Clinic Rev Allerg Immunol (2012) 42:5–15
DOI 10.1007/s12016-011-8285-8

Hygiene Hypothesis and Autoimmune Diseases

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Published online: 17 November 2011
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Abstract Throughout the twentieth century, there were striking increases in the incidences of many chronic inflammatory disorders in the rich developed countries. These included autoimmune disorders such as Type 1 diabetes and multiple sclerosis. Although genetics and specific triggering mechanisms such as molecular mimicry and viruses are likely to be involved, the increases have been so rapid that any explanation that omits environmental change is incomplete. This chapter suggests that a series of environmental factors, most of them microbial, have led to a decrease in the efficiency of our immunoregulatory mechanisms because we are in a state of evolved dependence on organisms with which we co-evolved (and that had to be tolerated) as inducers of immunoregulatory circuits. These organisms ("Old Friends") are depleted from the modern urban environment. Rather than considering fetal programming by maternal microbial exposures, neonatal programming, the hygiene hypothesis, gut microbiota, and diet as separate and competing hypotheses, I attempt here to integrate these ideas under a single umbrella concept that can provide the missing immunoregulatory environmental factor that is needed to explain the recent increases in autoimmune disease.

Keywords Immunoregulation · "Old Friends" · Microbiota · Treg

Introduction

The hygiene hypothesis, recently reformulated as the Old Friends Hypothesis to bring it in line with Darwinian medicine, and with the latest epidemiological and experimental evidence, suggests that since the start of modern "concrete and tarmac" urbanization in the early nineteenth century there has been a progressive increase in immunoregulatory problems attributable to depletion from the urban environment of organisms with which mammals co-evolved, and that had been tasked by co-evolutionary forces with a crucial role in setting up "normal" background levels of immunoregulation (this will be explained, expanded and referenced below). It would be foolish to suggest that this mechanism is the whole explanation for the striking increases in certain autoimmune diseases (notably Type 1 diabetes (T1D) and multiple sclerosis (MS)) in the twentieth century. On the other hand, the recent nature of these increases makes it certain that the major underlying cause is environmental. The role of genetic factors cannot be more than to determine which individuals develop the disease after the environmental changes have occurred… a classic example of gene-environment interaction. In addition there are other potential environmental factors that I consider to be subcomponents of the Old Friends hypothesis (such as *delayed* exposure to viruses), and others that are entirely separate in nature (such as deficient Vitamin D3). All of these will exacerbate the immunoregulatory deficit. Figure 1 lists some relevant factors, and also emphasizes the immunoregulatory role of the gut. One of the most important discoveries in recent years is the fact that manipulations of the immune system (or loss of the Old Friends!) may act indirectly via changes in the gut flora…the microbiota. Wen and colleagues showed that specific-pathogen free (SPF) non-obese diabetic (NOD) mice that spontaneously develop a condition resembling T1D, are protected from the disease following knockout of the gene encoding MyD88 (an adaptor for multiple Toll-like receptors) [1]. However, this did not mean that MyD88 was directly involved in the autoimmune response to β cells in the pancreas. Rather, it emerged that the modification of the immune system resulting from knocking out MyD88 caused profound changes in the interactions between the immune system and the microbiota. Consequent changes in the composition of the microbiota were responsible for the immunoregulatory

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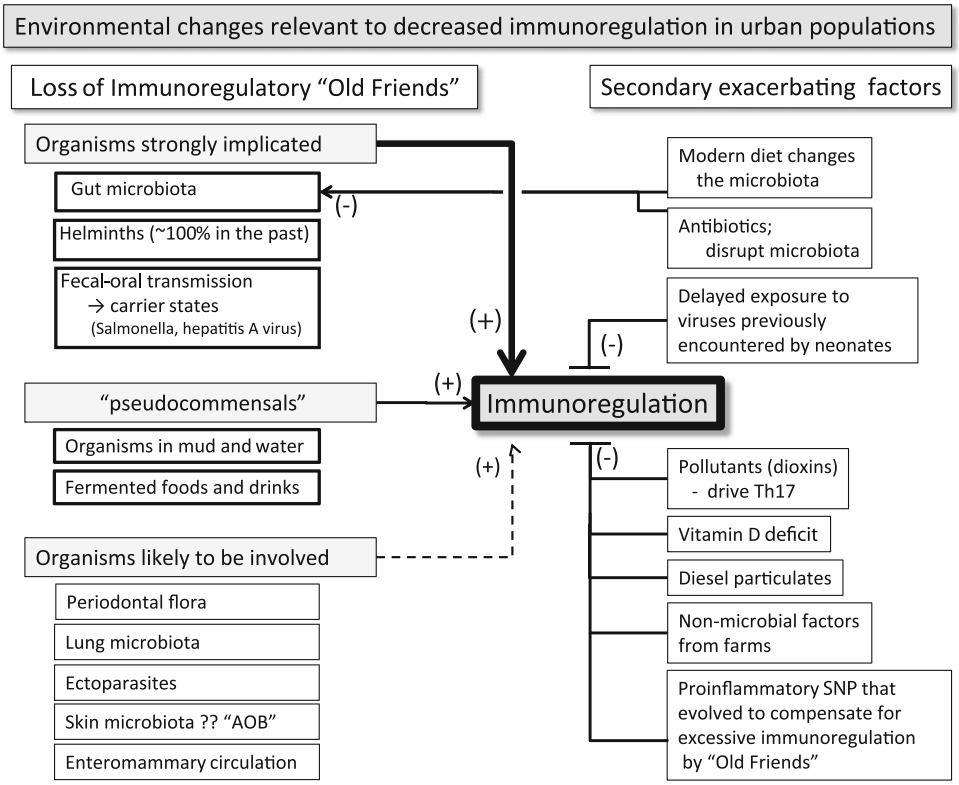

Fig. 1 Factors that affect immunoregulation. The most strongly implicated, (epidemiology, experimental models and some clinical trials) are the gut microbiota, helminths and faecal–oral transmission of other "Old Friends" (other microbiota have received little attention but are likely to be involved). A number of secondary exacerbating factors are listed on the right. Loss of the Old Friends is the most likely environmental factor underlying the rapid increases in autoimmune (and other chronic inflammatory) diseases

effect that blocked the autoimmune process. Thus changes in the microbiota, which is profoundly different in Europeans from that of people living in a traditional rural African village [2], must be regarded as part of the Old Friends hypothesis. Clearly diminished exposures to intestinal helminths, faecal organisms of other humans and environmental fermenting species such as *Lactobacilli* etc. (i.e. the "Old Friends" mentioned above) will have direct effects on the nature of the microbiota. Changes in the systemic load of "Old Friends" such as helminths, by changing the immune system will also indirectly modulate the host–microbiota relationship.

Another major modulator of the microbiota is diet [3]. Figure 1 summarises the relationships between these interacting factors and lists those that can be regarded as subcomponents of the hygiene hypothesis, those that are separate factors which exacerbate the resulting immunoregulatory problem, becoming relevant only once immunoregulation is impaired. The theme of this review is therefore rather broad because it makes no sense to claim that the increase in autoimmune disease is caused entirely by the hygiene hypothesis, or entirely by vitamin D deficiency, or entirely by exposure to self-cross-reactive epitopes or entirely by viruses, or dioxins, or diet or whatever….. All of these factors play a role, but the fundamental underlying problem is immunoregulation, and our changing microbial exposures are fundamental aspects of the immunoregulatory deficit.

Hygiene, or "Old Friends" Hypothesis

The hygiene hypothesis, or as we prefer to call it, the "Old Friends" hypothesis, can be traced back to the 1870s when Charles Harrison Blackley noticed that aristocrats and city-dwellers were more likely to get hayfever than were farmers [4]. Similarly, in 1966, Leibowitz and colleagues noted that the incidence of multiple sclerosis in Israel was positively related to levels of sanitation [5]. However the phrase "hygiene hypothesis" was coined—and hit the media—in 1989 when Strachan noted that hayfever was less frequent in families with many siblings [6]. This led to a focus on allergic disorders despite the clear evidence that other chronic inflammatory disorders were increasing in parallel in western societies [7–9]. More seriously, the findings were interpreted, particularly by the media, as suggesting that the common infections of childhood, and/or poor hygiene, were for some reason needed by the developing immune system.

The expression "Old Friends hypothesis" was coined to provide a "Darwinian" synthesis, and to focus attention on the fact that modern domestic hygiene is not the important issue. The chronic inflammatory disorders that started to increase in Europe in the mid-nineteenth century (allergies, inflammatory bowel diseases, autoimmunity [Type 1 diabetes, multiple sclerosis]) all show evidence of defective immunoregulation, often at the level of regulatory T cells

[10–12]. The Old Friends hypothesis suggests that one reason for the increasing incidences of chronic inflammatory disorders, (both Th2-mediated and Th1/Th17-mediated [8]), in developed countries since the mid-nineteenth century is the depletion from the urban environment of organisms that accompanied mammalian evolution and had to be tolerated [13]. Some of these organisms had to be tolerated because they were essential symbiotic intestinal microbiota. Others, like helminths, had to be tolerated because once established they cannot be removed by the immune system, which is therefore downregulated to avoid pointless immunopathology. Other relevant organisms were species that established carrier states soon after birth. Because these all had to be tolerated, co-evolutionary forces ensured that they came to play essential roles in the priming and optimal functioning of immunoregulatory pathways that are involved in tolerance [13]. We know that a failure of immunoregulatory mechanisms really can lead to simultaneous increases in diverse types of pathology. For example, genetic defects of Foxp3 lead to the X-linked autoimmunity–allergic dysregulation syndrome (XLAAD) that includes aspects of allergy, autoimmunity and enteropathy [14].

Genetics

In parts of the world where there was 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. This is seen for several proinflammatory cytokines [15], IgE [16] and STAT6, a transcription factor involved in Th2 responses [17]. There is also an increased frequency of a truncated form of the serotonin transporter that also has a marked proinflammatory effect [18]. The problem here is clear (Fig. 2). As soon as the immunoregulation-inducing organisms are withdrawn by the modern lifestyle, these genetic variants lead to excessive inflammation, and become risk factors for chronic inflammatory disorders [15–18]. This constitutes a second layer of evolved dependence on the continuing presence of the "Old Friends" (Fig. 2).

This is important because work that identifies proximate "causes" for diseases that were rare or nonexistent before the Second Epidemiological Transition may merely be unravelling a problem that would be irrelevant if the microbial status could be returned to that seen in the paleolithic. For instance gluten-associated enteropathies

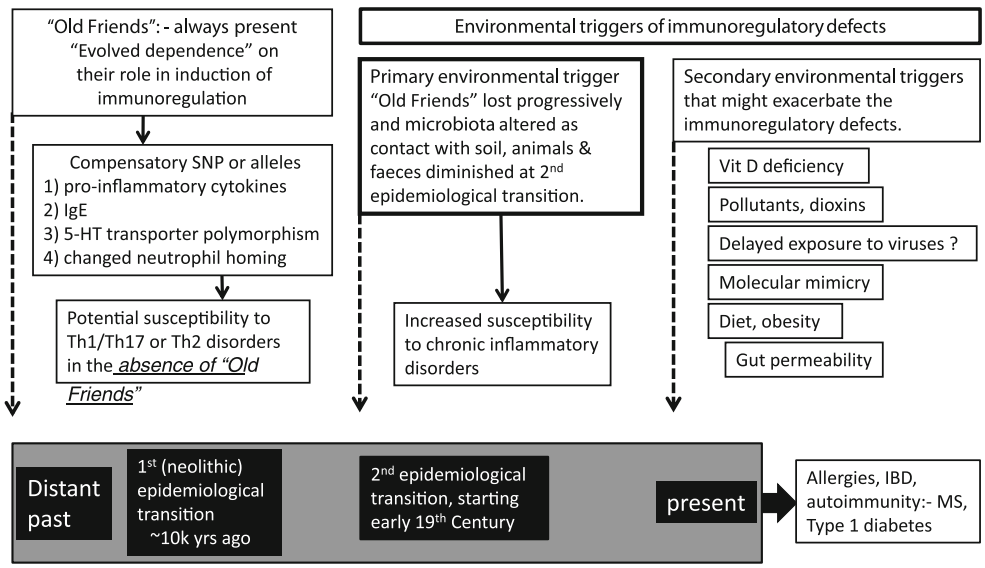

Fig. 2 Interaction of genetics and loss of the "Old Friends". The Old Friends had to be tolerated and so co-evolved roles as triggers of immunoregulatory pathways. In areas with very high loads of these and other organisms, particularly helminths, compensatory genetic variants accumulated, to partially restore inflammatory responses. In the absence of the Old Friends, not only is immunoregulation inadequately primed, but also these genetic variants cause excessive inflammation and become risk factors for chronic inflammatory disorders. Genetic variants that were advantageous, and did not cause disease in the past, start to do so in the absence of the Old Friends (referenced in main text). Several aspects of modern life are potentially interacting with the lack of "Old Friends" at the level of immunoregulation. Obesity is associated with altered gut microbiota and excessive release of proinflammatory cytokines. Stress also alters gut microbiota, and drives corticotropin-releasing hormone (CRH) which increases permeability of the gut mucosa. Increased absorption of LPS and other microbial components drives further release of proinflammatory cytokines. Lack of vitamin D exacerbates immuno-dysregulation, as does the triggering of Th17 cells by dioxins. Meanwhile, the changes in the gut are also likely to impact on Th17 development. Viruses that used to be encountered harmlessly in early infancy (under cover perhaps of maternal antibody) can trigger autoimmunity if encountered for the first time later in life. Raised levels of proinflammatory cytokines trigger depression in some individuals, and this feeds back into the CRH/gut circuits

might be an "artefact" of poorly immunoregulated guts. Similarly, the recent claim to have discovered that the "cause" of Crohn's disease is a genetically determined defect in the homing of neutrophils [19] is difficult to reconcile with the fact that 100 years ago the disease barely existed. It is the recent environmental changes that have caused this phenotype to become a risk factor (Fig. 2).

Epidemiological Transitions

Which organisms are involved? The bottom line is that they are organisms associated with faeces (microbiota, helminths and faecal–oral transmission of infections/carrier states), animals (farm or pet) and mud [2, 6, 20–23]. Humans were continuously exposed to these from early on in evolution. In 1971, Omran coined the term "Epidemiological Transition" to describe the major watersheds in human development (discussed in 24). Paleolithic populations carried the organisms that they inherited from their primate ancestors ("heirloom" species), including many viruses (Fig. 3) [24, 25]. In addition, they would have been exposed to zoonoses that they picked up as they scavenged carrion [24]. Finally, they will have consumed several milligrams of harmless environmental saprophytes ("pseudocommensals") every day, since these are ubiquitous in soil and water. The organisms that have been found to be important for the hygiene hypothesis belong within these categories, as described and references later.

About 10,000 years ago, the shift to agriculture and husbandry created the First (Neolithic) Epidemiological Transition (Fig. 3) [24]. This will have had little effect on exposure to the "pseudocommensals" or to the heirloom species. However, the more sedentary lifestyle increased faecal–oral transmission, and caused prolonged contact with animals. The latter led to adaptation to man of a number of animal viruses shown in Fig. 3 [25]. However, the viruses acquired during the Neolithic such as influenza (B and C), smallpox, mumps and measles do not become endemic until there are communities of several hundreds of thousands, which did not occur until the appearance of cities 2–3000 years ago. Since this represents only 100–150 generations, extremely strong selection pressure would have been required for evolved dependence to appear, and this seems unlikely. Moreover, most humans did not live in such large groups, and these viruses were, for example, absent from pre-columbian American populations.

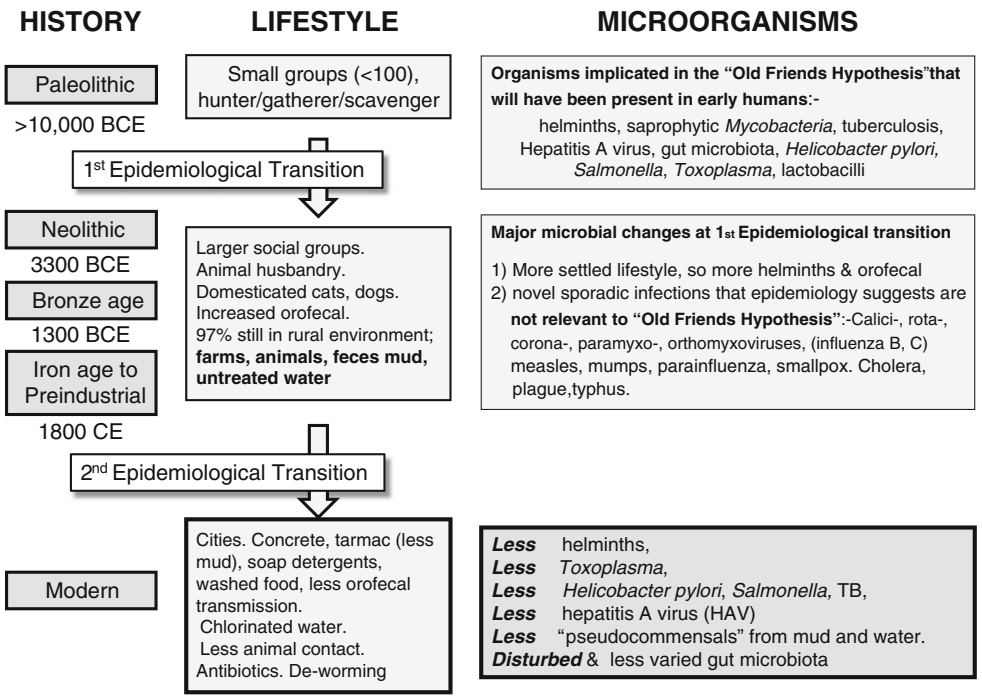

Fig. 3 Aspects of man's microbiological history that are most relevant to the hygiene hypothesis. Epidemiological data, laboratory and animal models, and preliminary clinical trials investigating the hygiene hypothesis implicate organisms that are thought to have accompanied mammalian and human evolution. This relationship persisted for long enough for the establishment of evolved dependence on these organisms. They must be tolerated, and so have developed roles in the initiation of regulatory pathways. Organisms that evolved during the Neolithic are less likely to be relevant in this context, and the First Epidemiological Transition did not reduce human contact with organisms associated with animals, faeces and mud. On the other hand the Second Epidemiological Transition has led to gene-environment misfit, as the "Old Friends" from the Paleolithic have been progressively removed from the modern environment

In short, there were dramatic changes to man's microbial environment after the First Epidemiological Transition, but this *did not* result in loss of exposure to the organisms implicated by epidemiology in the hygiene hypothesis because until the modern era more than 97% of the population still lived in rural environments, close to mud, animals and faeces which were the sources of these organisms. The situation did not change until the mid-nineteenth century. Since then some populations have undergone a Second Epidemiological Transition in which public health measures and more recently, antibiotics, have resulted in diminished (or *delayed*) exposure to many of the organisms that were present in earlier eras.

The Critical Immunoregulation-Inducing Organisms

Against this evolutionary and historical background, we can take a closer look at the organisms that have been implicated as inhibitors of the chronic inflammatory disorders that are increasing in the developed countries. We can propose the following criteria for the identification of relevant organisms, which we designate "Old Friends" to emphasise the duration of these interactions, and their evolutionary significance. Importantly, the "Old Friends" all satisfy the following criteria:

1. Abundant during mammalian evolution (i.e. going back much further than the ~500 generations since the start of agriculture)
2. Virtually absent … and increasingly so over the last century.....from the modern environment
3. Proven to have therapeutic effects in animal models of chronic inflammatory disorders
4. Some proven to have therapeutic effects in human clinical trials

Ideally, there should also be some understanding of the molecular mechanisms underlying the immunoregulatory effects. The organisms implicated, and the reasons and references are listed in Table 1. A few particularly important examples are discussed below in the context of their immunoregulatory mode of action.

Intestinal Microbiota

Germ-free mice have poorly developed lymphoid systems, and are susceptible to inflammatory disorders. Colonisation with certain organisms can reverse or exacerbate these defects. The effects depend on the organism used [26]. For example, segmented filamentous bacteria strongly induce intestinal Th17 cells, which play a role in host resistance against intestinal pathogens and promote systemic autoimmunity [27–29]. In sharp contrast, colonisation of germ-free mice with a defined intestinal flora resulted in the generation, expansion and activation of Treg in the colonic lamina propria [30]. Similarly, a mixture of 46 different Clostridia species was shown to be a potent inducer of colon lamina propria Treg cells [31]. These reconstituted animals were subsequently resistant to colitis and systemic IgE responses [31]. There has been a detailed study of *Bacteroides fragilis*. A polysaccharide from this organism acts directly via TLR2 on Treg to increase their numbers and state of activation, and so restrain Th17 activity [32]. It was established 10 years ago that the intestinal microbiota are required for successful induction, by the oral route, of tolerance to ovalbumin [33]

The proof, using modern methods, that the microbiota of city-dwelling European (EU) children differs dramatically from that of rural Africans was mentioned above [2]. The faecal flora of children from Burkina Faso (BF) had more Bacteroidetes and less Firmicutes and Enterobacteriaceae. Only the BF children had organisms (*Prevotella* and *Xylanibacter*) known to contain for hydrolysis of cellulose and xylan which may have contributed to the fact that BF children had more short-chain fatty acids (SCFA) than did EU children [2]. SCFA have a protective anti-inflammatory role in the gut [34, 35]. There was also significantly greater microbial richness and biodiversity in BF samples than in EU samples [2]. This is important because a decrease in the abundance and biodiversity of Firmicutes has been observed repeatedly in Crohn's disease patients [36]. Interestingly, *Faecalibacterium prausnitzii*, an anti-inflammatory commensal bacterium that also contributes to SCFA generation, was present in the microbiota of the BF children [2], but is often lacking in European CD patients [36].

The virome of the microbiota is now also being studied. Most of the viruses are bacteriophages but how they vary or affect the immunoregulatory effects of the bacterial microbiota is not yet known [37].

Orofaecally Transmitted Chronic Infections

The orofaecally transmitted organisms highlighted in recent studies of the hygiene hypothesis include *Helicobacter pylori*, *Salmonella*, Hepatitis A virus (HAV), enteroviruses and *Toxoplasma gondii* [38–41]. Protozoa not yet considered in this context, to my knowledge, include the very ancient *Entamoeba*, *Giardia*, and *Trichomonas* all of which have lost their mitochondria and have a close association with humans.

Many helminths (considered below) are also orofaecally transmitted or rapidly picked up from the environment and must be regarded as part of the co-evolved microbiota.

Helminths

The immunoregulatory role of helminths is best established for allergic disorders [42]. It is estimated that in 1947 about 36% of the population of Europe carried helminths such as

Table 1 Organisms considered protective in the Old Friends Hypothesis

Organism or location	Disease or model or effect	References
• Gut microbiota		
Segmented filamentous bacteria	Th17 cells	[27–29]
Clostridia species	Treg in lamina propria	[30]
Bacillus fragilis	IL-10 and Treg	[32]
Faecalibacterium prausnitzii	Crohn's disease	[36]
• Faecal–oral transmission		
Helicobacter pylori	Allergies	[39]
Salmonella	Allergies	[38]
Toxoplasma	Allergies	[39, 77]
–Viruses		
Enteroviruses	Allergies	[41]
Hepatitis A virus	Asthma	[76, 77]
–Viruses protective if infected very early, but trigger disease if late [85]		
Coxsackievirus B	Type 1 diabetes	[86]
Rotavirus	Type 1 diabetes	[87]
• Helminths		
Many species	Allergies	[42, 50, 51, 53, 55]
Assorted natural infection	Multiple sclerosis	[59, 60]
Trichuris trichiura	Multiple sclerosis (correlation)	[57]
Enterobius vermicularis	Type 1 diabetes (correlation)	[44]
Various species	Inflammatory bowel disease	[61, 62]
–Animal models treated with helminths		
Heligmosomoides polygyrus	Allergy, T1D, colitis	Reviewed in [67]
Schistosoma mansoni	Allergy, T1D, EAE, colitis, arthritis	Reviewed in [67]
Strongyloides stercoralis	Allergy	Reviewed in [67]
Fasciola hepatica	EAE	Reviewed in [67]
Trichinella spiralis	T1D, EAE	Reviewed in [67]
Hymenolepsis diminuta	Colitis, arthritis	Reviewed in [67]
–Clinical trials with helminths		
Trichuris suis	Multiple sclerosis	[95]
Trichuris suis	Inflammatory bowel disease	[96, 97]
Necator americanus	Asthma	[98]
• Other natural microbial flora		
Skin flora; ammonia-oxidising bacteria	Nitrite, nitric oxide	[68]
Lung flora	Asthma	[69]
Oral and periodontal flora	Inflammatory bowel disease	[70]
Gut organisms transported to breast milk	? Immunoregulation	[71]
• Environmental saprophyte		
Mycobacterium vaccae	Allergy (mouse, dog)	[73, 74]
• Ectoparasites		
Various	Response to TLR agonists in vitro	[72]

Enterobius vermicularis, *Trichuris trichiura*, and *Ascaris lumbricoides* [43]. Now even pinworm (*E. vermicularis*) has become a rarity in Europe [44]. A number of studies have reported inverse correlations between indicators of helminth burden, and allergic sensitisation to environmental allergens [45–49]. More importantly, the risk of wheeze was reduced in individuals with hookworm (*Necator americanus*) infection in Ethiopia [50], and *Enterobius* infestation was negatively correlated with asthma and rhinitis in primary school children in Taiwan [51]. Similarly, it was suggested that infection with *Schistosoma mansoni* was associated with milder forms of asthma [52]. Similarly, deworming Vietnamese schoolchildren for 12 months [53], and still more prolonged treatment of

children in Venezuela or Gabon, all led to increased allergen sensitization and skin prick test responses [54, 55]. A meta-analysis, and an account of some discordant studies was published recently [33 studies reviewed in 56].

Helminths in Human Autoimmunity and Inflammatory Bowel Disease

The rise in Type 1 diabetes (an autoimmune destruction of the insulin-secreting β cells in the pancreas) in Western Europe and the USA during the twentieth century correlates strikingly with the decline of helminth infections, particularly *E. vermicularis* [44]. Direct proof of a link is not yet available.

The prevalence of multiple sclerosis (MS) was shown many years ago to correlate inversely with sanitation [5]. MS is extremely rare in countries with a prevalence of *T. trichiura* of more than 10%, but rises dramatically in areas with lower prevalence of this parasite [57]. This is entirely correlative, circumstantial evidence. However, Correale and colleagues have shown that patients with MS who become infected with helminths have a strikingly diminished rate of disease progression, and develop circulating myelin-specific Treg that release IL-10 and TGF-β in response to a peptide from myelin basic protein [58]. Helminth infection also induces a population of IL-10-secreting regulatory B cells in these patients [59]. If the helminth infection has to be treated, exacerbation of the MS rapidly follows [60].

As far as IBD is concerned, the epidemiological data are less strong than for allergic disorders, because IBD, like MS, is much less common. Nevertheless, analyses of the available data conclude that exposure to helminths is one of the environmental factors most convincingly associated with a low risk of IBD [61, 62].

Most studies of the immunoregulatory mechanisms of helminths have been performed in mouse models. One intensely studied species in rodent models is *Heligmosomoide polygyrus*. This helminth exerts immunoregulatory effects via at least three pathways: modulation of the bacterial microbiota [63]; modulation of the maturation of intestinal dendritic cells (DC) [64]; and, like *B. fragilis*, by directly driving proliferation of Treg [65]. These and other points are illustrated in Fig. 4. Other mechanisms include induction of regulatory B cells [59], regulatory macrophages [66], and modulation of the Treg/Th17 ratio in the gut [20]. In animal models, helminth infections will prevent or treat arthritis, multiple sclerosis, Type 1 diabetes, colitis and allergies and this also is listed in Table 1, and was extensively reviewed recently [67].

Other Classes of Organism Likely to have Immunoregulatory Roles

Table 1 lists and provides references for the growing evidence that other microbiota, in addition to that of the gut, are also involved in immunoregulation, and also likely

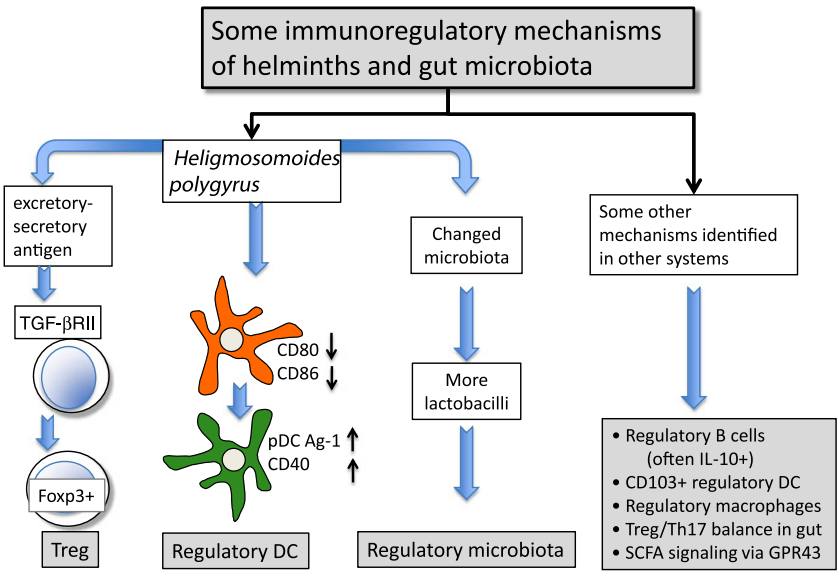

Fig. 4 Some mechanisms involved in the immunoregulatory properties of the "Old Friends" and microbiota. A helminth (*Heligmosomoides polygyrus)* that has been intensively studied in a variety of mouse models is used as an example. This helminth secretes a molecule that directly drives Treg via the receptor for TGF-β. Another key pathway is the modification of DC so that they tend to drive Treg (other organisms drive regulatory CD103+ DC). Such DC also process self antigens, allergens etc., and so drive crucial specific regulatory cell populations. The intestinal helminths also exert indirect effects by modulating the microbiota. The individual pathways are referenced in the main text. *SCFA* short-chain fatty acids, *GPR43* G-protein-coupled receptor 43, *CD103* an integrin and marker of intestinal regulatory DC

to be disrupted in modern urban environments. These include the flora of the skin [68] and lung [69], periodontal and oral flora [70], and the flora of milk [71]. There is an "entero-mammary" circulation of bacteria-laden immature dendritic cells due to increased translocation from the gut to Peyer's patches during lactation [71]. There are several other neglected areas. In a study of wild rodents, it emerged that various aspects of the innate immune response were profoundly affected by the load of ectoparasites [72]. Is the loss of ectoparasites important for decreased immunoregulation in man? There are no studies. Similarly, what about the loss of *Entamoeba*, *Giardia* and *Trichomonas* all of which have lost their mitochondria and have a very close evolutionary association with humans?

Some environmental saprophytes in mud and untreated water were so abundant both in the hunter–gatherer farming and farming environment as to become "pseudocommensals", consumed regularly and inevitably in milligram quantities. These too turn out to have immunoregulatory roles and can be potent inducers of regulatory T cells [73–75].

Virus Infections; Protective or Triggers of Disease?

Most childhood virus infections do not protect from chronic inflammatory disorders (an exception is Hepatitis A virus, which in the past was picked up early in the neonatal period and modulates T cell subsets in a way that protects from allergic disorders [76, 77]). As predicted on evolutionary grounds above, the commonly recognized childhood virus infections, most of which were picked up by some humans rather "recently" after the First Epidemiological Transition about 10,000 years ago, but were often not endemic until much more recently, do not fulfil the criteria for establishing a state of evolved dependence. It was the observations on family size and birth order that first gave impetus to the hygiene hypothesis [6, 78, 79], so childhood infections were initially considered to be likely candidates. However, when considered in the light of the evolutionary considerations it becomes clear that the contemporary childhood virus infections are extraordinarily unlikely to be important in this context. They were not part of the hunter–gatherer environment in which humans evolved, and in any case, being sporadic and dangerous, they were not appropriate for the setting up of a relationship such as evolved dependence.

Thus as might be expected, the common infections of childhood do not protect from allergic disorders [39, 80, 81]. Although the studies are less numerous, similar conclusions are being drawn from investigation of the relationship between childhood infections and susceptibility to other members of the groups of chronic inflammatory disorders that are increasing in the rich countries. Thus, no evidence could be found that the childhood infections exert protective effects against Type 1 diabetes [82], or inflammatory bowel disease [61, 83, 84].

Enteroviruses such as Coxsackievirus B (CVB) and other faecal–oral viruses such as rotavirus have been implicated as triggers of type 1 diabetes [85]. Weaker evidence implicates mumps virus, cytomegalovirus and rubella virus [85]. However, timing is crucial, and coxsackie viruses [86] or rotaviruses [87] or LCM that provoke autoimmunity when given late (for instance at weaning) can be protective when given very early [85–87]. These findings suggest another twist to the hygiene hypothesis. Modern hygiene may cause *delayed faecal–oral transmission*, so that the virus infection occurs later than was normal during human evolution. It is possible that autoimmune disease can then results, because the immune system is at an inappropriate stage of maturation, and levels of antibody obtained transplacentally are lower.

Attempts to relate viruses to MS have a long history and will be discussed in detail in other chapters in this volume. The link with Epstein–Barr virus (EBV) remains controversial [88], as does the recent interest in local activation of endogenous retroviruses [89]. The only point to be made here is that neither of these can explain the massive recent increases in incidence, so environmental factors have to be invoked. One interesting idea is a synergy between EBV and vitamin D deficiency [90].

Role of Secondary Factors

As suggested in the previous section, even if some viruses do trigger autoimmune disease, it seems that they rarely did so in the past. If they tend to do so now, there must have been relevant recent environmental changes.

Other factors can be shown to have a secondary role via similar historical analysis. The Neolithic revolution (First Epidemiological Transition) about 10,000 years ago led to changes in diet, with progressive increases in consumption of foods containing saponins, lectins, gliadin and capsaicin, all of which can increase intestinal permeability [91] (moreover, increased permeability can lead to increased uptake of endotoxin, and so to further inflammation [3]). Increased permeability is seen by some authors as likely to be critical for the subsequent increases in autoimmunity because it could result in increased intake of epitopes cross-reactive with self [91]. However, the increase in autoimmunity with which this volume is concerned did not occur for another 10,000 years! Therefore, if the switch from the hunter–gatherer diet to the early agricultural diet does contribute to the rise in autoimmunity, it does not do so until the underlying immunoregulatory deficit is established at the Second Epidemiological Transition. These diseases are rare in contemporary traditional agricultural communities in developing countries that live on grain, chillies and tomatoes!

Other authors assume that the human diet has become more diverse so that we are now exposed to a flood of "novel" antigens with greater likelihood of cross-reactivity with self. But humans have always eaten absolutely anything available. There is no evidence at all that our diet is more diverse that it used to be [92]. There is however evidence that a very fatty diet can increase permeability and that a heavy input of sugars can cause overgrowth of inappropriate intestinal flora [3], so these very recent trends might be further exacerbating the immunoregulatory problems caused by loss of Old Friends.

Similarly, it is likely that low levels of vitamin D contribute to immunoregulatory problems, but again this can only be relevant in the presence of other factors. It was normal for young ladies in Victorian England to avid sunlight in order to maintain a pale white complexion, but autoimmunity appears to have been rare. Interestingly, the Karelians living in Russia have very low levels of Type 1 diabetes, which is six times more prevalent in Karelians living in adjoining Finland [93]. Thus, although genetically identical (including their HLA-DQ alleles) and living at the same Northern latitude, the thoroughly modern Westernized Finnish Karelians suffer from this problem, while the backward, rural, undeveloped Russian Karelians do not [93]. The latter have quite different microbial exposures [94].

The Future

There is a widespread view that the hygiene hypothesis is in conflict with the obvious need for constant improvements in hygiene. In reality there is no conflict. Not even the most extreme adherent of the Old Friends hypothesis would claim that we are too clean for our own good. We cannot go back to the hunter–gatherer lifestyle. Relaxing domestic hygiene in a modern urban environment would not expose us to Old Friends…. only to new enemies like *E. coli* O104! We can however research the mechanisms that enable the Old Friends to enhance immunoregulation, and exploit these as drugs, vaccines or probiotics to combat the rising epidemic of chronic inflammatory disorders. The recent clinical trial in MS using *Trichuris suis* is encouraging [95], and if further trials can prove the principle that "Old Friends" are therapeutic via induction of immunoregulation, then there will be renewed efforts to unravel the mechanisms and the molecules responsible so that the use of the organisms themselves can be circumvented. Similarly, manipulation of the bowel flora is in its infancy but has enormous potential for the future.

This concept is in danger of being split into several different competing hypotheses: fetal programming by maternal microbial exposures, neonatal programming, the hygiene hypothesis, gut microbiota and diet. I have attempted to integrate these ideas under the single umbrella concept that our microbial histories, from fetal life and throughout adulthood, modulate our immunoregulatory circuits.

Finally, this chapter is not intending to suggest that the Old Friends hypothesis is the whole explanation for the rise in autoimmune diseases. But the genetic, molecular mimicry and viral hypotheses are incoherent without a major simultaneous environmental change to weaken background immunoregulation, so that certain genotypes, in the presence of certain triggers, can develop these diseases.

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