact with co-evolved, tolerogenic microorganisms and the pathophysiology and treatment of major depression. *Arch Gen Psychiatry* 2010; **67**:1211–24.
- 128 Raison CL, Rutherford RE, Woolwine BJ *et al.* A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. *Arch Gen Psychiatry* 2012; **Supp 3**:1–11.

- 129 McDade TW, Hoke M, Borja JB, Adair LS, Kuzawa CW. Do environments in infancy moderate the association between stress and inflammation in adulthood? Preliminary evidence from a birth cohort in the Philippines. *Brain Behav Immun* 2012; **31**:23–30.
- 130 McDade TW, Borja JB, Adair L, Kuzawa CW. Depressive symptoms are not associated with inflammation in younger and older adults in the Philippines. *Evol Med Public Health* 2012; **2013**(1):18–23.
- 131 Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med* 2009; **71**:171–86.
- 132 Stanwell-Smith R, Bloomfield SF, Rook GAW. The hygiene hypothesis and its implications for home hygiene, lifestyle and public health. *International Scientific Forum on Home Hygiene* 2012. Available at: <http://www.ifh-homehygiene.org/reviews-hygiene-hypothesis> (accessed 10 February 2014).