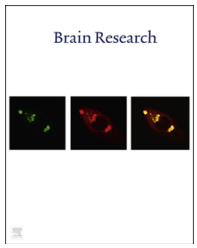
BRAIN RESEARCH ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research



Research Report

Hygiene and other early childhood influences on the subsequent function of the immune system

Graham A.W. Rook[a,*], *Christopher A. Lowry*[b], *Charles L. Raison*[c]

[a]*Centre for Clinical Microbiology, Department of Infection, University College London (UCL), London, UK*
[b]*Department of Integrative Physiology and Center for Neuroscience, University of Colorado Boulder, Boulder, CO 80309-0354, USA*
[c]*Department of Psychiatry, College of Medicine and Norton School of Family and Consumer Sciences, College of Agriculture and Life Sciences, University of Arizona, Tucson, AZ, USA*

ARTICLE INFO

Article history:
Accepted 5 April 2014

Keywords:
Microbiota
Immunoregulation
Co-evolution
Old Friends
Hygiene
Depression

ABSTRACT

The immune system influences brain development and function. Hygiene and other early childhood influences impact the subsequent function of the immune system during adulthood, with consequences for vulnerability to neurodevelopmental and psychiatric disorders. Inflammatory events during pregnancy can act directly to cause developmental problems in the central nervous system (CNS) that have been implicated in schizophrenia and autism. The immune system also acts indirectly by "farming" the intestinal microbiota, which then influences brain development and function via the multiple pathways that constitute the gut–brain axis. The gut microbiota also regulates the immune system. Regulation of the immune system is crucial because inflammatory states in pregnancy need to be limited, and throughout life inflammation needs to be terminated completely when not required; for example, persistently raised levels of background inflammation during adulthood (in the presence or absence of a clinically apparent inflammatory stimulus) correlate with an increased risk of depression. A number of factors in the perinatal period, notably immigration from rural low-income to rich developed settings, caesarean delivery, breastfeeding and antibiotic abuse have profound effects on the microbiota and on immunoregulation during early life that persist into adulthood. Many aspects of the modern western environment deprive the infant of the immunoregulatory organisms with which humans co-evolved, while encouraging exposure to non-immunoregulatory organisms, associated with more recently evolved "crowd" infections. Finally, there are complex interactions between perinatal psychosocial stressors, the microbiota, and the immune system that have significant additional effects on both physical and psychiatric wellbeing in subsequent adulthood.

This article is part of a Special Issue entitled Neuroimmunology in Health And Disease.

*Corresponding author.
E-mail address: g.rook@ucl.ac.uk (G.A.W. Rook).

http://dx.doi.org/10.1016/j.brainres.2014.04.004
0006-8993/

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

1. Introduction

This special issue addresses the relevance of the immune system to the development and function of the brain. However this chapter has the narrower remit of outlining factors in the perinatal and early childhood period that modulate subsequent function of the immune system. The ways in which the immune system is relevant to brain development and psychopathology are addressed in detail in other chapters of this volume, but some aspects need to be summarized here in order to put what follows into context. First, CD4+T lymphocytes (Th2-like, and also regulatory cells) are required in the meninges and choroid plexus for normal brain development and function, probably because they release necessary trophic and immunoregulatory factors (Baruch and Schwartz, 2013; Derecki et al., 2010; Rattazzi et al., 2013). Second, the immune system signals to the brain via cytokines and afferent nerves (Lowry et al., 2007; Miller et al., 2013). Third, we know that inflammatory events during pregnancy (whatever their cause) can cause developmental problems in the CNS that have been implicated in schizophrenia and autism (reviewed in Meyer et al., 2011). Clearly the nature and extent of the inflammatory episode is partly determined by the effector immune systems of mother and fetus, and by the immunoregulatory pathways that limit or terminate inflammation. Fourth, the immune system plays a crucial role in "farming" the intestinal microbiota, as explained later. This is important because the microbiota influences brain development and function via multiple pathways that constitute the gut–brain axis (Desbonnet et al., 2014; Heijtz et al., 2011; Stilling et al., 2013). Moreover, the microbiota also plays a major role in the regulation of the immune system. This brings us to the fifth major role of the immune system in brain function. Inflammatory states need to be restrained to the minimum effective level, and to be terminated completely when inflammation is not required, because inflammation is metabolically costly and also damages the host if allowed to persist. For example, poor regulation of inflammation will contribute to increased inflammatory responses to psychosocial stressors (Aschbacher et al., 2012; Pace et al., 2006; Rook et al., 2013), and persistently raised levels of background inflammation, manifested as raised C-reactive protein (CRP) in the absence of a clinically apparent inflammatory stimulus, correlate with an increased risk of depression 12 years later (Gimeno et al., 2009). The ways in which chronic inflammation can lead to CNS dysfunction have been extensively reviewed elsewhere (Miller et al., 2013). Finally, there are complex interactions between perinatal psychosocial stressors, the microbiota, the hypothalamic–pituitary–adrenal (HPA) axis and the immune system that have significant effects on HPA axis stress responses and wellbeing in subsequent adulthood.

2. Mammalian evolution and microorganisms

Humans interact with macro- and micro-organisms in ways that were mostly not appreciated until recently. All mammals are colonised internally and externally by a vast range of symbiotic species including viruses, archaea, bacteria, fungi, protozoa and even multicellular mites found in hair follicles and sebaceous glands. These diverse organisms constitute the microbiotas of epithelial linings, including skin, genitourinary system, airways, oropharynx and gut. Less than 10% of our cells are human, and the symbionts with which we co-evolved are essential components of our physiology. Indeed these symbionts contain at least 150-fold more genes that does the human genome itself (O'Hara and Shanahan, 2006), and recent studies of human metabolomics reveal that much of "our" metabolism is in fact microbial (Wikoff et al., 2009). Germ-free animals survive well in a germ-free environment, but they have multiple developmental, immunological, metabolic and behavioural abnormalities that are discussed and referenced throughout this chapter.

For example, these microbiotas provide signals that drive and modulate development of organs such as the gut, bones, immune system and brain (reviewed in McFall-Ngai et al., 2013). The brains of germ-free mice have altered chemistry and gene expression, and the animals behave abnormally (Heijtz et al., 2011). The HPA axis of germ-free animals is also abnormal, manifested as altered CNS gene expression and abnormal responses to stress (Sudo et al., 2004). To correct these abnormalities in mice it is necessary to reconstitute the gut microbiota with appropriate organisms within the first 6 weeks of life (Heijtz et al., 2011; Sudo et al., 2004). As adolescence in rodents is considered to be approximately postnatal days 28–42 (P28–42), the timing of this early critical period corresponds to human mid-adolescence (Spear, 2000). This suggests that early life events that modulate the microbiota of human babies, and the ability of the immune system to "farm" that microbiota, might be relevant to brain function and psychiatric health later in life.

The various microbiotas, together with other organisms from our evolutionary past to be discussed later, also drive development and maturation of the immune system. The understanding that the human is in reality a human-microbe symbiotic ecosystem has totally changed our concept of the immune system. In the past the immune system was thought to "police" self/non-self discrimination, but this is only part of a more complex reality. In fact the role of the immune system is to "farm" the symbionts (notably the gut microbiota), while rejecting infections, which might be defined as organisms that damage the host, either directly, or by upsetting the symbiotic ecosystems. Thus "self" to the immune system is not the genetically human component, but rather the entire "holobiont" including the symbionts that are part of our physiology (Gilbert et al., 2012). At least part of this "farming" of the microbiota is genetically encoded. It has been possible to demonstrate heritable genetic factors that control the composition of the gut microbiota in mice (Benson et al., 2010), and the same is probably true in humans. The gut microbiotas of monozygotic twins are more similar to each other than to unrelated subjects even when they are discordant for obesity and so must have experienced different diets and lifestyles (Tims et al., 2013). This implies a genetic influence, which is supported by some (Stewart et al., 2005) but not all other studies (Turnbaugh et al., 2009). Some understanding of how genes and the immune system might modulate the microbiota has come from experiments in gene

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

knockout mice. When genes encoding various components of the innate immune system such as MYD88, T-bet, IL-18 or TLR5 are inactivated, there can be increased (Garrett et al., 2007) or decreased (Wen et al., 2008) inflammatory responses, as well as metabolic changes leading to metabolic syndrome (Henao-Mejia et al., 2012; Vijay-Kumar et al., 2010). However in each of these diverse experimental systems, the altered phenotype can be transferred from the gene-knockout mice to wild-type mice by transferring microbiota from the knockout mice. Thus genetically encoded functions within the immune system modulate the microbiota, which in turn modulates the regulation of the immune system.

Thus a crucial function of the immune system is immunoregulation, which is tuned during development via inputs from microorganisms. The immune system must know when *not* to attack, because inflammatory responses to microbiota might disturb the commensal ecosystem, or eliminate physiologically essential partners, while causing tissue damage, manifested, for example as inflammatory bowel disease (IBD). Throughout this review the terms "immunoregulation" and "immunoregulatory" refer to those mechanisms that inhibit responses to inappropriate targets (such as self, gut contents and allergens) and that turn off inappropriate background inflammation, often manifested as raised C-reactive protein (CRP) in the absence of any clinically apparent need for an inflammatory response.

This chapter therefore focuses mostly on microbial factors acting on the mother, fetus or neonate that modulate subsequent control of inflammation. The discussion is based on an evolutionary approach, and in particular the human holobiont concept outlined above.

3. The hygiene hypothesis or "Old Friends" mechanism

The brain and the immune system are both learning systems that can only function correctly if they receive the appropriate data inputs both before and after birth. The immune system acquires most of its data from exposure to certain subsets of micro- and macroorganisms. The disruption of our exposure to these organisms is at least partly responsible for the immunoregulatory deficits that lie behind the increased prevalence of chronic inflammatory disorders (allergy, autoimmunity and IBD) in developed high-income countries (Bach, 2002). This mechanism is often called the "hygiene hypothesis" (Strachan, 1989) but we prefer terms such as the biodiversity hypothesis (von Hertzen et al., 2011)) or the Old Friends mechanism (Rook, 2010) because it is no longer an hypothesis, and it has little to do with hygiene, but is rather associated with broad changes in lifestyle to be described below.

Which organisms are involved in driving the immunoregulatory circuits that are the concern of this paper? Humans evolved as small hunter-gatherer groups colonized by the various microbiotas described in the introduction (Fig. 1). They were also exposed to microorganisms from the natural

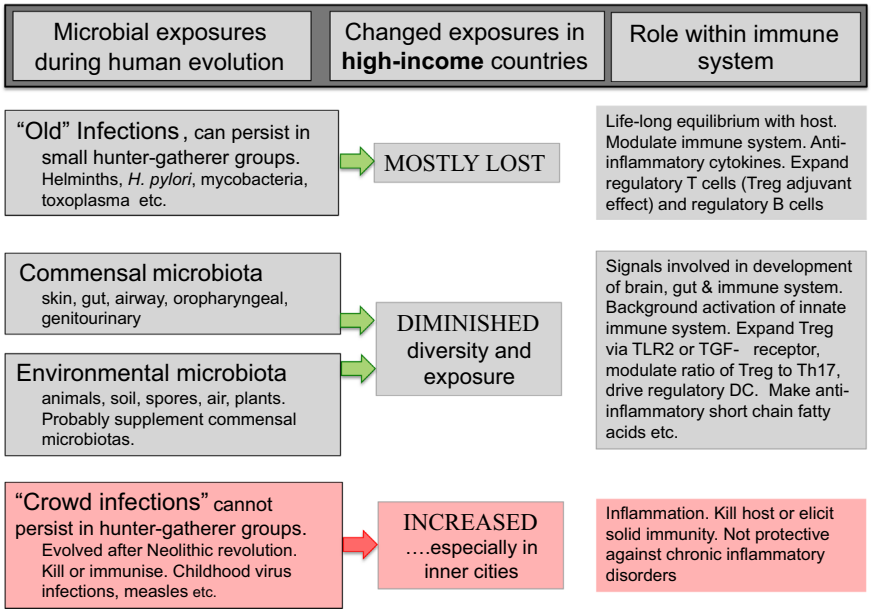


Fig. 1 – A simple categorization of organisms according to their source, and whether they co-evolved with humans and so developed immunoregulatory roles because they had to be tolerated. The "old" infections were able to persist in isolated hunter-gatherer groups as carrier states and latent infections. They neither killed nor immunized, but they modulated the immune system so as to ensure their own persistence, and the survival of the host. 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 after the Neolithic revolution when settlements arose, and populations increased. They kill the host or induce solid immunity, so could not persist in small hunter-gatherer groups. The crowd infections constitute the only category that is increased rather than decreased in some high-income settings (unless relevant vaccines are available, effective and widely used). Epidemiological studies show that the crowd infections are not immunoregulatory and fail to protect from the chronic inflammatory disorders.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

environment, some of which would have been able to establish themselves within the microbiotas (Mulder et al., 2009). Finally, there are certain "Old" infections that established lifelong carrier states or subclinical infections and so were able to survive within small hunter-gatherer groups. This term "Old" infections was used by Jared Diamond and his colleagues in their classic paper in 2007 (Wolfe et al., 2007). In order to be able to persist in small hunter-gatherer groups the Old infections had to avoid inducing effective immunity, or killing the host. And in order to maintain the health of the host, they had to be tolerated. Thus they drive regulatory anti-inflammatory responses. (The Old infections must not be confused with "Old Friends", a term used to include all the categories of organism with which human co-evolved, including notably the symbiotic microbiota (Fig. 1)). Ancestral strains of *Mycobacterium tuberculosis*, *Helicobacter pylori*, gut helminths and blood nematodes, hepatitis A virus (HAV) all fall into this category. Analysis of their phylogenetic trees, and comparison with the human phylogenetic tree, reveal how the Old infections co-evolved and spread over the globe with human populations (Comas et al., 2013; Linz et al., 2007; Wolfe et al., 2007). All these categories of organism were constantly present, and had to be tolerated and so co-evolved roles in setting up immunoregulatory pathways. For example, blood nematodes are powerfully immunoregulatory, and relatively harmless if tolerated, but aggressive immune responses that attempt (unsuccessfully) to eliminate them destroy the lymphatic system and result in elephantiasis (Babu et al., 2006). Immunoregulation by these three categories of organisms (Old infections, microbiotas and organisms from the natural environment), collectively known as the "Old Friends" has been reviewed in detail elsewhere (Rook, 2010; Rook et al., 2013), but briefly, they can be shown to block or treat a wide range of chronic inflammatory disorders in animal models (Osada and Kanazawa, 2010), and although many more mechanisms remain to be revealed, many of them secrete molecules that expand regulatory T cell (Treg) populations (Atarashi et al., 2011; Grainger et al., 2010; Round et al., 2011), or cause dendritic cells to drive Treg rather than inflammatory effector cells (Correale and Farez, 2013; Smits et al., 2005). The latter "Treg adjuvant" function might explain the observation that patients with early relapsing/remitting multiple sclerosis (MS) who picked up helminth infections were found to develop circulating populations of Treg specific for myelin basic protein that coincided with a halt in disease progression (Correale and Farez, 2007). These Treg disappeared when the helminth infections were treated, and disease progression then returned (Correale and Farez, 2011).

When human populations expanded after the Neolithic revolution, and urbanization commenced, humans began to be infected by the "crowd" infections such as measles. Because these organisms either kill the host or induce solid immunity, they could not have survived in isolated Paleolithic hunter-gatherer groups (Wolfe et al., 2007). As anticipated, therefore, epidemiological studies show that the "crowd infections" do not drive immunoregulation, and do not protect from the chronic inflammatory disorders that are increasing in developed high-income countries (Benn et al., 2004; Bremner et al., 2008; Dunder et al., 2007). Meanwhile the crowd infections are common in high-income urban communities, while modern air travel and population growth increase the threat from new crowd infections, such as avian influenza viruses.

In contrast to the crowd infections, the immunoregulatory Old Friends (microbiotas, Old infections and organisms from the natural environment) are depleted to varying extents from the modern high-income urban environment by a whole range of mechanisms that are discussed below.

4. Microbial factors in the perinatal period

Against this background we can consider the aspects of modern pregnancy and childhood that modulate the function of the immune system in ways that have consequences for vulnerability to psychiatric disorders later in life. We will do this in relation to the categorization of organisms described above. Much of the evidence cited will be derived from studies of chronic inflammatory disorders. This is justified because inflammation during pregnancy, whatever the cause (Meyer et al., 2011), is associated with brain developmental abnormalities, and because chronic inflammatory disorders during adulthood are associated with an increased risk of depression (Dhabhar et al., 2009; Graff et al., 2009; Raison et al., 2010). This risk tends to correlate with plasma levels of inflammatory mediators rather than with symptoms of the disease itself. If the immune system is poorly regulated, and also not capable of shutting itself down completely when no inflammatory response is required, there is an increased risk of psychopathology (reviewed in Rook et al., 2013). This failure of immunoregulation is seen in high-income developed countries where chronically raised CRP is common (Gimeno et al., 2009; Hemingway et al., 2003; McDade, 2012), as is an exaggerated inflammatory response to psychosocial stressors (Aschbacher et al., 2012; Pace et al., 2006). These persistent or labile inflammatory responses are associated with cardiovascular disease and depression (Aschbacher et al., 2012; Gimeno et al., 2009). In sharp contrast, persistently raised CRP was not seen in a longitudinal study of a low-income developing country setting, where episodes of inflammation driven by infection are followed by a return of the CRP levels to normal, suggesting that in this setting inflammation occurs when needed, but is successfully regulated when no longer needed (McDade et al., 2012b).

4.1. *Old infections*

In high-income developed countries most of the immunoregulatory Old infections are now rare. This depletion varies from almost total absence (e.g. helminths) to considerably diminished (e.g. *H. pylori*). For example, it is estimated that in 1947 about 36% of the population of Europe carried helminths such as *Enterobius vermicularis*, *Trichuris trichiura*, and *Ascaris lumbricoides*, but these have almost totally disappeared (Stoll, 1947). Since it is standard practice in many developing countries to deworm pregnant women, the consequences for the child of removing these organisms from the mother can be monitored. Deworming in pregnancy increases the risk of eczema and wheeze in the resulting infant (Mpairwe et al., 2011). Latent tuberculosis provides another example.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

Tuberculin-positive children are less likely to have allergic rhinitis or positive allergen skin prick tests (Obihara et al., 2005), but although tuberculosis is still a problem in poor areas of modern cities, most high socioeconomic status (SES) citizens of Europe or the USA are no longer infected (World_Health_Organization (2012)). Similarly rates of hepatitis A virus infection have fallen in high-income settings where the chronic inflammatory immunoregulatory disorders are increasing (Jacobsen and Wiersma, 2010; Seiskari et al., 2007).

A remarkable recent study recruited all pregnant women in an area of the Philippines, studied their homes and lifestyles, and then looked for correlations with health in the resulting children when they were in their early twenties. It was noted that high levels of exposure to animal feces during infancy correlated with *lower* levels of background CRP in adulthood (McDade et al., 2010), and with diminished inflammatory responses to psychosocial stressors (McDade et al., 2012a), consistent with the hypothesis that childhood exposure to "Old Friends" drives effective immunoregulation that persists into adulthood.

4.2. *Crowd infections*

In high-income settings pregnant women might have poor immunoregulation as a result of reduced contact with immunoregulation-inducing Old Friends. On the other hand exposure to the non-immunoregulatory crowd infections is common in high-income urban settings. Crowd infections, such as rubella or measles, can cause inflammatory events during pregnancy that lead to fetal CNS developmental abnormalities associated with the subsequent appearance of autism and schizophrenia (extensively reviewed and referenced in Meyer et al., 2011) (Crespi and Thiselton, 2011; Schwarz et al., 2011; Zerbo et al., 2012). In fact, it may be the convergence of a lack of contact with Old Friends, together with exposure to crowd infections that has the most potential for damaging effects. The concept that inflammatory mediators can drive developmental CNS abnormalities is supported by animal models (rodents and monkeys) showing that inflammation in the mother during pregnancy induced by injecting lipopolysaccharide (LPS) or poly (I:C) (which partly mimics virus infections) or by direct injection of IL-6, causes changes in the grey and white matter of the fetuses and behavioural changes that are reminiscent of autism and schizophrenia (Brown and Derkits, 2010; Smith et al., 2007; Willette et al., 2011). When virus-induced inflammation was mimicked during pregnancy in mice by injecting poly (I:C), the pregnancy resulted in offspring with increased expression of IL-6 mRNA and IL-6 protein in their colonic epithelium, increased gut permeability, altered gut microbiota particularly in the Bacteroidal and Clostridial operational taxonomic units (OTU), and behavioral abnormalities reminiscent of autism spectrum disorders (ASD) (Hsiao et al., 2013). The behavioral effect was at least partly due to a 46-fold increase in production and uptake of 4-ethylphenylsulfate, a metabolite dependent upon the microbiota. Remarkably, all of these abnormalities could be reversed by administration of *Bacteroides fragilis* (Hsiao et al., 2013), a probiotic organism previously shown to have potent immunoregulatory properties (Round and Mazmanian, 2010), despite the fact that the *B. fragilis* did not colonise the guts of the recipients.

Thus an important question is whether the risk that maternal infection will lead to fetal brain damage is itself influenced by the state of immunoregulation in the mother or the child? There is evidence for this. First, autism is associated with a family history of other chronic inflammatory immunoregulatory disorders such as autoimmunity and allergies (reviewed and referenced in Meyer et al., 2011), that have been strongly linked to the Old Friends mechanism (Correale and Farez, 2007; Ege et al., 2006), and the patients themselves also have increased risk of autoimmunity and IBD (Kohane et al., 2012). Second, we also know that prenatal exposure (i.e. of the pregnant mother) to the farming environment protects the infant against some allergic manifestations (Ege et al., 2008; Schaub et al., 2009). Thirdly, there is incontrovertible evidence of background inflammatory activity in autistics (Becker, 2007; Onore et al., 2012). Fourthly, genetic studies have revealed that some maternal genes involved in regulation of inflammation, such as *HLA-DR4*, are involved in modulating the risk of these disorders even when not inherited by the foetus (Johnson et al., 2009), suggesting the importance of genes involved in the regulation of inflammation in the mother. It is therefore reasonable to suggest that the Old Friends mechanism plays a role in susceptibility to developmental abnormalities of the brain.

4.3. *Organisms from the natural environment*

The gut microbiota of children from rural communities in low-income undeveloped countries is usually different from that of high-income Europeans (De Filippo et al., 2010) or Americans (Yatsunenko et al., 2012). The microbiota of children in Burkina Faso was dominated by Bacteroidetes, whereas microbiota from a matched control group of Italians was dominated by Firmicutes (De Filippo et al., 2010). Differences are not seen only at the phylum level. The biodiversity of microbiota from the USA was lower than that from Malawians, or Venezuelan Amerindians, and there were striking differences at the level of bacterial species and functional gene repertoires. Much of this variation might be attributable to diet, and, as outlined earlier, there is also a genetic component. What we do not know is the contribution of organisms from the natural environment. In fact we know remarkably little about the extent to which exposure to the natural environment can diversify the microbiota by colonization, because published data rarely identify organisms at the strain level, though this is changing as the methods develop. Moreover many gut organisms, particularly some Firmicutes, are spore-forming and these spores can persist in the environment for millennia. Spores from the natural environment, some of which will be derived from human or animal guts, can germinate in the small bowel (Hong et al., 2009). This is an unexplored area that needs attention (reviewed in Rook et al., 2014). Moreover, we are not aware of studies comparing the development of gut microbiota over time in human babies exposed or not exposed to the natural environment. However this has been attempted in pigs. Genetically similar piglets were housed in a natural outdoor environment, or reared in a very clean indoor facility.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

Firmicutes, in particular *Lactobacillus* strains were dominant in the gut microbiotas of the outdoor piglets, whereas the hygienic indoor piglets had reduced *Lactobacillus* and more potentially pathogenic phylotypes (Mulder et al., 2009). Moreover, the piglets reared in clean interiors had different patterns of gene expression in the ileum, much of it related to the immune system. They had increased Type 1 interferon activity, increased MHC Class 1, and upregulation of many chemokines (Mulder et al., 2009) implying a more inflammatory state in the guts of animals whose microbiota had not been modified by exposure to the natural environment. The issue of the immunoregulatory role of organisms from the natural environment was reviewed recently (Rook, 2013). It seems clear that modern human babies are brought up like the indoor rather than the outdoor piglets, which is likely to predispose them to poor control of inflammation.

The environment might contribute to microbial biodiversity. Reduced gut microbial biodiversity is often, but not always associated with poor control of inflammation. Mice exhibit at least two, partly genetically determined, enterotypes (bacterial ecosystems in the gut microbiota), one of which has low biodiversity, and correlates with biomarkers of inflammation (Hildebrand et al., 2013). Gut microbiota of limited diversity is also characteristic of several human inflammation-associated conditions such as obesity and inflammatory bowel disease (Rehman et al., 2010; Turnbaugh et al., 2009). Similarly, diminished microbiota biodiversity in institutionalized elderly people correlates with diminished health and raised levels of peripheral inflammatory markers such as IL-6 (Claesson et al., 2012).

Interestingly, the piglets reared in the clean indoor environment had reduced Lactobacilli and Firmicutes, and increased inflammatory biomarkers in their guts, but they did not have reduced overall biodiversity compared to the outdoor piglets (Mulder et al., 2009). Thus it remains uncertain whether biodiversity is important per se as has been suggested (von Hertzen et al., 2011), or merely increases the chances of encountering specific beneficial organisms.

4.4. *Microbiota and maternal behaviour*

In the perinatal period maternal factors such as birth mode, breast-feeding and birth order have large effects on the microbiotas of the infant that will inevitably translate into changes in immunoregulation (Penders et al., 2013). Interestingly, there is evidence that some organisms are passed from the mother to the fetus in utero before the start of the birth process (Funkhouser and Bordenstein, 2013). Such organisms have been reported not only in meconium, but also in fetal membranes, amniotic fluid and in cord blood of babies born by Caesarean section (Funkhouser and Bordenstein, 2013). But even if this proves to be correct it clearly does not result in adequate priming of the infant's microbiota.

4.4.1. *Caesarean section*

Data comparing Caesarean section with normal birth have to be interpreted with caution because of possible effects of the condition that led to the Caesarean, or effects of trauma experienced by the baby during the natural birth process. Nevertheless, it appears that birth by Caesarean section delays transfer of additional maternal microbiota and alters the course of colonization (Dominguez-Bello et al., 2010). This might explain the observations that Caesarean birth increases the risk of allergy (Guibas et al., 2013; Magnus et al., 2011; Thavagnanam et al., 2008), autoimmunity (Bonifacio et al., 2011; Cardwell et al., 2008), and, to a more modest extent, both coeliac (Decker et al., 2010), and inflammatory bowel disease (Bager et al., 2012; Malmborg et al., 2012). A nice example of the importance of transfer of maternal microbiota comes from the observation that if the mother thoroughly washes and boils the baby's pacifier (dummy) after it has fallen on the floor, that baby has an increased risk of developing asthma, eczema or allergic sensitization compared to babies whose mothers merely suck the pacifier and put it back in the baby's mouth (Hesselmar et al., 2013). The babies protected by exposure to pacifiers sucked clean by their mothers had demonstrably different oral microbiota (Hesselmar et al., 2013).

4.4.2. *Birth order*

The observation that birth order (i.e. having older siblings) can protect from allergic disorders (Strachan, 1989) is also now thought to be due to increased transfer of microbiota (Penders et al., 2013), and, as explained above, cannot be explained by exposure to crowd infections from those siblings, as now demonstrated epidemiologically (Benn et al., 2004; Bremner et al., 2008; Dunder et al., 2007). In a recent study in the USA, Type 1 diabetes (T1D) was also negatively associated with having older siblings (D'Angeli et al., 2010), but similar studies of the effects of birth order on T1D, and on other inflammatory conditions such as IBD and MS have painted a less consistent picture (Cardwell et al., 2011; Hampe et al., 2003; Van Kruiningen et al., 2007; Zilber et al., 1988). The effect of birth order is also rather variable for psychiatric disorders. For depression an association with birth order is sometimes reported, but the relationship is inconsistent (Bergeron et al., 2007; Schmidt and Tolle, 1977; Wells et al., 1985). The effect may be more pronounced for autism and schizophrenia. A comprehensive Finnish study of families with at least two children, one of whom was schizophrenic, found that being the firstborn was a significant risk factor for schizophrenia, but the protective effect of older siblings was complex and depended on how much older they were (Haukka et al., 2004). The relevance of birth order to autism has been reviewed in detail elsewhere (Becker, 2007). Briefly, the risk of autism has been shown to fall as the number of older siblings rises in studies in the United States, Western Australia and England, though not every study shows this (Becker, 2007). In view of the significant epidemiological association with familial allergic disorder, where the birth order effect is clear, this is of great interest (discussed in Meyer et al., 2011; Onore et al., 2012).

4.4.3. *Breastfeeding*

Breastfeeding also modulates the microbiota (Stark and Lee, 1982), and this might explain the protective effects of breastfeeding against eczema, and possibly against other allergic disorders (Kramer, 2011). There is mounting evidence for an entero-mammary pathway that transfers a wide range of microorganisms from the maternal gut to the baby via the breast milk (Donnet-Hughes et al., 2010; Hunt et al., 2011; Jost et al., 2013a, 2013b). Moreover human milk contains complex

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

polysaccharides that act as selective prebiotics and so encourage the colonization of the infant gut with appropriate microbiota (Garrido et al., 2012; Zivkovic et al., 2011), though breast milk is also biologically active in other ways that do not necessarily involve the microbiota (Labeta et al., 2000; Saarinen et al., 1999; van Neerven et al., 2012). However, whether as a consequence of immunoregulatory, microbiota-induced or other socio-environmental mechanisms, breast-feeding is important for the brain. Duration of breast-feeding is related to verbal and nonverbal intelligence later in life (Belfort et al., 2013), and to better cognitive and motor development (Bernard et al., 2013), and to greater social mobility (Sacker et al., 2013).

4.4.4. *Antibiotics, smoking, medication and diet*

Several other variables during pregnancy have effects on the incidence of immunoregulatory disorders, and might be operating via the immune system and/or microbiota. Use of antibiotics in the perinatal period delays colonization by *Bifidobacter* and *Lactobacillus* species (Faa et al., 2013; Westerbeek et al., 2006). This probably has long-term consequences because allergies (Droste et al., 2000; Metsala et al., 2013; Russell et al., 2012; Stensballe et al., 2013), irritable bowel syndrome (IBS) (Villarreal et al., 2012) and IBD (Hviid et al., 2011; Shaw et al., 2010) are all more frequent in antibiotic-exposed children.

The risk of IBD is increased in children of mothers who smoked (Roberts et al., 2011). This could also be due to changes to the microbiota, because cessation of smoking leads to increased microbial biodiversity, increased Firmicutes and Actinobacteria and a lower proportion of Bacteroidetes and Proteobacteria (Biedermann et al., 2013).

The child's microbiota might also be altered by prenatal exposure to valproate, an anticonvulsant drug and teratogen, administration of which during pregnancy is a risk factor for autism spectrum disorders (ASD) in the offspring (reviewed in de Theije et al., 2013a). In a mouse model, treating the pregnant mother with valproate caused changes in male offspring including epithelial cell loss and neutrophil infiltration in the intestinal tract, increased expression of neuroinflammatory markers in the brain and ASD-like behaviour. These inflammatory and behavioral symptoms were accompanied by significant changes in OTUs within the Bacteroidetes and Firmicutes (de Theije et al., 2013a, 2013b). The precise sequence of events is unclear but the concurrence of altered microbiota, inflammation and behavioral changes raises the possibility that this is also the sequence of causation.

Diet, apart from the issue of duration (if any) of breastfeeding, has received little attention in infants, but it has profound effects on all components of the gut microbiota (Hoffmann et al., 2013; Wu et al., 2011). For example it has been proposed that human microbiota can cluster in one of three different "enterotypes", dominated by Bacteroides, Prevotella, and Ruminococcus, respectively (Arumugam et al., 2011). The Bacteroides enterotype was associated with consumption of animal protein and saturated fats, whereas the Prevotella enterotype was associated with carbohydrates and simple sugars (Wu et al., 2011). In the mouse, at least, similar enterotypes show some correlation with background intestinal inflammation (Hildebrand et al., 2013), and a high fat diet encourages raised intake of endotoxin (LPS), weight gain and diabetes (Cani et al., 2007). Some authors consider diet-induced changes in microbiota to be the main factor causing immunoregulatory deficits in high-income countries (Maslowski and Mackay, 2011). For example the traditional lifestyle in low-income countries results in consumption of less saturated fat, and low ratios of proinflammatory *n*-6 polyunsaturated fatty acids (PUFA) to anti-inflammatory *n*-3 PUFA, whereas in Western high-income settings the diet often contains a ratio of *n*-6 to *n*-3 PUFA that exceeds 16:1 (Shen et al., 2013). This encourages complex changes in the microbiota and proinflammatory states (reviewed in Shen et al., 2013). However, even if diet is important, much of the immunoregulatory effect of diet is likely to be mediated via changes to the microbiota.

5. Immigration

Immigration from a low-income developing country to a high-income urban environment leads to progressive loss of many of the Old infections, reduced exposure to organisms from the natural environment, and changed microbiota. By contrast, exposure to many of the non-immunoregulatory crowd infections is likely to increase, except for those for which vaccines are provided in rich societies (Fig. 1). The loss of the Old Friends might be relevant to the fact that the chronic inflammatory disorders tend to be more common in immigrants to high-income urban environments than in the birth population from which the immigrant was derived (Ahlgren et al., 2011; Hou et al., 2009; Rottem et al., 2005; Soderstrom et al., 2012). However, age at immigration is crucial, suggesting the importance of early events. Iranians who migrate to Sweden have twice the prevalence of MS seen in their birth country (Ahlgren et al., 2011). Interestingly, if the 2nd (or later) generation immigrants return to their developing country of origin, they retain their increased susceptibility to MS, which remains higher than in the local population that was not born abroad (Cabre, 2009). A similar phenomenon was seen when people born in the United Kingdom (UK: a high MS country) migrated to South Africa (SA: a low MS country). Migration from the UK to SA was protective when the migrant was a child, whereas adult migrants retained their high UK prevalence of MS (Dean, 1967). Analysis of this and other studies suggests that the environmental factors that protect from or predispose to MS act during the first two decades of life (Gale and Martyn, 1995; Milo and Kahana, 2010). The same is true for T1D. Here the crucial factor is to have been *born* in the receiving developed country, again suggesting that relevant environmental factors act very early, or even in the prenatal period (Soderstrom et al., 2012). Similar observations exist for IBD (Carr and Mayberry, 1999; Li et al., 2011), and allergic disorders (Eldeirawi et al., 2009; Hjern et al., 1999).

5.1. *Immigration and psychiatric disorders*

In view of the relationship between inflammation and psychiatric disorders outlined above, it is interesting that the immigrant effect is also seen in psychiatric disorders, including depression and anxiety (Breslau et al., 2011), schizophrenia

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

(Dealberto, 2010) and autism (Keen et al., 2010). Again the age of the individual at the time of immigration turns out to be important. Depression is particularly interesting in this respect (Breslau et al., 2009; Vega et al., 2004). Mexicans, Cubans and African/Caribbean peoples have a 2–3-fold increase in the prevalence of depression if immigration to the USA occurred when the individual was less than 13 years old, or was born in the USA, compared to the prevalence in those who migrated after the age of 13 (Breslau et al., 2009). But this is not likely due to psychosocial stress related to skin color, because white Eastern European immigrants show the same effect. In sharp contrast, the effect is not seen in immigrants from Western Europe, or from Puerto Rico, which is closely associated with the USA. (These last two populations already have a high prevalence of depression that is not increased by immigrating to, or being born in, the USA) (Breslau et al., 2009). These findings imply that influences important for determining vulnerability to depression occur perinatally, or in the early years of life.

Immigration also increases the risk of psychotic disorders (Coid et al., 2008). A large Danish study noted that immigration into Denmark when less than 4 years old was associated with a strikingly increased risk for psychotic disorders, whereas the increased risk gradually decreased with older age at migration and disappeared in those immigrating when more than 29 years old (Veling et al., 2011). Similarly a large meta-analysis confirmed that schizophrenia was increased amongst 1st generation immigrants, and further increased amongst 2nd generation immigrants, particularly when the country of origin was a developing one (Cantor-Graae and Selten, 2005). Again, early events seem crucial.

Age at immigration is irrelevant to an early onset condition such as autism, but autism is strikingly (as much as 10-fold) increased in 2nd generation Caribbean or African immigrants born in the UK, compared to children of white UK-born mothers (Keen et al., 2010), implying that the damage is done very early in prenatal development.

These findings implicate crucial early events in the perinatal period or early childhood as risk factors for chronic inflammatory disorders, but also for depression, schizophrenia and autism. However, it is difficult to disentangle the effects of immunoregulatory problems attributable to changed microbial inputs to the immune system, from the multiple other factors that affect immigrants, particularly stress.

6. Perinatal psychosocial stress and immunoregulation

Although immigrants certainly meet a changed microbial environment that will have immunoregulatory consequences, it is equally certain that they face psychosocial stressors. Such stressors will cause further immunoregulatory changes because of well-documented effects of perinatal stress on inflammation, the HPA axis, and the composition of the microbiota.

6.1. *Perinatal stress and inflammation*

Many studies in animals and humans have shown that psychosocial stressors during pregnancy activate inflammation (Haroon et al., 2012; Howerton and Bale, 2012), detectable as raised circulating cytokines or CRP (Coussons-Read et al., 2005, 2007). The crucial point in the current context is that such perinatal inflammatory episodes result in adults who themselves show exaggerated inflammatory responses to stress (Carpenter et al., 2010; Danese et al., 2007, 2008) (Fig. 2). For example, peripheral blood mononuclear cells

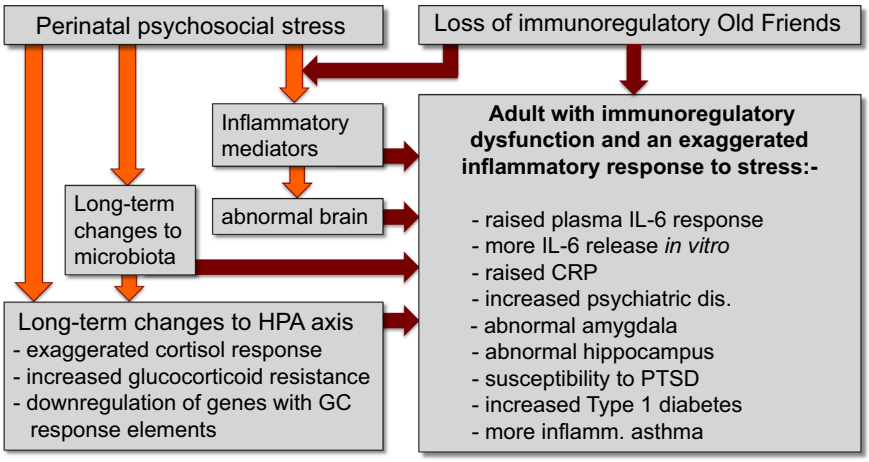


Fig. 2 – Perinatal influences on adult immunoregulation. Multiple factors in the perinatal period influence the developing brain, immune system, microbiota and HPA axis. Withdrawal of immunoregulation-inducing Old Friends (from the mother and from the neonate) is often exacerbated by immigration from a low-income to a high-income developed urban setting. Exposure to perinatal psychosocial stressors can also result in immunoregulatory defects that are apparent in the adult. Such adults have increased risk of chronic inflammatory disorders, and increased inflammatory responses to psychosocial stressors, resulting in susceptibility to depression and other psychiatric conditions. Since much of the effect of psychosocial stressors is mediated via inflammatory pathways, the lack of immunoregulatory Old Friends probably exacerbates the consequence of such stressors. The figure does not show the additional effects, via the microbiota, of birth order, lack of breastfeeding, and abuse of antibiotics that are described in the main text.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

from healthy young women whose mothers had experienced major negative life events during pregnancy showed altered responses to phytohemagglutinin compared to cells from a control group (Entringer et al., 2008). Adverse events early in childhood have a similar effect. 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 (Carpenter et al., 2010; Pace et al., 2006), and maltreated children tend to have higher levels of CRP 20 years later (Danese et al., 2007), and similar observations have been made in children from low socioeconomic status (SES) backgrounds (Miller et al., 2009).

Interestingly, negative life events in the neonatal period, whether they affect the child directly or indirectly via traumatic experiences of the mother, also predispose to the autoimmune disease T1D (reviewed in Peng and Hagopian, 2006; Sepa et al., 2005; Vlajinac et al., 2006). It is likely that this reflects the influence of perinatal negative life events on subsequent immunoregulation described above. However a study of children born in the Philippines suggested that traumatic childhood events did not lead to raised CRP in adulthood in those children who had experienced heavy microbial exposures during infancy (McDade et al., 2010, 2012a). Perhaps the risk that perinatal stress will lead to an immunoregulatory deficit in adulthood can be attenuated by the immunoregulatory effect of the Old Friends? As we have pointed out elsewhere, lack of contact with immunoregulatory Old Friends may lead to decreased stress resilience (Rook et al., 2013).

But why does immune activation during pregnancy lead to increased background inflammatory activity in adulthood? One mechanism may be altered development of the immune response itself. Pups born to mice that received inflammatory stimuli during pregnancy developed immune systems in adulthood that were biased towards maturation of inflammatory Th17 cells, and more prone to induction of the autoimmune condition Experimental Autoallergic Encephalomyelitis (EAE), often considered to mimic MS (Mandal et al., 2013). However some of the developmental change in the immune system might be secondary to stress-induced changes in the HPA axis and the microbiota.

6.2. *Perinatal stress and long-term changes to the HPA axis*

Numerous animal models have demonstrated associations between prenatal stress and long-term alterations in HPA axis function (Coe et al., 1996, 1999; Kapoor et al., 2006; Weinstock, 2005). Healthy young adult humans who had been exposed to prenatal stress, because their mothers had experienced severe negative life events during pregnancy, responded differently to a standardized social stressor (TSST) when compared to an age-matched comparison group of healthy young adults who had not been exposed to prenatal stress. The prenatal stress group had lower cortisol levels ($p=0.007$) before the TSST, and a larger increase in response to the TSST ($p=0.03$) (Entringer et al., 2009b). Similar changes have been associated with severe stress in early childhood (Heim et al., 2000). Moreover, adults with post-traumatic stress disorder (PTSD) symptoms who were abused as children show increased nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and decreased glucocorticoid sensitivity and these two findings are highly correlated (Pace et al., 2012). This is consistent with the idea that HPA axis changes as a result of early abuse or neglect contribute to increased inflammatory drive. The persistence into adult life of HPA axis effects triggered in the perinatal period might be explained by epigenetic changes, such as altered DNA methylation patterns (Guo et al., 2011; Miller et al., 2008; Weaver et al., 2004), or by shortened telomere length, and reduced telomerase activity (Choi et al., 2008; Entringer et al., 2011; Jacobs et al., 2011; Ornish et al., 2008). Reduced telomere length is associated with inflammation and autoimmunity as well as with premature immunosenescence (Carrero et al., 2008; Fitzpatrick et al., 2007; Hohensinner et al., 2011), though whether as cause or consequence is not certain.

Interestingly, a transcriptional profiling of adults whose childhood background had been of low or high socioeconomic status (SES) revealed that those from a low childhood SES background had up-regulation of genes bearing response elements for the cAMP response element binding (CREB)/activating transcription factors (ATF) family of transcription factors involved in signaling to leukocytes, heightened expression of transcripts bearing response elements for NF-κB, and down-regulation of genes with response elements for the glucocorticoid receptor (GR) involved in anti-inflammatory function (Miller et al., 2009), suggesting a proinflammatory bias in adults who had a low SES childhood. A similar proinflammatory bias in gene expression emerged from a comparison of asthmatic children from low or high SES backgrounds (Chen et al., 2009). In conclusion, perinatal and early neonatal stressors are likely to induce long-term changes in HPA axis function with obvious consequences for immune function.

6.3. *Perinatal stress and long-term changes to the microbiota*

Stress alters the microbiota of experimental animals (Bailey et al., 2011; Kiliaan et al., 1998), and the same is true of the microbiota of severely stressed critically ill humans, where the changes are rapid and prolonged (Hayakawa et al., 2011). *Prenatal* stressors have been shown to alter the microbiome in rhesus monkeys by reducing the overall numbers of bifidobacteria and lactobacilli during adulthood (Bailey et al., 2004). In a rat model the stress of maternal separation in the neonatal period had long-term effects on the diversity of the microbiota that were still apparent when the pups became adults (O'Mahony et al., 2009).

This might be an important mechanism because the nature of the microbiota during the first weeks of life has a profound effect on development of the CNS and the HPA axis. For example, germ-free mice have increased motor activity, reduced anxiety, altered gene expression in several brain areas, and increased turnover of noradrenaline, dopamine and serotonin in the striatum (Heijtz et al., 2011). These abnormalities persist into adulthood, and cannot be corrected by reconstitution of the microbiota of adult animals (Heijtz et al., 2011). Moreover, the nature of the microbiota is crucial.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

In another study it was noted that germ-free mice had abnormal responses to restraint stress, specifically increased adrenocorticotrophic hormone (ACTH) and corticosterone responses, together with reduced expression of brain-derived neurotrophic factor (BDNF) in cortex and hippocampus, reduced glucocorticoid receptors (GR) in the cortex, and raised corticotrophin-releasing hormone (CRH) in the hypothalamus (Sudo et al., 2004). Oral reconstitution with normal microbiota normalised the HPA axis function if done at 6 weeks, but not if done later. Early mono-association with *Bifidobacterium infantis* also normalized HPA axis function, but mono-association with enteropathogenic *Escherichia coli* made the abnormalities more severe. Thus, not only is the microbiota modified by stress, but it is also involved in development of the CNS, so it is possible that perinatal stress exerts physiological effects on the brain in adulthood at least in part via its impact on the microbiota.

6.4. *Perinatal stress and the developing brain*

In view of these findings we should not be surprised that prenatal psychosocial stress (i.e. experienced by the pregnant mother) or early postnatal stress are associated with long-term changes in neurogenesis (reviewed in Korosi et al., 2012), in cognition and memory (Entringer et al., 2009a) and in HPA axis function (Entringer et al., 2009b). Moreover perinatal maternal anxiety affects early development of the hippocampus (Qiu et al., 2013), and a history of childhood trauma is manifested as a smaller hippocampal volume in women with major depression (Vythilingam et al., 2002). Early-life stress also induces persistent alterations in amygdala circuitry and function in mice and humans (Malter Cohen et al., 2013).

7. Genetics, epigenetics and future therapies

In parts of the world where there has been a heavy load of organisms causing immunoregulation (such as helminths) there has been selection for single nucleotide polymorphisms (SNP) or other variants that partially compensate for the immunoregulation and so restore the inflammatory response (Fumagalli et al., 2009). If immunoregulation-inducing organisms are withdrawn by the modern lifestyle, these genetic variants lead to excessive inflammation, and become risk factors for chronic inflammatory disorders (Barnes et al., 2005; Fredericks et al., 2010; Fumagalli et al., 2009; Moller et al., 2007). This suggests that to some extent the presence of the Old Friends is a genetically determined necessity and that humans are in a state of evolved dependence on them.

On the other hand it is possible that the immunoregulatory role of some of these organisms becomes incorporated epigenetically or developmentally, and that this therefore occurs only in those individuals who are exposed to the relevant organisms *in utero* or in early postnatal life. For example, deworming pregnant women increases the risk of allergic problems in the infant (Mpairwe et al., 2011), but deworming older children does not always have this effect (Cooper et al., 2006; Flohr et al., 2010; van den Biggelaar et al., 2004). Similarly, the reduced prevalence of allergic disorders after exposure to the farm environment is most evident if the exposure is during pregnancy or the neonatal period (Ege et al., 2008; Riedler et al., 2001). Moreover, in a helminth-endemic country (Argentina) allowing patients with early MS to become infected with helminths appears to stop progression of the disease, and drive formation of Treg specific for myelin basic protein (Correale and Farez, 2007, 2011). But it remains to be seen whether helminths will also treat MS in the current generation of Americans or Europeans who had no perinatal exposure to helminths. Their immune systems developed without "anticipating" helminth-mediated anti-inflammatory immunoregulation. Without this developmental and epigenetic programming, the helminths might not be necessary or even effective. The recent failure of trials attempting to treat IBD with *Trichuris suis* in Americans or Europeans (http://tinyurl.com/nodazuu) is disappointing, and should alert us to this possibility, though it is also possible that the repeated dosing required when using this organism, which cannot establish itself in the human gut, eventually drives immunity rather than the systemic immunoregulation seen with natural human infections that become established and tolerated (Correale and Farez, 2013).

Some authors believe that the future lies in developing "domesticated" variants of the Old infections, such as genetically modified helminths, that could be administered to all children, and carried for life (Parker and Ollerton, 2013). Interestingly there are signs that the Bacille Calmette & Guérin (BCG) which is an attenuated strain of the organism causing bovine tuberculosis (which itself evolved from human tuberculosis strains), has beneficial effects in patients with very early MS (Ristori et al., 2014), in which case perhaps we already have one "domesticated" version of an Old infection with which we co-evolved (Comas et al., 2013).

However, where the microbiota is concerned, the situation might be different. The presence of the microbiota appears to be a true genetically determined physiological and metabolic necessity, because of the developmental and metabolic abnormalities that occur in its absence (Bailey et al., 2011; Heijtz et al., 2011; Sudo et al., 2004; Wikoff et al., 2009). Moreover, the organisms comprising the gut microbiota are themselves a form of epigenetic inheritance as pointed out by others (Stilling et al., 2013). Since microbiota can drive potent immunoregulatory responses (Atarashi et al., 2011; Hsiao et al., 2013; Round and Mazmanian, 2010), appropriate modulation of the gut microbiota may be able to compensate for the faulty immunoregulation that partly explains the increases in chronic inflammatory and psychiatric disorders in developed high-income countries. Such therapies might stop gut inflammation, correct the poorly defined signals of the gut–brain axis, and terminate the persistently raised levels of circulating inflammatory mediators commonly seen in high-income settings. It is encouraging that consumption by healthy individuals of a probiotic-rich fermented milk product for 4 weeks was able to modulate activity in brain regions that control central processing of emotion and sensation (Tillisch et al., 2013). Similarly a probiotic formulation taken daily for 30 days reduced anxiety, depression and perceived stress in subjects recruited from the general population (Messaoudi et al., 2011).

In conclusion, in developed high-income settings there is an epidemic of chronic inflammatory disorders, and of

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

persistently raised circulating levels of inflammatory mediators. These inflammatory states are associated with psychiatric disorders. In this paper we outline the factors in the perinatal period and early childhood that influence the way in which the immune system regulates and terminates these inflammatory states. The perinatal factors discussed all directly or indirectly influence the microbial inputs to the immune system. The future may lie either in development of domesticated versions of the Old infections (helminths, tuberculosis etc), or in exploitation of the regulatory role of the microbiota. It will be particularly helpful if those performing clinical trials of these strategies in the fields of allergy, autoimmunity and inflammatory bowel disease include psychiatric assessments.

Acknowledgments

GAWR is supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. CLR receives grant support from the National Center for Complementary and Alternative Medicine (R01AT004698, R01AT004698-01A1S1), the Depressive and Bipolar Disorder Alternative Treatment Foundation and The Brain and Behavior Research Foundation. He reports the following activities for the previous two 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. CAL receives grant support from the National Institute of Mental Health (R01MH065702, R01DA019921), the National Science Foundation (NSF-IOS 0921969), the Depressive and Bipolar Disorder Alternative Treatment Foundation, and is the recipient of a NARSAD, Brain & Behavior Research Foundation 2010 Young Investigator Award.

REFERENCES

Ahlgren, C., et al., 2011. A nationwide survey of the prevalence of multiple sclerosis in immigrant populations of Sweden. Mult. Scler..

Arumugam, M., et al., 2011. Enterotypes of the human gut microbiome. Nature 473, 174–180.

Aschbacher, K., et al., 2012. Maintenance of a positive outlook during acute stress protects against pro-inflammatory reactivity and future depressive symptoms. Brain Behav. Immun. 26, 346–352.

Atarashi, K., et al., 2011. Induction of colonic regulatory T cells by indigenous clostridium species. Science 331, 337–341.

Babu, S., et al., 2006. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. J. Immunol. 176, 3248–3256.

Bach, J.F., 2002. The effect of infections on susceptibility to autoimmune and allergic diseases. N. Engl. J. Med. 347, 911–920.

Bager, P., et al., 2012. Cesarean section and offspring's risk of inflammatory bowel disease: a national cohort study. Inflamm. Bowel Dis. 18, 857–862.

Bailey, M.T., et al., 2004. Prenatal stress alters bacterial colonization of the gut in infant monkeys. J. Pediatr. Gastroenterol. Nutr. 38, 414–421.

Bailey, M.T., et al., 2011. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. Brain Behav. Immun. 25, 397–407.

Barnes, K.C., et al., 2005. A review of the genetic epidemiology of resistance to parasitic disease and atopic asthma: common variants for common phenotypes? Curr. Opin. Allergy Clin. Immunol 5, 379–385.

Baruch, K., Schwartz, M., 2013. CNS-specific T cells shape brain function via the choroid plexus. Brain Behav. Immun. 34, 11–16.

Becker, K.G., 2007. Autism, asthma, inflammation, and the hygiene hypothesis. Med. Hypotheses 69, 731–740.

Belfort, M.B., et al., 2013. Infant feeding and childhood cognition at ages 3 and 7 years: effects of breastfeeding duration and exclusivity. JAMA Pediatr 167, 836–844.

Benn, C.S., et al., 2004. Cohort study of sibling effect, infectious diseases, and risk of atopic dermatitis during first 18 months of life. Br. Med. J. 328, 1223.

Benson, A.K., et al., 2010. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc. Nat. Acad. Sci. U.S.A. 107, 18933–18938.

Bergeron, L., et al., 2007. Correlates of depressive disorders in the Quebec general population 6 to 14 years of age. J. Abnorm. Child Psychol. 35, 459–474.

Bernard, J.Y., et al., 2013. Breastfeeding duration and cognitive development at 2 and 3 years of age in the EDEN mother-child cohort. J. Pediatr. 163, 36-42 e1.

Biedermann, L., et al., 2013. Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans. PLoS One 8, e59260.

Bonifacio, E., et al., 2011. Cesarean section and interferon -induced helicase gene polymorphisms combine to increase childhood type 1 diabetes risk. Diabetes 60, 3300–3306.

Bremner, S.A., et al., 2008. Infections presenting for clinical care in early life and later risk of hay fever in two UK birth cohorts. Allergy 63, 274–283.

Breslau, J., et al., 2009. Immigration to the USA and risk for mood and anxiety disorders: variation by origin and age at immigration. Psychol. Med. 39, 1117–1127.

Breslau, J., et al., 2011. Migration from Mexico to the United States and subsequent risk for depressive and anxiety disorders: a cross-national study. Arch. Gen. Psychiatry 68, 428–433.

Brown, A.S., Derkits, E.J., 2010. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am. J. Psychiatry 167, 261–280.

Cabre, P., 2009. Environmental changes and epidemiology of multiple sclerosis in the French West Indies. J. Neurol. Sci. 286, 58–61.

Cani, P.D., et al., 2007. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56, 1761–1772.

Cantor-Graae, E., Selten, J.P., 2005. Schizophrenia and migration: a meta-analysis and review. Am. J. Psychiatry 162, 12–24.

Cardwell, C.R., et al., 2008. Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. Diabetologia 51, 726–735.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

Cardwell, C.R., et al., 2011. Birth order and childhood type 1 diabetes risk: a pooled analysis of 31 observational studies. Int. J. Epidemiol. 40, 363–374.
Carpenter, L.L., et al., 2010. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. Neuropsychopharmacology 35, 2617–2623.
Carr, I., Mayberry, J.F., 1999. The effects of migration on ulcerative colitis: a three-year prospective study among Europeans and first- and second- generation South Asians in Leicester (1991–1994). Am. J. Gastroenterol. 94, 2918–2922.
Carrero, J.J., et al., 2008. Telomere attrition is associated with inflammation, low fetuin-A levels and high mortality in prevalent haemodialysis patients. J. Intern. Med. 263, 302–312.
Chen, E., et al., 2009. Genome-wide transcriptional profiling linked to social class in asthma. Thorax 64, 38–43.
Choi, J., et al., 2008. Reduced telomerase activity in human T lymphocytes exposed to cortisol. Brain Behav. Immun. 22, 600–605.
Claesson, M.J., et al., 2012. Gut microbiota composition correlates with diet and health in the elderly. Nature 488, 178–184.
Coe, C.L., et al., 1996. Prenatal endocrine activation alters postnatal cellular immunity in infant monkeys. Brain Behav. Immun. 10, 221–234.
Coe, C.L., et al., 1999. Prenatal stress and immune recognition of self and nonself in the primate neonate. Biol. Neonate 76, 301–310.
Coid, J.W., et al., 2008. Raised incidence rates of all psychoses among migrant groups: findings from the East London first episode psychosis study. Arch. Gen. Psychiatry 65, 1250–1258.
Comas, I., et al., 2013. Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans. Nat. Genet.
Cooper, P.J., et al., 2006. Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. Lancet 367, 1598–1603.
Correale, J., Farez, M., 2007. Association between parasite infection and immune responses in multiple sclerosis. Ann. Neurol. 61, 97–108.
Correale, J., Farez, M.F., 2011. The impact of parasite infections on the course of multiple sclerosis. J. Neuroimmunol. 233, 6–11.
Correale, J., Farez, M.F., 2013. Parasite infections in multiple sclerosis modulate immune responses through a retinoic acid-dependent pathway. J. Immunol. 191, 3827–3837.
Coussons-Read, M.E., et al., 2005. Prenatal stress alters cytokine levels in a manner that may endanger human pregnancy. Psychosom. Med. 67, 625–631.
Coussons-Read, M.E., et al., 2007. Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. Brain Behav. Immun. 21, 343–350.
Crespi, B.J., Thiselton, D.L., 2011. Comparative immunogenetics of autism and schizophrenia. Genes Brain Behav. 10, 689–701.
D'Angeli, M.A., et al., 2010. Environmental factors associated with childhood-onset type 1 diabetes mellitus: an exploration of the hygiene and overload hypotheses. Arch. Pediatr. Adolesc. Med. 164, 732–738.
Danese, A., et al., 2007. Childhood maltreatment predicts adult inflammation in a life-course study. Proc. Nat. Acad. Sci. U.S.A. 104, 1319–1324.
Danese, A., et al., 2008. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Arch. Gen. Psychiatry 65, 409–415.
De Filippo, C., et al., 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc. Nat. Acad. Sci. U.S.A. 107, 14691–14696.
de Theije, C.G., et al., 2013a. Intestinal inflammation in a murine model of autism spectrum disorders. Brain Behav. Immun.
de Theije, C.G., et al., 2013b. Altered gut microbiota and activity in a murine model of autism spectrum disorders. Brain Behav. Immun.
Dealberto, M.J., 2010. Ethnic origin and increased risk for schizophrenia in immigrants to countries of recent and longstanding immigration. Acta Psychiatr. Scand. 121, 325–339.
Dean, G., 1967. Annual incidence, prevalence, and mortality of multiple sclerosis in white South-African-born and in white immigrants to South Africa. Br. Med. J. 2, 724–730.
Decker, E., et al., 2010. Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. Pediatrics 125, e1433–e1440.
Derecki, N.C., et al., 2010. Regulation of learning and memory by meningeal immunity: a key role for IL-4. J. Exp. Med. 207, 1067–1080.
Desbonnet, L., et al., 2014. Microbiota is essential for social development in the mouse. Mol. Psychiatry 19, 146–148.
Dhabhar, F.S., et al., 2009. Low serum IL-10 concentrations and loss of regulatory association between IL-6 and IL-10 in adults with major depression. J. Psychiatr. Res. 43, 962–969.
Dominguez-Bello, M.G., et al., 2010. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc. Nat. Acad. Sci. U.S.A. 107, 11971–11975.
Donnet-Hughes, A., et al., 2010. Potential role of the intestinal microbiota of the mother in neonatal immune education. Proc. Nutr. Soc. 69, 407–415.
Droste, J.H., et al., 2000. Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease? Clin. Exp. Allergy 30, 1548–1553.
Dunder, T., et al., 2007. 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. 161, 972–977.
Ege, M.J., et al., 2006. 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. 117, 817–823.
Ege, M.J., et al., 2008. Prenatal exposure to a farm environment modifies atopic sensitization at birth. J. Allergy Clin. Immunol. 122 (407-12), 412 e1 (412e4).
Eldeirawi, K., et al., 2009. Associations of doctor-diagnosed asthma with immigration status, age at immigration, and length of residence in the United States in a sample of Mexican American School Children in Chicago. J. Asthma 46, 796–802.
Entringer, S., et al., 2008. Influence of prenatal psychosocial stress on cytokine production in adult women. Dev. Psychobiol. 50, 579–587.
Entringer, S., et al., 2009a. Prenatal psychosocial stress exposure is associated with subsequent working memory performance in young women. Behav. Neurosci. 123, 886–893.
Entringer, S., et al., 2009b. Prenatal exposure to maternal psychosocial stress and HPA axis regulation in young adults. Horm. Behav. 55, 292–298.
Entringer, S., et al., 2011. Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. Proc. Nat. Acad. Sci. U.S.A. 108, E513–E518.
Faa, G., et al., 2013. Factors influencing the development of a personal tailored microbiota in the neonate, with particular emphasis on antibiotic therapy. J. Matern. Fetal Neonatal Med. 26 (Suppl. 2), 35–43.
Fitzpatrick, A.L., et al., 2007. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. Am. J. Epidemiol. 165, 14–21.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

Flohr, C., et al., 2010. Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. Clin. Exp. Allergy 40, 131–142.
Fredericks, C.A., et al., 2010. Healthy young women with serotonin transporter SS polymorphism show a pro-inflammatory bias under resting and stress conditions. Brain Behav. Immun. 24, 350–357.
Fumagalli, M., et al., 2009. Parasites represent a major selective force for interleukin genes and shape the genetic predisposition to autoimmune conditions. J. Exp. Med. 206, 1395–1408.
Funkhouser, L.J., Bordenstein, S.R., 2013. Mom knows best: the universality of maternal microbial transmission. PLoS Biol. 11, e1001631.
Gale, C.R., Martyn, C.N., 1995. Migrant studies in multiple sclerosis. Prog. Neurobiol. 47, 425–448.
Garrett, W.S., et al., 2007. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. Cell 131, 33–45.
Garrido, D., et al., 2012. A molecular basis for bifidobacterial enrichment in the infant gastrointestinal tract. Adv. Nutr. 3, 415S–421S.
Gilbert, S.F., et al., 2012. A symbiotic view of life: we have never been individuals. Q. Rev. Biol. 87, 325–341.
Gimeno, D., et al., 2009. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. Psychol. Med. 39, 413–423.
Graff, L.A., et al., 2009. Depression and anxiety in inflammatory bowel disease: a review of comorbidity and management. Inflamm. Bowel Dis. 15, 1105–1118.
Grainger, J.R., et al., 2010. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-beta pathway. J. Exp. Med. 207, 2331–2341.
Guibas, G.V., et al., 2013. Conception via in vitro fertilization and delivery by Caesarean section are associated with paediatric asthma incidence. Clin. Exp. Allergy 43, 1058–1066.
Guo, J.U., et al., 2011. Neuronal activity modifies the DNA methylation landscape in the adult brain. Nat. Neurosci. 14, 1345–1351.
Hampe, J., et al., 2003. Association of inflammatory bowel disease with indicators for childhood antigen and infection exposure. Int. J. Colorectal. Dis. 18, 413–417.
Haroon, E., et al., 2012. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 37, 137–162.
Haukka, J.K., et al., 2004. Family structure and risk factors for schizophrenia: case-sibling study. BMC Psychiatry 4, 41.
Hayakawa, M., et al., 2011. Dramatic changes of the gut flora immediately after severe and sudden insults. Dig. Dis. Sci. 56, 2361–2365.
Heijtz, R.D., et al., 2011. Normal gut microbiota modulates brain development and behavior. Proc. Nat. Acad. Sci. U.S.A. 108, 3047–3052.
Heim, C., et al., 2000. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. JAMA 284, 592–597.
Hemingway, H., et al., 2003. Social and psychosocial influences on inflammatory markers and vascular function in civil servants (the Whitehall II study). Am. J. Cardiol. 92, 984–987.
Henao-Mejia, J., et al., 2012. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 482, 179–185.
Hesselmar, B., et al., 2013. Pacifier cleaning practices and risk of allergy development. Pediatrics 131, e1829–e1837.
Hildebrand, F., et al., 2013. Inflammation-associated enterotypes, host genotype, cage and inter-individual effects drive gut microbiota variation in common laboratory mice. Genome Biol. 14, R4.
Hjern, A., et al., 1999. Age at adoption, ethnicity and atopic disorder: a study of internationally adopted young men in Sweden. Pediatr. Allergy Immunol. 10, 101–106.
Hoffmann, C., et al., 2013. Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. PLoS One 8, e66019.
Hohensinner, P.J., et al., 2011. Telomere dysfunction, autoimmunity and aging. Aging Dis 2, 524–537.
Hong, H.A., et al., 2009. Bacillus subtilis isolated from the human gastrointestinal tract. Res. Microbiol. 160, 134–143.
Hou, J.K., et al., 2009. Distribution and manifestations of inflammatory bowel disease in Asians, Hispanics, and African Americans: a systematic review. Am. J. Gastroenterol. 104, 2100–2109.
Howerton, C.L., Bale, T.L., 2012. Prenatal programing: at the intersection of maternal stress and immune activation. Horm. Behav. 62, 237–242.
Hsiao, E.Y., et al., 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155, 1451–1463.
Hunt, K.M., et al., 2011. Characterization of the diversity and temporal stability of bacterial communities in human milk. PLoS One 6, e21313.
Hviid, A., et al., 2011. Antibiotic use and inflammatory bowel diseases in childhood. Gut 60, 49–54.
Jacobs, T.L., et al., 2011. Intensive meditation training, immune cell telomerase activity, and psychological mediators. Psychoneuroendocrinology 36, 664–681.
Jacobsen, K.H., Wiersma, S.T., 2010. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. Vaccine 28, 6653–6657.
Johnson, W.G., et al., 2009. HLA-DR4 as a risk allele for autism acting in mothers of probands possibly during pregnancy. Arch. Pediatr. Adolesc. Med. 163, 542–546.
Jost, T., et al., 2013a. Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. Br. J. Nutr. 110, 1253–1262.
Jost, T., et al., 2013b. Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding. Environ. Microbiol..
Kapoor, A., et al., 2006. Fetal programming of hypothalamo-pituitary-adrenal function: prenatal stress and glucocorticoids. J. Physiol. 572, 31–44.
Keen, D.V., et al., 2010. Autism, ethnicity and maternal immigration. Br. J. Psychiatry 196, 274–281.
Kiliaan, A.J., et al., 1998. Stress stimulates transepithelial macromolecular uptake in rat jejunum. Am. J. Physiol. 275, G1037–G1044.
Kohane, I.S., et al., 2012. The co-morbidity burden of children and young adults with autism spectrum disorders. PLoS One 7, e33224.
Korosi, A., et al., 2012. Early-life stress mediated modulation of adult neurogenesis and behavior. Behav. Brain Res. 227, 400–409.
Kramer, M.S., 2011. Breastfeeding and allergy: the evidence. Ann. Nutr. Metab. 59 (Suppl. 1), 20–26.
Labeta, M.O., et al., 2000. Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed pattern recognition receptor, soluble CD14. J. Exp. Med. 191, 1807–1812.
Li, X., et al., 2011. Risk of inflammatory bowel disease in first- and second-generation immigrants in Sweden: a nationwide follow-up study. Inflamm. Bowel Dis. 17, 1784–1791.
Linz, B., et al., 2007. An African origin for the intimate association between humans and *Helicobacter pylori*. Nature 445, 915–918.
Lowry, C.A., et al., 2007. Identification of an immune-responsive mesolimbocortical serotonergic system: potential role in regulation of emotional behavior. Neuroscience 146, 756–772.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

Magnus, M.C., et al., 2011. Delivery by Cesarean section and early childhood respiratory symptoms and disorders: the Norwegian mother and child cohort study. Am. J. Epidemiol. 174, 1275–1285.
Malmborg, P., et al., 2012. Cesarean section and the risk of pediatric Crohn's disease. Inflamm. Bowel Dis. 18, 703–708.
Malter Cohen, M., et al., 2013. Early-life stress has persistent effects on amygdala function and development in mice and humans. Proc. Nat. Acad. Sci. U.S.A. 110, 18274–18278.
Mandal, M., et al., 2013. Maternal immune stimulation during pregnancy shapes the immunological phenotype of offspring. Brain Behav. Immun. 33, 33–45.
Maslowski, K.M., Mackay, C.R., 2011. Diet, gut microbiota and immune responses. Nat. Immunol. 12, 5–9.
McDade, T.W., et al., 2010. Early origins of inflammation: microbial exposures in infancy predict lower levels of C-reactive protein in adulthood. Proc. Biol. Sci. 277, 1129–1137.
McDade, T.W., 2012. Early environments and the ecology of inflammation. Proc. Nat. Acad. Sci. U.S.A. 109 (Suppl. 2), 17281–17288.
McDade, T.W., et al., 2012a. 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. 31, 23–30.
McDade, T.W., et al., 2012b. Analysis of variability of high sensitivity C-reactive protein in lowland ecuador reveals no evidence of chronic low-grade inflammation. Am. J. Hum. Biol. 24, 675–681.
McFall-Ngai, M., et al., 2013. Animals in a bacterial world, a new imperative for the life sciences. Proc. Nat. Acad. Sci. U.S.A. 110, 3229–3236.
Messaoudi, M., et al., 2011. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. Br. J. Nutr. 105, 755–764.
Metsala, J., et al., 2013. Mother's and offspring's use of antibiotics and infant allergy to cow's milk. Epidemiology 24, 303–309.
Meyer, U., et al., 2011. Schizophrenia and autism: both shared and disorder-specific pathogenesis via perinatal inflammation? Pediatr. Res 69, 26R–33R.
Miller, A.H., et al., 2013. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. Depress. Anxiety 30, 297–306.
Miller, C.A., et al., 2008. DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. Neurobiol. Learn. Mem. 89, 599–603.
Miller, G.E., et al., 2009. Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. Proc. Nat. Acad. Sci. U.S.A. 106, 14716–14721.
Milo, R., Kahana, E., 2010. Multiple sclerosis: geoepidemiology, genetics and the environment. Autoimmun. Rev. 9, A387–A394.
Moller, M., et al., 2007. Genetic haplotypes of Th-2 immune signalling link allergy to enhanced protection to parasitic worms. Hum. Mol. Genet. 16, 1828–1836.
Mpairwe, H., et al., 2011. Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. Pediatr. Allergy Immunol. 22, 305–312.
Mulder, I.E., et al., 2009. Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. BMC Biol. 7, 79.
O'Hara, A.M., Shanahan, F., 2006. The gut flora as a forgotten organ. EMBO Rep. 7, 688–693.
O'Mahony, S.M., et al., 2009. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol. Psychiatry 65, 263–267.
Obihara, C.C., et al., 2005. Inverse association between Mycobacterium tuberculosis infection and atopic rhinitis in children. Allergy 60, 1121–1125.
Onore, C., et al., 2012. The role of immune dysfunction in the pathophysiology of autism. Brain Behav. Immun. 26, 383–392.
Ornish, D., et al., 2008. Increased telomerase activity and comprehensive lifestyle changes: a pilot study. Lancet Oncol. 9, 1048–1057.
Osada, Y., Kanazawa, T., 2010. Parasitic helminths: new weapons against immunological disorders. J. Biomed. Biotechnol. 2010, 743–758.
Pace, T.W., et al., 2006. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. Am. J. Psychiatry 163, 1630–1633.
Pace, T.W., et al., 2012. Increased peripheral NF-kappaB pathway activity in women with childhood abuse-related posttraumatic stress disorder. Brain Behav. Immun. 26, 13–17.
Parker, W., Ollerton, J., 2013. Evolutionary biology and anthropology suggest biome reconstitution as a necessary approach toward dealing with immune disorders. Evol. Med. Public Health 2013 (1), 89–103.
Penders, J., et al., 2013. Establishment of the intestinal microbiota and its role for atopic dermatitis in early childhood. J. Allergy Clin. Immunol..
Peng, H., Hagopian, W., 2006. Environmental factors in the development of Type 1 diabetes. Rev. Endocr. Metab. Disord. 7, 149–162.
Qiu, A., et al., 2013. Maternal anxiety and infants' hippocampal development: timing matters. Transl. Psychiatry 3, e306.
Raison, C.L., et al., 2010. Inflammation, sanitation and consternation: loss of contact with co-evolved, tolerogenic micro-organisms and the pathophysiology and treatment of major depression. Arch. Gen. Psychiatry 67, 1211–1224.
Rattazzi, L., et al., 2013. CD4(+) but not CD8(+) T cells revert the impaired emotional behavior of immunocompromised RAG-1-deficient mice. Transl. Psychiatry 3, e280.
Rehman, A., et al., 2010. Transcriptional activity of the dominant gut mucosal microbiota in chronic inflammatory bowel disease patients. J. Med. Microbiol. 59, 1114–1122.
Riedler, J., et al., 2001. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet 358, 1129–1133.
Ristori, G., et al., 2014. Effects of Bacille Calmette-Guerin after the first demyelinating event in the CNS. Neurology 82, 41–48.
Roberts, S.E., et al., 2011. Perinatal and early life risk factors for inflammatory bowel disease. World J. Gastroenterol. 17, 743–749.
Rook, G.A., et al., 2014. Microbial "Old Friends", immunoregulation and socio-economic status. Clin. Exp. Immunol.
Rook, G.A.W., 2010. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: darwinian medicine and the 'hygiene' or 'old friends' hypothesis. Clin. Exp. Immunol. 160, 70–79.
Rook, G.A.W., 2013. Regulation of the immune system by biodiversity from the natural environment: an ecosystem service essential to health. Proc. Nat. Acad. Sci. U.S.A. 110, 18360–18367.
Rook, G.A.W., et al., 2013. Microbial old friends, immunoregulation and stress resilience. Evolution, medicine and public health. EMPH (2013), 46–64, http://dx.doi.org/10.1093/emph/eot004.
Rottem, M., et al., 2005. Atopy and asthma in migrants. Int. Arch. Allergy Immunol. 136, 198–204.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

Round, J.L., Mazmanian, S.K., 2010. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc. Nat. Acad. Sci. U.S.A. 107, 12204–12209.

Round, J.L., et al., 2011. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 332, 974–977.

Russell, S.L., et al., 2012. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. EMBO Rep. 13, 440–447.

Saarinen, K.M., et al., 1999. Transforming growth factor-beta1 in mothers' colostrum and immune responses to cows' milk proteins in infants with cows' milk allergy. J. Allergy Clin. Immunol. 104, 1093–1098.

Sacker, A., et al., 2013. Breast feeding and intergenerational social mobility: what are the mechanisms? Arch. Dis. Child 98, 666–671.

Schaub, B., et al., 2009. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J. Allergy Clin. Immunol. 123, 774–782 (e5).

Schmidt, C.W., Tolle, R., 1977. Family constellation in mental disorder. results and methods of statistical investigations of family size, birth order and sib position (author's transl). Fortschr. Neurol. Psychiatr. Grenzgeb 45, 20–52.

Schwarz, E., et al., 2011. Identification of a biological signature for schizophrenia in serum. Mol. Psychiatry 17, 494–502.

Seiskari, T., et al., 2007. Allergic sensitization and microbial load–a comparison between Finland and Russian Karelia. Clin. Exp. Immunol. 148, 47–52.

Sepa, A., et al., 2005. Mothers' experiences of serious life events increase the risk of diabetes-related autoimmunity in their children. Diabetes Care 28, 2394–2399.

Shaw, S.Y., et al., 2010. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. Am. J. Gastroenterol. 105, 2687–2692.

Shen, W., et al., 2013. Influence of dietary fat on intestinal microbes, inflammation, barrier function and metabolic outcomes. J. Nutr. Biochem..

Smith, S.E., et al., 2007. Maternal immune activation alters fetal brain development through interleukin-6. J. Neurosci. 27, 10695–10702.

Smits, H.H., et al., 2005. 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. 115, 1260–1267.

Soderstrom, U., et al., 2012. Being born in Sweden increases the risk for type 1 diabetes—a study of migration of children to Sweden as a natural experiment. Acta Paediatr. 101, 73–77.

Spear, L.P., 2000. The adolescent brain and age-related behavioral manifestations. Neurosci. Biobehav. Rev. 24, 417–463.

Stark, P.L., Lee, A., 1982. The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year. J. Med. Microbiol. 15, 189.

Stensballe, L.G., et al., 2013. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. J. Pediatr. 162, 832–838 (e3).

Stewart, J.A., et al., 2005. Investigations into the influence of host genetics on the predominant eubacteria in the faecal microflora of children. J. Med. Microbiol. 54, 1239–1242.

Stilling, R.M., et al., 2013. Microbial genes, brain & behaviour—epigenetic regulation of the gut–brain axis. Genes Brain Behav. http://dxdoi.org/10.1111/gbb.12109.

Stoll, N.R., 1947. This wormy world. J. Parasitol. 33, 1–18.

Strachan, D.P., 1989. Hay fever, hygiene, and household size. Br. Med. J. 299, 1259–1260.

Sudo, N., et al., 2004. Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. J. Physiol. 558, 263–275.

Thavagnanam, S., et al., 2008. A meta-analysis of the association between Caesarean section and childhood asthma. Clin. Exp. Allergy 38, 629–633.

Tillisch, K., et al., 2013. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology 144 (1394-401), 1401 e1 (1401 e4).

Tims, S., et al., 2013. Microbiota conservation and BMI signatures in adult monozygotic twins. ISME J. 7, 707–717.

Turnbaugh, P.J., et al., 2009. A core gut microbiome in obese and lean twins. Nature 457, 480–484.

van den Biggelaar, A.H., et al., 2004. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J. Infect. Dis. 189, 892–900.

Van Kruiningen, H.J., et al., 2007. Familial Crohn's disease in Belgium: pedigrees, temporal relationships among cases, and family histories. J. Clin. Gastroenterol. 41, 583–590.

van Neerven, R.J., et al., 2012. Which factors in raw cow's milk contribute to protection against allergies? J. Allergy Clin. Immunol 130, 853–858.

Vega, W.A., et al., 2004. 12-month prevalence of DSM-III-R psychiatric disorders among Mexican Americans: nativity, social assimilation, and age determinants. J. Nerv. Ment. Dis. 192, 532–541.

Veling, W., et al., 2011. Age at migration and future risk of psychotic disorders among immigrants in the Netherlands: a 7-year incidence study. Am. J. Psychiatry 168, 1278–1285.

Vijay-Kumar, M., et al., 2010. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 328, 228–231.

Villarreal, A.A., et al., 2012. Use of broad-spectrum antibiotics and the development of irritable bowel syndrome. WMJ 111, 17–20.

Vlajinac, H., et al., 2006. The Belgrade childhood diabetes study - comparison of children with type 1 diabetes with their siblings. Paediatr. Perinat. Epidemiol. 20, 238–243.

von Hertzen, L., et al., 2011. Natural immunity. Biodiversity loss and inflammatory diseases are two global megatrends that might be related. EMBO Rep. 12, 1089–1093.

Vythilingam, M., et al., 2002. Childhood trauma associated with smaller hippocampal volume in women with major depression. Am. J. Psychiatry 159, 2072–2080.

Weaver, I.C., et al., 2004. Epigenetic programming by maternal behavior. Nat. Neurosci. 7, 847–854.

Weinstock, M., 2005. The potential influence of maternal stress hormones on development and mental health of the offspring. Brain Behav. Immun. 19, 296–308.

Wells, V.E., et al., 1985. Risk factors for depression in adolescence. Psychiatr. Dev. 3, 83–108.

Wen, L., et al., 2008. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 455, 1109–1113.

Westerbeek, E.A., et al., 2006. The intestinal bacterial colonisation in preterm infants: a review of the literature. Clin. Nutr. 25, 361–368.

Wikoff, W.R., et al., 2009. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc. Nat. Acad. Sci. U.S.A. 106, 3698–3703.

Willette, A.A., et al., 2011. Brain enlargement and increased behavioral and cytokine reactivity in infant monkeys following acute prenatal endotoxemia. Behav. Brain Res. 219, 108–115.

Wolfe, N.D., et al., 2007. Origins of major human infectious diseases. Nature 447, 279–283.

World_Health_Organization, 2012. Global Tuberculosis Report 2012. World Health Organization Document 2012; WHO/HTM/TB/. 2012.6, 1–272.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004

Wu, G.D., et al., 2011. Linking long-term dietary patterns with gut microbial enterotypes. Science 334, 105–108.

Yatsunenko, T., et al., 2012. Human gut microbiome viewed across age and geography. Nature 486, 222–227.

Zerbo, O., et al., 2012. Is maternal influenza or fever during pregnancy associated with autism or developmental delays? Results from the CHARGE (CHildhood Autism Risks from Genetics and Environment) study. J. Autism Dev. Disord. 43, 25–33.

Zilber, N., et al., 1988. Multiple sclerosis and birth order. Acta Neurol. Scand. 78, 313–317.

Zivkovic, A.M., et al., 2011. Microbes and health sackler colloquium: human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc. Nat. Acad. Sci. U.S.A. 108, 4653–4658.

Please cite this article as: Rook, G.A.W., et al., Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Research (2014), http://dx.doi.org/10.1016/j.brainres.2014.04.004