Reconstitution of the human biome as the most reasonable solution for epidemics of allergic and autoimmune diseases

Staci D. Bilbo [a], Gregory A. Wray [b], Sarah E. Perkins [c], William Parker [d,*]

[a] Department of Psychology and Neuroscience, Systems and Integrative Neuroscience Group, Duke University, Durham, NC 27710, United States
[b] Department of Biology, Duke Institute for Genome Sciences and Policy, Duke University, Durham, NC 27710, United States
[c] Cardiff School of Biosciences, Biomedical Sciences Building, Museum Avenue, Cardiff CF10 3AX, UK
[d] Department of Surgery, Duke University Medical Center, Durham, NC 27710, United States

ARTICLE INFO

ABSTRACT

A wide range of hyperimmune-associated diseases plague post-industrial society, with a prevalence and impact that is staggering. Strong evidence points towards a loss of helminths from the ecosystem of the human body (the human biome) as the most important factor in this epidemic. Helminths, intestinal worms which are largely eradicated by elements of post-industrial culture including toilets and water treatment facilities, have an otherwise ubiquitous presence in vertebrates, and have co-evolved with the immune system. Not only do helminths discourage allergic and autoimmune reactions by diverting the immune system away from these pathologic processes and stimulating host regulatory networks, helminths release a variety of factors which down-modulate the immune system. A comprehensive view of hyperimmune-related disease based on studies in immunology, parasitology, evolutionary biology, epidemiology, and neurobiology indicates that the effects of biome depletion may not yet be fully realized, and may have an unexpectedly broad impact on many areas of human biology, including cognition. Fortunately, colonization with helminths results in a cure of numerous autoimmune and allergic diseases in laboratory rodents, and clinical studies in humans have indicated their utility for treatment of both multiple sclerosis and inflammatory bowel disease. Based on these considerations, commitment of considerable resources toward understanding the effects of "biome depletion" and systematically evaluating the most effective approach toward biome reconstitution is strongly encouraged.

© 2011 Elsevier Ltd. All rights reserved.

Introduction

The field of clinical immunology is faced with epidemics of a wide range of allergic, autoimmune, and other inflammation-associated diseases with an impact that is staggering. These epidemics, which appear to be products of post-industrial society, have strongly resisted ever-increasing efforts focused on the development of pharmaceuticals to combat the problem [1]. In this paper, we examine evidence from the fields of evolutionary biology, ecology, immunology, parasitology, neurobiology, and medicine for the hypothesis that the only reasonable solution to these epidemics is to reconstitute the ecosystem of the human body, the "human biome", with factors, especially helminths, that have been depleted in post-industrial society. A range of arguments which might be mounted against this hypothesis are addressed, as well as the implications for biomedical research and the field of clinical immunology.

## Epidemiology of hyperimmune-associated disease

Epidemics of diseases associated with a hyperactive immune system occur much more frequently in countries that enjoy the widespread use of modern medical care, sanitation practices, and water treatment technology [2–4]. These diseases can be associated with an increased propensity for inflammation, and often involve pathologic immune reactivity directed against self and non-self antigens, producing autoimmune and allergic diseases, respectively. The wide range of these non-infectious conditions is striking, and includes lupus, multiple sclerosis, inflammatory bowel disease, hay fever, appendicitis, eczema, Graves' disease, asthma, and dangerously allergic reactions against antigens in common food substances including nuts, milk and grains [5,6]. The potential that some neurological disorders such as autism may also be hyperimmune-associated [7] adds further to the milieu. The prevalence of these diseases in post-industrial societies is staggering [6,8]. For example, more than one person in 1000 acquires lupus [9], approximately one in 600 acquires multiple sclerosis in some areas of the world [10], another one in 600 acquires type 1 diabetes [11], one in 40 suffers from food allergies [12], approximately one in 20 acquires autoimmune thyroid disease [13], about one in 18

* Corresponding author. Address: Department of Surgery, Duke University Medical Center, Box 2605, Durham, NC 27710, United States. Tel.: +1 919 681 3886; fax: +1 919 681 7263.
E-mail address: bparker@duke.edu (W. Parker).

reports symptoms of asthma [14], about one in 13 acquires appendicitis [15], and approximately half of the population in the United States reacts against common allergens based on a skin test [16] and experiences symptoms such as a runny nose and itchy eyes that are consistent with allergic reactions [17]. The most widely supported view of the cause of this epidemic can be termed "The Biome Depletion Theory". This theory states that a profound depletion of components from the ecosystem of the human body, the "human biome", in post-industrial society has left our immune systems profoundly over-reactive, with a strong propensity to react against a wide range of non-pathogenic self and non-self antigens.

The human biome and the immune system: a single, functional unit

The Biome Depletion Theory is based on the intimate connection between the human immune system and those organisms that live in and on the human body. Studies in which animals are completely separated from all microorganisms have been instrumental in our understanding of the connection between the immune system and the rest of the human biome. Decades of experiments have reproducibly demonstrated that microbe-free animals, known as gnotobiotic or germ free animals, have an immune system which is grossly undeveloped relative to the immune system of animals that are colonized with a normal bacterial flora [18–21]. The addition of bacteria to previously germ free animals results in restoration of the immune system [21], and demonstrates the dependence of the immune system on the microbial flora. Ongoing work on the relationship between the microbial flora and the immune system has led to the understanding that the immune system is better described as an interface with the environment more so than as a defensive wall against the environment. The immune system's role as an environmental interface is illustrated by its support of mutualistic microorganisms that deter pathogenic microorganisms, a process which potentially provides a more effective barrier to contagious disease than does anti-microbial measures carried out directly by the immune system [22].

Understanding the connection between the microorganisms normally associated with our bodies and the immune system has garnered great interest, and knowledge in this field is expanding quickly. These human-associated microorganisms, collectively known as the "microbiome", comprise an important part of the human biome. The importance of this part of the human biome extends far beyond the immune system, affecting metabolism and potentially the development of other parts of the biome, including worms, or helminths, that inhabit the gut of all vertebrate species [23]. Modern sanitation and water treatment practices, the widespread use of antibiotics, modern practices in obstetrics (e.g., delivery by caesarian section and the use of antiseptics), and the use of substitutes for mother's milk, have all profoundly affected the microbiome. These changes almost certainly contribute to hyper-immune-associated disease [24,25], but it is profound changes in colonization of the gut by helminths rather than changes in colonization of the gut by microorganisms that has been identified as the single most important factor impacting epidemics of hyperimmune-associated disease [26–29]. Helminths interact in a complex manner with the immune system of the host as well as with the microorganisms of the human biome, forming a key component of the highly interdependent network normally associated with the human biome (Fig. 1). This key component of the biome has largely been eradicated in post-industrial society, and a wide range of evidence suggests that this depletion leaves the remaining components of the biome in an unbalanced, relatively unstable, and disease-prone state.
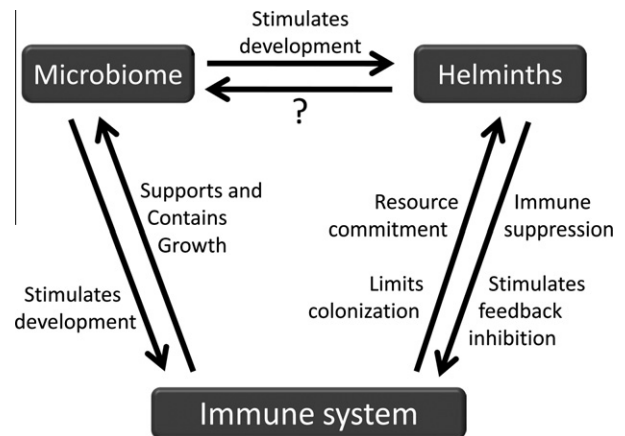
Fig. 1. Interdependence of the microbiome (microorganisms colonizing the human body), helminths colonizing the human body, and the immune system. Factors having a strong effect on any one component potentially have both direct and indirect effects on all other components. Each of these three major components is itself a complex and interdependent set of subcomponents, and potentially interacts with other systems (e.g., endocrine, nervous, cardiovascular) in the human biome.

Coevolution of vertebrates with helminths

The ancient coevolutionary history of the immune system with helminths provides insight into the entrenched nature of the immune system-helminth connection. The evolutionary origin of jawed fishes more than 400 million years ago marked not only the appearance of immune systems containing all of the major components found in humans, but also presumably provided suitable vertebrate hosts for flatworm parasites [30]. Although it remains unknown when in evolutionary history helminths took up residence in the vertebrate gut, several lines of evidence point toward helminths residing in the guts of vertebrates more than 100 million years ago. For example, the observation that several clades (groups of related species) of invertebrates are composed entirely of species that are obligate associates of vertebrate guts suggests that gut colonization has been present for as long as these clades have been in existence [31]. Thus, gut colonization probably dates back to the origin of tapeworms (cestodes, with more than 1000 species), spiny-headed worms (acanthocephalans, with more than 1100 species), and several clades of flukes (trematodes) and roundworms (nematodes) [32]. Additional evidence for long-term associations between helminths and vertebrates comes from phylogenetic analyses that have uncovered cases where host and helminth groups show similar patterns of speciation [31,33], suggesting that helminths and hosts have co-evolved. Hoberg et al., for example, considering the coevolutionary history of tapeworms and vertebrates, estimated that these helminths have co-evolved with their vertebrate hosts for a minimum of 350–420 million years [34]. Additional assessments of helminth–vertebrate gut associations by molecular clocks or by the geographic distribution of living species relative to tectonic events indicates that helminth–vertebrate associations are at least 100–200 million years old [32,35,36].

"Typical" association of helminths with humans

An understanding of "typical" helminth colonization in humans living in a Neolithic culture might provide some insight into the environment for which our immune systems are evolved to function. For several reasons, however, defining such colonization remains problematic. First, epidemiological data on helminth associations is often geographically patchy and gathered using

different methods, making generalization difficult. Second, most of the data in humans are not derived from populations living in a Neolithic culture, but rather are based on populations living in towns and cities, where sanitation and anti-helminthics alter the incidence of colonization and may affect which species are present. Third, colonization status differs enormously among human populations. Climate and socioeconomic status are two of the largest determinants of these differences, but other factors can have a strong influence [37,38]. Because many helminths have complex life cycles involving intermediate hosts such as snails or insects, the local ecology of these other species has a major impact. Activities such as farming and hunting increase the likelihood of contact with vectors of early life history stages of some helminths, and can also influence colonization rates and species composition. As a consequence, the epidemiological data we have are likely representative primarily of the past tens to hundreds of years of human history; they are almost certainly not characteristic of the vast sweep of time humans have been living in association with helminths.

Despite these uncertainties, it seems clear that colonization by multiple helminth species would have been nearly universal throughout human evolutionary history [39]. The most ancient direct evidence comes from two frozen humans dating to the fifth century BCE that contain embryos of hookworms [40]. However, physical evidence of this kind is rare, and the most extensive evidence comes from epidemiological studies. Over 340 species of helminths colonizing humans have been recorded [41], with about 30 of those species regarded as medically relevant. Rates of colonization are very high for some helminth species even today [41,42]. For example, more than one-sixth of the world's population is inhabited by just two soil-transmitted helminths, *Ascaris lumbricoides* and *Trichuris trichiura*, and several other helminths each inhabit more than 100 million humans [41,43]. In some parts of the world, colonization by multiple helminths is the norm rather than the exception [37,42]. Perhaps the most useful information regarding normal colonization status comes from indigenous populations, where the influence of confounding factors such as sanitation, anti-helminthics, and exotic food sources are minimal. For example, a study of people living in a pre-industrial culture in Rwanda revealed serological evidence of helminth colonization (average IgE levels in serum above normal for post-industrial society, despite the absence of allergy), even in those individuals which did not test positive for helminths based on a fecal analysis [44,45]. Collectively, the epidemiological evidence strongly suggests that humans have been living with multiple helminths throughout their evolutionary history, and that these associations are a normal part of our ecology.

Although the typical colonization of humans by helminths might be difficult to access with certainty, the number and diversity of helminth species has been assessed in a variety of wild (i.e., not laboratory bred or domesticated) animals, and may provide insight into what is typical of a mammalian species. Most individual animals in natural environments are colonized by both a wide diversity and a larger number and intensity of helminth species. Although geographic and ecological variations exist in the patterns of colonization, what is clear is that helminth colonization is the norm. From a broad dataset consisting of 56 mammalian species, in any given population, the number of helminth species was found to range from 2 to 39 species [46,47]. Further, rates of colonization by helminths are typically high in mammalian species closely related to humans, including the great apes [48]. For example, it is clear that our closest living relatives, common chimpanzees and bonobos, are both host to a diverse set of helminths in their natural habitats [31,48]. Each helminth species may be present in up to one-third of the individuals in these great ape populations, although sampling is exclusively based on feces

and therefore may underestimate true colonization rates [49,50]. Thus, studies evaluating colonization of non-human mammals by helminths suggests strongly that colonization by helminths is the norm for mammals, despite the fact that humans living in post-industrial societies have come to regard the absence of resident helminths as "normal".

Well-studied ecological biomes provide parallels that might help understand the dynamics of the human biome and the effects of biome depletion. Ecological biomes such as grasslands and tropical forests reach new, unstable equilibria as a consequence of rapid and prolonged loss of co-evolved components [51]. These biomes often contain "keystone species" that, relative to their biomass or density, have a disproportionately heavy impact on the ecosystem when lost [52]. We propose that the human biome follows the same properties and has been rendered unstable by the recent loss of a critical, co-evolved component: helminths. This instability is apparent in the widespread, aberrant function of the immune system in the absence of 'typical' helminth colonization, and suggests that helminths might collectively be considered as the equivalent of a keystone species of the human biome.

Modulation of the immune system by helminths

Helminths have evolved to secrete dozens if not hundreds of molecules that down regulate their host's immune system [53,54]. The range of immunoregulatory molecules includes a variety of protease inhibitors, including metalloprotease inhibitors, toll-like receptor signaling inhibitors, a variety of antioxidant enzymes, cytokine homologs, and a wide range of other molecules that inhibit or regulate the function of T-cells, macrophages, and dendritic cells [53]. Each species of helminth secretes different proteins that often exhibit strong host specificity, and many helminths secrete different proteins at different stages of their life cycle. Helminths also produce non-protein molecules such as phosphorylcholine, glycans, and lipids which down-regulate the immune system. It seems inevitable that the host has adapted to the presence of these immunosuppressive "drugs" [28].

Immunosuppression of the immune system by helminth-derived factors is only one means by which helminths might avert allergic and autoimmune reactions. Another very important property of helminth colonization that seems almost at odds with the immunosuppressive effect is that it causes a substantial and chronic immune response, which probably has several effects on the host. For example, by inducing chronic commitment of the immune system toward helminth-derived factors, helminths leave the immune system with fewer resources available for allergic or autoimmune responses. Further, chronic stimulation of the immune system by helminths results in the induction of a substantial feedback inhibition system [55,56]. In laboratory rodents, this is readily observed as an increased production of T-regulatory cells following colonization by helminths [53,57]. With these observations in mind, it seems evident that the ubiquitous presence of helminths and their omnipresence during the evolution of the immune system have profoundly shaped the biology of both helminth and immune system [58], although many of the effects of this coevolution probably remain to be discovered.

Biome depletion as the cause of epidemics of hyperimmune-associated disease, but not the sole cause of hyperimmune-associated disease

Almost paradoxically, The Depleted Biome Theory does not imply that biome depletion is the primary cause of allergies or autoimmune diseases. In addition to biome depletion, two other equally important factors are often or perhaps even usually found to contribute to hyperimmune-associated diseases (Fig. 2). First,
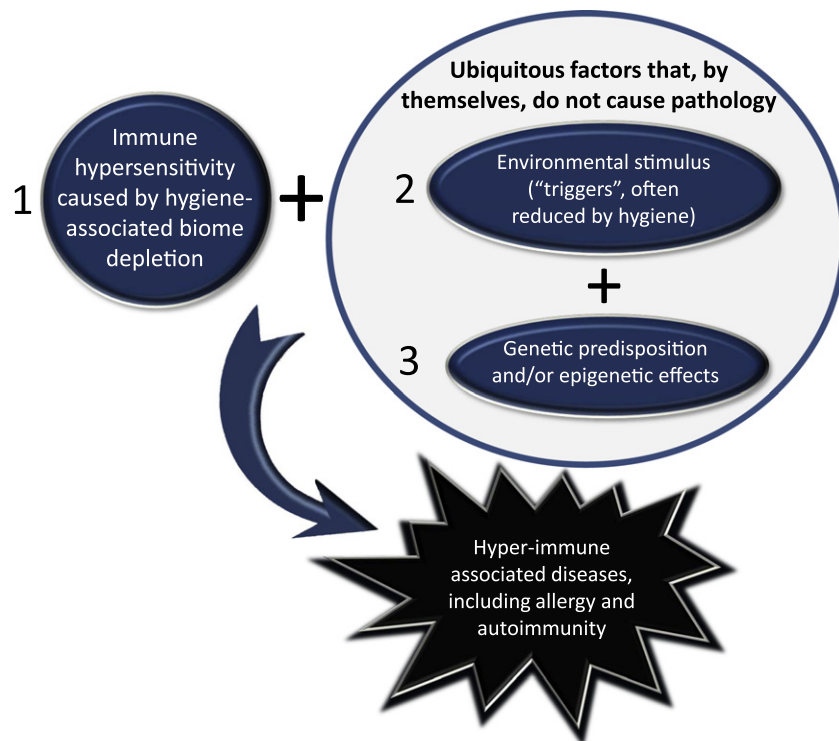
hyperimmune-associated diseases are often associated with a degree of heritability, having genetic and possibly epigenetic factors that predispose people to disease. Second, hyperimmune-associated diseases are often triggered by environmental factors that include viral infections, chemical pollutants, or common antigens produced by a wide range of organisms, ranging from ragweed to cockroaches to cows and dust mites. Thus, the Biome Depletion Theory involves a "three-hit paradigm" that is gaining increasing support. Hay fever, for example, is associated with genetic factors [59], and is, of course, triggered by exposure to antigens such as those produced by ragweed. Further, chemical pollutants probably affect the incidence and the severity of hay fever [60]. Thus, biome depletion by itself does not actually cause hay fever. However, hay fever is a disease only associated with post-industrial society [61], and ongoing studies indicate that it is biome depletion which positions the immune system to react toward harmless (non-pathogenic) antigens [62,63]. Thus, the epidemic of hay fever, like other hyperimmune-associated diseases, is apparently caused by biome depletion, even though biome depletion is not sufficient by itself to cause hay fever.

Multiple sclerosis is an example of a hyperimmune-associated disease that can be triggered by viral infection [64], is associated with certain genetic markers [65], but yet can be effectively treated by colonization with helminths [55,56]. Similarly, type 1 diabetes is potentially associated with specific proteins found in the diet [66,67] and is associated with certain genetic factors [68]. Yet, helminth colonization is an effective treatment for type 1 diabetes in autoimmune prone mice [69], and the increase in prevalence of type 1 diabetes in post-industrial cultures has not been explained by the introduction of new proteins into the diet or by a change in the genetics of the population. Thus, epidemics of multiple sclerosis and type 1 diabetes, as well as other autoimmune diseases and a variety of allergic diseases, are explained well by The Biome Depletion Theory, despite the presence of environmental triggers and genetic and/or epigenetic factors associated with the disease. Indeed, it is counterintuitive to attribute recent epidemics of disease solely to factors that have remained relatively unchanged for hundreds or even thousands of years, such as human genetics, ragweed pollen, wheat proteins, and common viruses. Rather, acute increases in disease must be attributed to factors such as biome depletion that are very recent occurrences. Unfortunately, failure to resolve this issue has resulted in considerable confusion in the biomedical literature: The identification of neither genetic factors that predispose to disease nor environmental factors that trigger disease rules out The Biome Depletion Theory as applicable (Fig. 2). Rather, it is (a) the role of "overly aggressive" immune reactivity, and (b) the increased incidence of disease in post-industrial society relative to pre-industrial agrarian or hunter gatherer societies that are the hallmarks of diseases associated with biome depletion.

Hygiene and the roots of The Biome Depletion Theory

The Biome Depletion theory evolved from an idea first developed in the 1980s that has long been known as "the hygiene hypothesis". This hypothesis was based on the understanding that certain hyperimmune diseases, allergies in particular, were associated with components of modern culture that generally increase hygiene, such as the use of modern, flushing toilets. As the understanding of hyperimmune-associated diseases evolved, it was eventually recognized that epidemics of allergies share a common cause with epidemics of autoimmune diseases. More importantly for our understanding of the nature of hyperimmune-associated diseases, changes in the microbiome and, more importantly,

Fig. 2. "The Biome Depletion Theory", with a three-hit paradigm of hyperimmune-associated disease. In this model, three factors interact to induce pathogenic allergic and autoimmune responses. While hygiene generally reduces the presence of one factor, "triggers", hygiene is the source of another factor, biome depletion and subsequent immune hypersensitivity. Of importance are the ideas that (a) ancient factors (triggers, genetic and epigenetics) interact with relatively recent biome depletion to cause epidemics of hyperimmune-associated disease, and (b) the effects of epigenetic factors on disease might not be evident until one or possibly more generations after biome depletion first occurs.

depletion of helminths from the human biome were identified as the leading suspects affecting epidemics of these diseases [29,70,71]. This final revelation clarified the particular component of hygiene responsible for epidemics of hyperimmune-associated disease. It was, in fact, not hygiene, *per se*, that was responsible. Indeed, increased exposures to a variety of components associated with an unhygienic environment, including viral infections, mold, dust mite-derived allergens, and cockroach-derived allergens, are known to be "triggers" for the induction of allergy and/or autoimmune disease. Thus, a particular house might be considered unhygienic in some regards because of high concentrations of mold or cockroach-derived allergens, which increase the propensity for allergic disease. At the same time, in the same house, the environment might be considered relatively hygienic because a modern, flushing toilet and running water are used regularly, which, as discussed below, effectively disrupts the life cycle of almost all helminths, further increasing the propensity for allergic disease. Therefore, hygiene *per se* does not have a predictable effect on hyperimmune-associated disease: Hygiene in the form of a toilet increases the risk for hyperimmune-associated disease, while hygiene in the form of preventing buildup of mold and cockroach derived allergens reduces the risk of hyperimmune-associated disease. Failure to understand this distinction has unfortunately retarded progress in the field.

The timing of biome depletion and hyperimmune-associated epidemics

The use of toilets effectively blocks the life cycle of almost all helminths, rendering humans in post-industrial societies as the only vertebrates free of association with these organisms to a large extent. The few helminths with the ability to reproduce despite the use of toilets, such as pinworms, are easily defeated by modern medicine because their ability to evolve resistance to pharmaceuticals is relatively limited by their long life cycle and small population size in comparison to bacteria and viruses that readily evolve drug resistance. Despite the fact that many individuals in post-industrial societies today take for granted the presence of toilets, the introduction of that technology is very recent, occurring, for example, in urban environments less than 100 years ago in the United States, and much more recently in rural environments. Most importantly, the impact of biome-depleting technologies such as the toilet on the immune system is expected to be delayed rather than immediate. This delay between cause and effect is anticipated for several reasons. First, many helminths have a long life span, some living for decades, and thus many individuals are expected to remain colonized for extended periods even after the transmission of the helminth has been halted. Second, some aspects of immunity may be strongly influenced by colonization with helminths early in life, and these influences may not be entirely reversed by loss of colonization at a later time. Thirdly, an individual's immune system is strongly influenced by their mother's immune system, and the extent to which the biome of the mother might protect the offspring from hyperimmune-associated disease remains unknown. The profound impact of epigenetics on human biology is only now beginning to be understood [72,73], and, although speculative, epigenetic effects resulting from colonization with helminths might be seen for multiple generations, thus delaying at least some of the effects of biome depletion (Fig. 2). With these factors in mind, it is not surprising that the effects of biome depletion are apparently increasing at the present time, despite the fact that for many, it was their grandparents, not themselves, which first enjoyed the convenience of biome-depleting technologies. In fact, we do not yet know if the potential effects of biome depletion have reached a climax.

Treatment of epidemics of allergy and autoimmune disease with biome reconstitution: results so far

It stands to reason that if biome depletion has caused epidemics of allergies and autoimmune diseases, then restoration of the biome should result in an end of those epidemics. Consistent with this reasoning, a number of experiments using animal models indicate that biome reconstitution does indeed prevent and even in some cases treat hyperimmune-associated disease [26–29]. For example, experimentally induced colitis, experimentally induced allergy, and type 1 diabetes can be effectively averted or treated in rodents by colonization with *Heligmosomoides polygyrus*, a roundworm commonly found in the small intestine of a variety of rodents [69,74,75]. Experimentally induced allergy in rodents can also be alleviated by colonization with another roundworm *Nippostrongylus brasiliensis* [76].

Even more encouraging than studies using animal models are two particular studies in humans. First, clinical trials demonstrate that exposure to a porcine helminth, *Trichuris suis*, provides an effective treatment for many patients with inflammatory bowel disease whose disease had proven untreatable with modern medicine [77]. More exciting results show that the progression of multiple sclerosis is halted by accidental helminth colonization [55].

A number of clinical trials addressing a variety of hyper-immune disorders are currently underway utilizing the porcine whipworm, *T. suis*, which has proven useful in treating inflammatory bowel disease, as mentioned above. Importantly, this helminth is adapted to colonize pigs, and does not colonize humans. Rather, it persists in the human intestine for a few days to weeks before being eliminated, and must be re-introduced to the human intestine on a frequent basis to maintain therapeutic levels. It is potentially difficult to place these pioneering clinical trials with *T. suis* within the broader perspective of biome reconstitution. It remains unknown, for example, how exactly exposure of a patient to *T. suis* might affect any subsequent attempts to reconstitute the biome of that patient with helminths that are adapted to colonize the human intestine. It also remains unknown how effective *T. suis* is in comparison to a variety of other helminths that are adapted to humans, and if *T. suis* might work in combination with other helminths as a therapy. Further, given the high cost of *T. suis*, due in large part to the need for frequent re-exposure to the organisms, it seems an unlikely candidate for use in clinical trials aimed at prophylactic reconstitution of the biome, if such trials are undertaken. Regardless of the utility or lack thereof that *T. suis* eventually affords modern medicine, work with *T. suis* represents the very first pioneering efforts in what promises to be a large and productive field of clinical medicine.

The health costs of biome depletion: how much?

Some might argue that treating hyperimmune-associated diseases such as hay fever and even food allergies is not worth the time, effort and energy that will be required to intentionally and systematically re-introduce organisms classified as "parasites" into the human population. Indeed, it can be argued that hay fever may be a worthwhile price to pay for avoiding these coevolutionary partners. Crohn's disease and multiple sclerosis are certainly less tolerable, but therapies which usually alleviate the symptoms of these diseases at least to some extent are available. Despite the generally high costs of many pharmaceuticals, the general consensus may be that some disease can be tolerated as a side effect of being parasite free. However, the apparently higher cost of biome depletion undermines this argument.

Although hyperimmune-associated diseases are not perceived as particularly life threatening, the death toll is not trivial. Allergic reactions to food alone kill an estimated 150–200 people every

year [78] Added to this number are the more than 3000 people who die from asthma each year [14,79]. In addition, if the current epidemic is not abated, more than one of every 300 people will have their lives strongly impacted if not shortened by lupus, multiple sclerosis, or type 1 diabetes. The number of deaths associated with colitis and other types of gastrointestinal inflammatory diseases adds to this number.

Another factor when considering the health costs of biome depletion is that we do not yet know the extent of pathology that may result. Diseases not obviously related to a hyperimmune response may be impacted by biome depletion. For example, biome reconstitution in mice attenuates helicobacter-induced gastric atrophy [80], suggesting that biome depletion may increase the propensity for gastric atrophy, which is a precursor to stomach cancer, the second leading cause of cancer-related deaths in humans. The overall analysis leaves us uncertain of the price we are currently paying for biome depletion, and in an uneasy "wait-and-see" position, not knowing what immune-associated epidemics are yet to come. This uneasy position is made worse by the realization that the effects of biome depletion may strongly impact human brain function.

Biome depletion and cognition

Our brain is intertwined with our immune system in ways that we are only beginning to understand [1,81]. Immunocompetent cells are located throughout every organ of the body, including the brain, and sophisticated interactions occur among these cells, primarily via soluble protein messengers called cytokines [82,83]. Notably, bidirectional communication between the brain and immune system by cytokines has significant consequences for neural function and behavior, including social, cognitive, and affective abilities. For instance, sick animals exhibit several well-characterized behavioral changes, including reductions in activity, exploration, and social and sexual interactions, as well as cognitive and affective changes [84,85]. Despite their maladaptive appearance, these behaviors are organized, adaptive strategies that are critical to host survival [86,87].

However, increasing evidence has linked chronic, *exaggerated* cytokine expression and resulting neuroinflammation within the brain to pathologic conditions as diverse as Alzheimer's disease, autism, and depression—notably, all conditions that share some striking similarities to sickness behaviors that have somehow become prolonged or pathological [88]. Neuroinflammation, for example, is strongly suspected as having a causal role in autism as well as a number of other developmental disorders. Further, neuroinflammation may be extremely important during the perinatal period, potentially altering the "program" of the normal course of development, with the result that adult outcomes, including behavior, are significantly and often permanently altered [89]. This idea accounts for the observation that perinatal exposure to infectious or other immune activating agents (e.g., stress, environmental toxins) have a number of enduring influences on physiology and behavior, including reactivity to stress, disease susceptibility, and notably, increased vulnerability to mental illness such as autism and schizophrenia [90–94]. Thus, to the extent that biome depletion results in an increased proinflammatory environment during the perinatal period, biome depletion is expected to cause an increase in the prevalence of mental illness. In support of this idea, a variety of indicators, including epidemiological, molecular, and genetic aspects of disease, suggest that biome depletion is responsible for the increasing incidence of autism [7].

A large number of neuroinflammatory changes have also been identified in normal brain aging, including upregulation of major histocompatibility (MHC II) expression on microglia, the resident immune cells of the brain [95], and increased expression of pro-inflammatory cytokines (e.g., interleukin [IL]-1β; [96]) which in turn may influence neural function (e.g., long-term-potentiation (LTP); [97]). Cognitive decline is one of the primary consequences of normal aging, although there is significant individual variability, as some individuals age more successfully than others, with a subset of the population eventually developing dementias such as Alzheimer's disease [98,99]. Again, the presence of neuroinflammatory changes in *normal* aging suggests the possibility that hyper-immune activity leading to excessive neuroinflammatory mechanisms can initiate poor cognitive aging. The potential influence of biome depletion on age-associated dementia remains unknown, but investigation of the possibility seems warranted.

Based on the above considerations, we suggest that biome depletion is changing the immune system at a population-wide level, which will undoubtedly impact the brain, particularly during sensitive periods of development and perhaps in the aging process. Importantly, this theory does not compete with any prior hypotheses or known risk factors for neuroinflammatory conditions, but rather complements them. For instance, biome depletion may set the stage for exaggerated levels of neuroinflammation, which interacts with other known risk factors (stress, infection, or genetics) in the induction of a given disease or disorder.

The physiologic effects of biome depletion

Although some of the consequences of biome depletion for health are becoming apparent, the exact nature of the effects of biome depletion on the immune system remains unknown, even in laboratory rodents. Virtually all research on the immune system has been conducted in biome-depleted (helminth free) laboratory animals or in humans living in a post-industrial society. Because of this, immunologists are now faced with the unsettling realization that the immune system they have spent all of their effort and energy dissecting over in the past 50 years is dramatically different than any immune system in a natural state of existence. In fact, "normal" is not helminth-free, and our coevolutionary partners must be included if we want to understand and appreciate the "normal" state of immunity.

Several factors potentially complicate efforts to determine the physiologic consequences of biome depletion. First, not only are the effects of biome depletion on a particular generation undoubtedly profound, the effects of biome depletion on subsequent generations, passed down by epigenetic mechanisms, might be considerable. The fact that the immune system of the fetus is strongly influenced by the immune system of the mother particularly supports this idea. In addition, the transfer of immune components through the mother's milk adds to the potential generational effects of biome depletion. A second factor which complicates efforts to determine the physiologic consequences of biome depletion is the fact that biome depletion is not an all-or-nothing proposition. The idea that specific components of the biome have different niches exponentially increases the complexity of efforts to evaluate the effects of biome depletion. The fact that those individual components of the biome interact with other components of the biome as well as with the host [100] adds even more complexity to the issue.

A wide range of studies in laboratory animals indicate that helminths exert a potent effect on virtually all components of the immune system [53]. Studies from our laboratory using a wild-caught rat versus laboratory rat model have provided insight into the potential extent of the impact of biome depletion on the immune system [45,101]. The differences between the immune systems of wild rats and those of laboratory rats exceed many inter-species differences in immunity. For example, wild rat derived lymphocytes

demonstrate an unresponsiveness that is not found in lymphocytes derived from laboratory rats, laboratory mice, or humans in a post-industrial society [101]. Similarly, studies comparing the immune systems of wild mice with laboratory mice [102] also show a wide range of differences in baseline immune parameters and immune responsiveness. To the extent that the biome is, in fact, the primary driving force behind the difference between "biome-normal" wild rodents and biome-depleted laboratory rodents, ongoing results indicate that biome depletion throws a wide range of parameters associated with cellular and humoral responses into disarray. For example, the overall maturation of the immune system as well as the balance between innate and adaptive immunity may be profoundly altered by biome depletion. As pointed out recently by Maizels and co-workers [53], helminths can regulate "every aspect of host immunity from initial recognition to end-stage effector mechanisms". Traditional paradigms associated with allergy and autoimmune disease (e.g., Treg versus Th1 versus Th2 versus Th17) do not adequately address this effect.

To reconstitute the biome or to develop helminth-inspired drugs that mimic a reconstituted biome?

Biomedical researchers often envision isolation and characterization of the individual components produced by helminths that modulate the immune system, with the goal of creating new helminth-inspired drugs to treat disease. On the one hand, this type of approach with other organisms has proven instrumental in the progress of modern medicine, giving us antibiotics and some important vaccines. Further, repeated injection of helminth-derived extracts or proteins in animal models of disease has already proven useful in alleviating such conditions as collagen-induced arthritis [103] and experimentally induced colitis [104], among others [105,106]. On the other hand, several factors are likely to limit the general use of this approach as a substitute for biome reconstitution.

First, it is difficult to imagine a single pharmaceutical or even a collection of pharmaceuticals that could recapitulate the vast complexity of the interaction between helminths and the host immune system. While pharmaceuticals are generally directed at one component in the immune apparatus, a single helminth species produces dozens if not more molecules that each target specific components of host immunity. The likely possibility that more than one species may be required to effectively reconstitute the biome adds a level of complexity that seems staggering to anyone interested in replicating the natural state using pharmaceuticals. Perhaps more importantly, our understanding of helminth immunoregulation is far from complete, with many gaps in our knowledge [53]. For example, many proteins secreted by helminths in low abundance may not have been identified, but yet may have important biological activity. Further, the biological role of some proteins secreted by helminths in high abundance, such as the glycolytic enzyme triose phosphate isomerase, remains poorly understood [53]. Thus, our present level of understanding of the vastly complex interactions between helminths and their hosts potentially adds a substantial hurdle to the design of therapeutics which will mimic those interactions.

Not only is the helminth/host interface vastly complex, it requires continuous input from the helminth. Helminths actively maintain a state of immunosuppression, and removal of helminths results in a loss of that regulation [53], suggesting that any pharmaceutical substitute would also need to be maintained at a relatively constant level for maximum efficacy. Such continuous maintenance is potentially difficult and costly to maintain using traditional pharmaceuticals, and regular injections of helminth-inspired drugs, even if effective, might prove burdensome and expensive. Further, the mechanism of delivery of helminth-derived

immunoregulatory molecules by living helminths may be important for their function, and it is possible that new drug delivery technologies may need to be devised which mimic the delivery of a living helminth. In other words, given the long coevolution of immune systems and helminths, it seems reasonable that the immune system may be adapted to and indeed dependent upon the nature, dose, and delivery method used by living helminths.

Probably it is the field of evolutionary biology that most strongly argues for living helminths and biome reconstitution in favor of pharmaceutical substitutes. Helminths represent a potential therapeutic that has been fine-tuned by hundreds of millions of years of natural selection not to encumber a healthy host. Consistent with this idea, several helminths are known to cause few if any symptoms in most cases [55,107,108]. The bovine tapeworm (*Taenia saginata*), for example, has been labeled a "commensal" rather than a parasite in humans [107]. To achieve this balance, natural selection has tested countless billions of combinations of molecular tools over millions of years, selecting those that are most effective for both helminth and host survival. Quite obviously, no pharmaceutical has ever been developed to match that record.

Another advantage that living helminths have over pharmaceuticals is their potential cost effectiveness. Helminths are typically long-term therapeutics if used in their natural host species, with a single dose lasting for years if not decades. Thus, costs will be limited by the long-term effectiveness of a single treatment. Further, the prospects for new technologies that facilitate cost effective and safe production of helminths in sufficient numbers for widespread use seem almost unlimited. Biotechnology involving in vitro culture of helminths or cultivation of human specific helminths in genetically modified mice (e.g., humanized or immuno-suppressed mice) are two examples of such biotechnologies which remain untapped. The technology and clinical implementation of biome reconstitution may eventually prove no more complex than that utilized for vaccinations, with biome reconstitution and maintenance being a routine part of a typical checkup at the doctor's office.

One potential advantage of living helminths as a therapeutic is that they might be genetically altered to enhance their beneficial nature. Currently available organisms can hypothetically be modified by selective breeding to enhance their beneficial nature, or may be modified in the laboratory to create specifically altered organisms, including transgenic helminths. In this manner, genetically modified helminths could be used as an effective drug delivery agent (vector) to treat a potentially unlimited variety of medical conditions. Helminths offer a number of advantages over viral or bacterial vectors for certain applications because of their long life span, built in resistance to destruction by the immune system, and, since they do not complete their life cycle in the host, a low propensity to mutate or evolve once introduced into the host.

One potential objection to the use of mutualistic helminths for biome reconstitution is that the introduction of organisms possessing "vastly complex and uncharacterized elements" into the human body might have unforeseen consequences. This argument is easily turned on its head, however, when we ask how it is rational to eliminate our coevolutionary partners that possess vastly complex and uncharacterized elements without expecting dire consequences. Indeed, many of the factors proven to be important for human survival, including the microbiome and even the food we eat, contain vastly complex and uncharacterized elements. Thus, complexity and a lack of understanding of that complexity do not rule out the importance of an element of the normal human biome for health. Further, many pharmaceuticals, molecules with substantially less complexity than helminths, cause exceedingly complex reactions within the human body, often with consequences that remain poorly understood. Thus, the relative simplicity of a

drug at the molecular level does not necessarily lead to a complete understanding of that drug's effects, and certainly does not impart safety for patients. Finally, the idea of biome reconstitution is not to modify human biology in a new way, but to restore the ecology of the post-industrial human biome to harmony with the way that our genes have evolved to function. In a nutshell, comfort with the idea of biome reconstitution requires only an understanding that natural selection has shaped our genes so that they are compatible with the environment. It does not require a complete and total understanding of that environment or how that environment interacts with the human body.

Another potential objection to the concept of biome reconstitution is the fact that helminths extract a horrible toll of suffering and death in developing countries [109]. Given this potential to cause harm, it could be argued that these organisms should not be reintroduced into post-industrial cultures. This argument, however, is entirely undermined by a consideration of the nature of parasitic disease in developing countries. In developing countries, at least one of the following three conditions are necessary ingredients for morbidity and mortality associated with helminth infection:

1. The widespread occurrence of starvation and malnourishment, conditions which add risk to colonization by helminths.
2. The absence of water treatment facilities and sewer systems, allowing uncontrolled infections and potentially high burdens of helminths.
3. The presence of helminths that are not well adapted to the host (e.g., parasites that are harmful to even a healthy host, such as *Dracunculus medinensis*, which causes dracunculiasis, *Taenia solium*, which causes cysticercosis, and *Loa loa*, which causes Loa loa filariasis).

Not one of these factors presents a difficult hurdle when considering the reintroduction of helminths into post-industrial societies, which generally enjoy over-nourishment rather than malnourishment. Given a proper selection of helminths for colonization, uncontrolled infections are impossible in the face of modern sewer systems and water treatment facilities. Further, modern medicine makes it possible to easily identify individuals with pre-existing conditions that could be contraindications for helminth therapy (e.g., anemia, malnutrition, immune deficiencies, coagulopathies, advanced aging, etc.). In addition, modern clinical research practices can readily be utilized to ensure that adverse side effects are reduced to far below acceptable levels prior to widespread therapeutic application. Thus, post-industrial cultures have the luxury of selecting controlled colonization with helminths, not pathologic infection. In essence, post-industrial society will have domesticated helminths. No restoration of the natural ecology of helminths is expected or even suggested, with the survival of the domesticated helminths being strictly dependent on the medical community. To maintain a reconstituted biome, patients would need periodic evaluation and maintenance, since natural transmission of therapeutic helminths would be non-existent given the proper selection of helminths and the sewer systems of post-industrial culture. Further, given the effectiveness of anti-helminthic drugs, therapy will be readily reversible should particular patients develop a condition in which the helminths are no longer warranted.

The loss of evolutionary partners leading to disease is not unique to the helminth/hyper-immune condition. The historically omnipresent struggle against predators and for food resources have left our bodies addicted to particular diets and a degree of exercise. The loss of predators and the loss of any challenge in finding food in post-industrial cultures have led to epidemics of obesity, type 2 diabetes, and other complications associated with a rich diet and little exercise. However, just as post-industrial societies do not need to face predation and possible starvation to avoid obesity and heart disease; neither do post-industrial cultures need to face the specter of helminth-associated disease in order to avoid allergy and autoimmune disease. Just as we have the option of safely utilizing proper diet and exercise regimens to give our cardiovascular system what it has evolved to require, it is expected that, far more effortlessly, we can safely utilize selected organisms to give our immune system what it has evolved to require.

Biome reconstitution and the public consciousness

A commonly expressed concern regarding biome reconstitution regards the ability of those who would benefit from the therapy to actually accept the therapy. Some speculate that the prospect of helminth colonization will prove too repulsive for most patients to accept. However, from several perspectives, it can be argued that the view of the public toward the concept of biome reconstitution will pose no obstacle whatsoever. Most importantly, it is expected that biome reconstitution will be the subject of extensive clinical trials, and will have a proven safety record prior to widespread use. In the face of clinical studies potentially showing that biome depletion can lead to lupus, multiple sclerosis, food allergies, asthma, and perhaps even an autistic child, it seems far more likely that concern over biome depletion rather than fear of biome reconstitution will be the hallmark of public opinion in the future. It seems inconceivable that an educated public would react with paranoia toward helminths rather than with concern for their health and the health of their children. Certainly the educated public has not reacted with paranoia toward probiotics. Indeed, the public is becoming aware of the dangers to their microbiome imposed by the heavy use of antibiotics. Further, no responsible individual plans to market helminths as a can of worms any more than probiotics or vaccinations have been marketed as a can of germs. There seems to be little evidence to support the idea that revulsion of helminths will be an impediment to biome reconstitution.

Reassuringly, negative views toward helminths that might be adopted by a few individuals are not expected to influence the efficacy of biome reconstitution for the population as a whole. As with diet and exercise programs, the health benefits of biome reconstitution will be limited to those who embrace the prospect, and one patient's health will not be influenced by another's decision to embrace or reject biome reconstitution. Fortunately, biome reconstitution should eventually prove much easier for the average individual to implement than does an effective diet and exercise program, since biome reconstitution is potentially a matter easily handled by a physician during a routine medical exam.

Approaching the solution

The approach to biome reconstitution needs to be systematic and rapidly executed. The following questions need to be addressed with the utmost urgency:

1. Which helminths or combination of helminths are safe and effective? Several species, including the bovine tapeworm (*T. saginata*), the human whipworm (*T. trichiura*), and the human hookworm (*Necator americanus*) are well adapted to humans, asymptomatic in low numbers, and thus seem to be obvious candidates for initial consideration. New technologies might be developed (e.g., irradiation of organisms to achieve sterility and eliminate the possibility of transmission, new culture methods, or cultivation of human-specific helminths in ultra-clean and immunodeficient rodents) which could improve on the initial answers to this question.

2. Which helminths should be utilized, at what dose, and under what conditions (patient age, disease state, gender, etc.)? Biome reconstitution for a 3-year old patient may be quite different that that required for a 30-year old patient, and the best approach to biome reconstitution for children may depend on the status of the mother's biome during pregnancy and the first years of the child's life.

3. Which hyperimmune-associated diseases can be cured or effectively treated with biome reconstitution, versus which can only be prevented by biome reconstitution? The answer to this question should drive efforts (or the lack thereof) to prophylactically restore the biome in the general population.

4. How important for children is the biome of the mother prior to and during pregnancy, and while breastfeeding?

5. What medical conditions (e.g., suppressed immune system, anemia, and coagulopathy) would be contraindications for biome reconstitution?

6. Are particular human genotypes better suited to particular helminth loads? Certainly hyperimmune-associated disease is connected strongly with genotype, and it is thus reasonable to expect that an optimized approach to biome reconstitution may need to consider the genotype of the patient.

7. Does modern medicine need to work toward reconstituting components of the biome other than helminths? For example, should intentional and controlled exposure to a normal microflora in a manner that recapitulates natural child birth replace current practices in obstetrics?

8. Will certain components of modern medicine (e.g., vaccines and immunosuppressive drugs) need to be modified so that they are effective on individuals with a healthy (reconstituted) biome? Modern medicine has been tailored to those with a depleted biome, and may require some adjustment for those with a healthy biome.

9. What are the effects of biome reconstitution on human biology in general? For example, how does it affect reproduction, aging, cancer, infectious disease, and cognition?

Obtaining the answers to these questions is the beginning of a new chapter in modern medicine and biotechnology, and potentially closes the door on a chapter aimed at treating hyperimmune associated diseases with pharmaceuticals. The answer to these questions will potentially drive a substantial arm of clinical medicine for the foreseeable future. Given the current mortality, morbidity, and expense inflicted by epidemics of hyperimmune-associated disease, speed seems paramount. At the same time, however, a systematic rather than a piecemeal approach to the solution is strongly encouraged.

Prognosis for modern medicine

One question in the minds of many who recognize the vast potential that biome reconstitution appears to offer is how quickly modern medicine will take advantage of this potential. From several perspectives, the prognosis for modern medicine is very good. Modern immunology has proven to be a highly flexible discipline as it struggles against widespread epidemics of immune related disease. Within a few short years of initial discovery, new findings often become "hot" topics, accruing well funded laboratories and hundreds of research papers. With that in mind, a tipping point in medicine can be seen fast approaching, in which colonization with helminths becomes the next hot topic of basic and clinical research. The critical events that mark this tipping point are not likely public opinion or even our capacity to solve the problem, but rather a change of paradigm within the minds of the policy makers of the biomedical community. When those who strongly influence the direction of the fields of clinical and basic immunology decide to take action, biomedical research can move with astonishing speed.

After that tipping point is reached for biomedical research, financial considerations could drive a very rapid evolution of clinical medicine. For example, if restoring the biome of a few hundred patients is shown to prevent the onset of even a few cases of such costly diseases as asthma, type 1 diabetes, or autism, strong pressures on financial resources could force the health care system to adapt quickly. With that in mind, the day when a doctor will prescribe not only a vaccination against a dreaded disease, but also colonization with a domesticated helminth, may not be so far distant in the future. The best news is that this type of treatment is expected to be both inexpensive and without major complications. If we keep open minds about the possibilities of biome reconstitution – the "tipping point" may be just around the corner.

Conclusions

It was only a very few generations ago, at most, that humans first began widespread use of indoor flushing toilets and acquired access to modern medicine. These technologies, which effectively interrupt most helminth life cycles, have, over time, resulted in a profound depletion of the human biome. Realizing the intimacy and strength of the connection between the biome and the immune system, it is possible to arrive at a remarkably simple conclusion that can account for a wide range of post-industrial disease: Biome depletion has left us with an overly reactive immune system, leading to epidemics of a wide range of allergic and autoimmune diseases.

A synthesis of epidemiology, modern medicine, evolutionary biology and immunology strongly suggests that biome reconstitution and maintenance should be a major thrust of biomedical research in the very near future, and a major component of medicine in the foreseeable future. Helminth-derived pharmaceuticals might offer an alternative to biome reconstitution, but given what is already known about the complex and multi-faceted nature of helminth–host interactions, pharmaceuticals which effectively mimic the effects of helminths might prove extremely difficult and expensive to develop and utilize. Further, the low costs, high efficacy, and very low rate of side effects that are expected to be associated with biome reconstitution make this approach extremely appealing. In addition, the potential for helminths to be used prophylactically and the potential for new helminth-supported biotechnology applications add to the attractiveness of biome reconstitution. The domestication of helminths, as with the domestication of other animals, may eventually provide a means for selection and breeding of organisms that have a greater utility for humans than those helminths found naturally.

The biology imposed by our evolution is, at present, inescapable, and medical science of the future will take that fact fully into account. To achieve this aim, intensive and systematic research should now be focused on the wide range of questions regarding the effects of biome depletion and the application of biome reconstitution. Hopefully, in the future, our biomes will be checked as regularly as our cholesterol is checked, with proactive as well as reactive biome reconstitution being routine.

Conflict of interest statement

The authors have no financial or other relationship with people or organizations that may inappropriately influence the author's work.

References

[1] Davis MM. A prescription for human immunology. Immunity 2008;29:835–8.

[2] I.S. Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee [see comment]. Lancet 2005;351:1225–32.

[3] Onkamo P, Vaananen S, Karvonen M, Tuomilehto J. Worldwide increase in incidence of Type I diabetes—the analysis of the data on published incidence trends. Diabetologia 1999;42:1395–403.

[4] Black P. Why is the prevalence of allergy and autoimmunity increasing? Trends Immunol 2001;22:354–5.

[5] Gergen PJ, Turkeltaub PC, Kovar MG. The prevalence of allergic skin test reactivity to eight common aeroallergens in the US population: results from the second National Health and Nutrition Examination Survey. J Allergy Clin Immunol 1987;80:669–79.

[6] Genuis SJ. Sensitivity-related illness: the escalating pandemic of allergy, food intolerance and chemical sensitivity. Sci Total Environ 2010;408:6047–61.

[7] Becker KG. Autism, asthma, inflammation, and the hygiene hypothesis. Med Hypotheses 2007;69:731–40.

[8] Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective. Am J Pathol 2008;173:600–9.

[9] Lahita RG. Special report: adjusted lupus prevalence. Results of a marketing study by the Lupus Foundation of America. Lupus 1995;4:450–3.

[10] Bostrom I, Callander M, Kurtzke JF, Landtblom AM. High prevalence of multiple sclerosis in the Swedish county of Varmland. Mult Scler 2009;15:1253–62.

[11] Vehik K, Hamman RF, Lezotte D, Norris JM, Klingensmith G, Bloch C, et al. Increasing incidence of type 1 diabetes in 0- to 17-year-old Colorado youth. Diabetes Care 2007;30:503–9.

[12] Liu AH, Jaramillo R, Sicherer SH, Wood RA, Bock SA, Burks AW, et al. National prevalence and risk factors for food allergy and relationship to asthma: results from the National Health and Nutrition Examination Survey 2005–2006. J Allergy Clin Immunol 2010;126:798–806. e713.

[13] Bagnasco M, Bossert I, Pesce G. Stress and autoimmune thyroid diseases. Neuroimmunomodulation 2006;13:309–17.

[14] Mannino DM, Homa DM, Akinbami LJ, Moorman JE, Gwynn C, Redd SC. Surveillance for asthma—United States, 1980–1999. MMWR: Surveill Summ 2002;51:1–13.

[15] Addiss DG, Shaffer N, Fowler BS, Tauxe RV. The epidemiology of appendicitis and appendectomy in the USA. Am J Epidemiol 1990;132:910–25.

[16] Arbes Jr SJ, Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the Third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 2005;116:377–83.

[17] Singh K, Axelrod S, Bielory L. The epidemiology of ocular and nasal allergy in the United States, 1988–1994. J Allergy Clin Immunol 2010;126:778–83. e776.

[18] Wilson KH, Sheagren JN, Freter R, Weatherbee L, Lyerly D. Gnotobiotic models for study of the microbial ecology of Clostridium difficile and Escherichia coli. J Infect Dis 1986;153:547–51.

[19] Cebra JJ. Influences of microbiota on intestinal immune system development. Am J Clin Nutr 1999;69:1046S–51S.

[20] Gordon HA, Pesti L. The gnotobiotic animal as a tool in the study of host microbial relationships. Bacteriol Rev 1971;35:390–429.

[21] Zaneveld J, Turnbaugh PJ, Lozupone C, Ley RE, Hamady M, Gordon JI, et al. Host-bacterial coevolution and the search for new drug targets. Curr Opin Chem Biol 2008;12:109–14.

[22] Parker W, Thomas AD. Cultivation of epithelial-associated microbiota by the immune system. Future Microbiol 2010;5:1483–92.

[23] Hayes KS, Bancroft AJ, Goldrick M, Portsmouth C, Roberts IS, Grencis RK. Exploitation of the intestinal microflora by the parasitic nematode Trichuris muris. Science 2010;328:1391–4.

[24] Michael P, Diane RG, Hassen A, Elaine H, Juan CC. Birth by cesarean section, allergic rhinitis, and allergic sensitization among children with a parental history of atopy. J Allergy Clin Immunol 2008;122:274–9.

[25] Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. Am J Gastroenterol 2010;105:2687–92.

[26] Capron A, Dombrowicz D, Capron M. Helminth infections and allergic diseases. Clin Rev Allergy Immunol 2004;26:25–34.

[27] Wilson MS, Maizels RM. Regulation of allergy and autoimmunity in helminth infection. Clin Rev Allergy Immunol 2004;24:35–50.

[28] Fumagalli M, Pozzoli U, Cagliani R, Comi GP, Riva S, Clerici M, et al. Parasites represent a major selective force for interleukin genes and shape the genetic predisposition to autoimmune conditions. J Exp Med 2009;206:1395–408.

[29] Rook GAW. Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. Immunology 2009;126:3–11.

[30] Freeman RS. Ontogeny of cestodes and its bearing on their phylogeny and systematics. In: Ben D, editor. Adv. Parasitol, vol. 11. Academic Press; 1973. p. 481–557.

[31] Brooks DR, McLennan DA. Extending phylogenetic studies of coevolution: secondary Brooks parsimony analysis, parasites, and the Great Apes. Cladistics: Int J Willi Hennig Soc 2003;19:104–19.

[32] Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A, et al. A molecular evolutionary framework for the phylum Nematoda. Nature 1998;392:71–5.

[33] Hugot JP. Primates and their pinworm parasites: the Cameron hypothesis revisited. Syst Biol 1999;48:523–46.

[34] Hoberg EP, Gardner SL, Campbell RA. Systematics of the Eucestoda: advances toward a new phylogenetic paradigm, and observations on the early diversification of tapeworms and vertebrates (2nd international workshop for tapeworm systematics, Lincoln, NE, USA, October 2–6, 1996). Syst Parasitol 1999;42:1–12.

[35] Verneau O, Bentz S, Sinnappah ND, du Preez L, Whittington I, Combes C. A view of early vertebrate evolution inferred from the phylogeny of polystome parasites (Monogenea: Polystomatidae). Proc R Soc Lond B: Biol Sci 2002;269:535–43.

[36] Olson PD, Tkach VV. Advances and trends in the molecular systematics of the parasitic platyhelminthes. Adv Parasitol 2005;60:165–243.

[37] Brooker S, Clements ACA, Bundy DAP. Global epidemiology, ecology and control of soil-transmitted helminth infections. Adv Parasitol 2006;62:221–61.

[38] Harhay MO, Horton J, Olliaro PL. Epidemiology and control of human gastrointestinal parasites in children. Expert Rev Anti-Infect Ther 2010;8:219–34.

[39] Hurtado AM, Frey MA, Hill K, Baker J. The role of helminths in human evolution. Implications for global health in the 21st century. In: Elton S, O'Higgins P, editors. Medicine and evolution: current applications, future prospects. New York: Taylor and Francis; 2008.

[40] Crubezy E, Magnaval JF, Francfort HP, Ludes B, Larrouy G. Herodotus, the Scythes and hookworm infection. Lancet 2006;367:1520.

[41] Crompton DWT. How much human helminthiasis is there in the world? J Parasitol 1999;85:397–403.

[42] Brooker S, Utzinger J. Integrated disease mapping in a polyparasitic world. Geospatial Health 2007;1:141–6.

[43] Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 2006;367:1521–32.

[44] Scaglia M, Tinelli M, Revoltella R, Peracino A, Falagiani P, Jayakar SD, et al. Relationship between serum IgE levels and intestinal parasite load in African populations. Int Arch Allergy Appl Immunol 1979;59:465–8.

[45] Devalapalli AP, Lesher A, Shieh K, Solow JS, Everett ML, Edala AS, et al. Increased levels of IgE and autoreactive, polyreactive IgG in wild rodents: implications for the hygiene hypothesis. Scand J Immunol 2006;64:125–36.

[46] Bordes F, Morand S. Helminth species diversity of mammals: parasite species richness is a host species attribute. Parasitology 2008;135:1701–5.

[47] Poulin R, Mouillot D. The evolution of taxonomic diversity in helminth assemblages of mammalian hosts. Evol Ecol 2004;18:231–47.

[48] Nunn CL, Altizer S, Sechrest W, Jones KE, Barton RA, Gittleman JL. Parasites and the evolutionary diversification of primate clades. Am Nat 2004;164:S90–S103.

[49] Howells ME, Pruetz J, Gillespie TR. Patterns of gastro-intestinal parasites and commensals as an index of population and ecosystem health: the case of sympatric western Chimpanzees (Pan troglodytes verus) and Guinea Baboons (Papio hamadryas papio) at Fongoli, Senegal. Am J Primatol 2011;73:173–9.

[50] Petrzelkova KJ, Hasegawa H, Appleton CC, Huffman MA, Archer CE, Moscovice LR, et al. Gastrointestinal parasites of the chimpanzee population introduced onto Rubondo Island National Park, Tanzania. Am J Primatol 2010;72:307–16.

[51] Foster D, Swanson F, Aber J, Burke I, Brokaw N, Tilman D, et al. The importance of land-use legacies to ecology and conservation. Bioscience 2003;53:77–88.

[52] Mills LS, Soule MF, Doak DF. The keystone-species concept in ecology and conservation. Bioscience 1993;43:219–24.

[53] Hewitson JP, Grainger JR, Maizels RM. Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. Mol Biochem Parasitol 2009;167:1–11.

[54] Zaccone P, Burton OT, Cooke A. Interplay of parasite-driven immune responses and autoimmunity. Trends Parasitol 2008;24:35–42.

[55] Correale J, Farez M. Association between parasite infection and immune responses in multiple sclerosis. Ann Neurol 2007;61:97–108.

[56] Correale J, Farez M, Razzitte G. Helminth infections associated with multiple sclerosis induce regulatory B cells. Ann Neurol 2008;64:187–99.

[57] Yazdanbakhsh M, van den Biggelaar A, Maizels RM. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease [see comment]. Trends Immunol 2001;22:372–7.

[58] Jackson JA, Friberg IM, Little S, Bradley JE. Review series on helminths, immune modulation and the hygiene hypothesis: immunity against helminths and immunological phenomena in modern human populations: coevolutionary legacies? Immunology 2009;126:18–27.

[59] Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD. Genetics of asthma and hay fever in Australian twins. Am Rev Respir Dis 1990;142:1351–8.

[60] Bhattacharyya N. Air quality influences the prevalence of hay fever and sinusitis. Laryngoscope 2009;119:429–33.

[61] Emanuel MB. Hay fever, a post industrial revolution epidemic: a history of its growth during the 19th century. Clin Allergy 1988;18:295–304.

[62] Fallon PG, Mangan NE. Suppression of TH2-type allergic reactions by helminth infection. Nat Rev Immunol 2007;7:220–30.

[63] Cooper PJ, Barreto ML, Rodrigues LC. Human allergy and geohelminth infections: a review of the literature and a proposed conceptual model to guide the investigation of possible causal associations. Br Med Bull 2006;79–80:203–18.

[64] Oleszak EL, Chang JR, Friedman H, Katsetos CD, Platsoucas CD. Theiler's virus infection: a model for multiple sclerosis. Clin Microbiol Rev 2004;17:174–207.

[65] Epplen C, Jackel S, Santos EJ, D'Souza M, Poehlau D, Dorzauer B, et al. Genetic predisposition to multiple sclerosis as revealed by immunoprinting. Ann Neurol 1997;41:341–52.

[66] Elliott RB, Harris DP, Hill JP, Bibby NJ, Wasmuth HE. Type I (insulin-dependent) diabetes mellitus and cow milk: casein variant consumption. Diabetologia 1999;42:292–6.

[67] Elliott RB, Wasmuth HE, Bibby NJ, Macgibbon AKH, Hill JP. The effect of beta-casein polymorphism on the stimulation of diabetes in the non-obese diabetic mouse and effect of beta-casomorphin-7 on immune cell activity. Int Dairy J 1998;8:580.

[68] Bonifacio E, Ziegler AG. Advances in the prediction and natural history of type 1 diabetes. Endocrinol Metab Clin N Am 2010;39:513–25.

[69] Saunders KA, Raine T, Cooke A, Lawrence CE. Inhibition of autoimmune type 1 diabetes by gastrointestinal helminth infection. Infect Immun 2007;75:397–407.

[70] Rook GA, Brunet LR. Microbes, immunoregulation, and the gut. Gut 2005;54:317–20.

[71] Helmby H. Helminths and our immune system: friend or foe? Parasitol Int 2009;58:121–7.

[72] Fernandez-Morera JL, Calvanese V, Rodriguez-Rodero S, Menendez-Torre E, Fraga MF. Epigenetic regulation of the immune system in health and disease. Tissue Antigens 2010;76:431–9.

[73] Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol 2010;28:1057–68.

[74] Wilson MS, Maizels RM. Regulatory T cells induced by parasites and the modulation of allergic responses. Chem Immunol Allergy 2006;90:176–95.

[75] Elliott DE, Setiawan T, Metwali A, Blum A, Urban Jr JF, Weinstock JV. Heligmosomoides polygyrus inhibits established colitis in IL-10-deficient mice. Eur J Immunol 2004;34:2690–8.

[76] Wohlleben G, Trujillo C, Muller J, Ritze Y, Grunewald S, Tatsch U, et al. Helminth infection modulates the development of allergen-induced airway inflammation. Int Immunol 2004;16:585–96.

[77] Summers RW, Elliott DE, Qadir K, Urban Jr JF, Thompson R, Weinstock JV. Trichuris suis seems to be safe and possibly effective in the treatment of inflammatory bowel disease [see comment]. Am J Gastroenterol 2003;98:2034–41.

[78] Sampson HA. Anaphylaxis and emergency treatment. Pediatrics 2003;111:1601–8.

[79] R.a.P.S. Epidemiology and Statistics Unit. Trends in asthma morbidity and mortality. American Lung Association; 2007.

[80] Fox JG, Beck P, Dangler CA, Whary MT, Wang TC, Shi HN, et al. Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy. Nat Med 2000;6:536–42.

[81] Bilbo SD, Schwarz JM. Early-life programming of later-life brain and behavior: a critical role for the immune system. Front Behav Neurosci 2009;3:14.

[82] Husband AJ. The immune system and integrated homeostasis. Immunol Cell Biol 1995;73:377–82.

[83] Vitkovic L, Bockaert J, Jacque C. "Inflammatory" cytokines: neuromodulators in normal brain? J Neurochem 2000;74:457–71.

[84] Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 1988;12:123–37.

[85] Kent S, Bluthe RM, Kelley KW, Dantzer R. Sickness behavior as a new target for drug development. Trends Pharmacol Sci 1992;13:24–8.

[86] Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun 2007;21:153–60.

[87] Nesse R, Williams G. Why we get sick. New York: Vintage Books; 1994.

[88] Streit WJ, Mrak RE, Griffin WS. Microglia and neuroinflammation: a pathological perspective. J Neuroinflam 2004;1:14.

[89] Bennet L, Gunn A. The fetal origins of adult mental illness. In: Wintour-Coghlan M, Owens J, editors. Early life origins of health and disease. Advances in experimental medicine and biology. New York: Springer; 2006. p. 204–11.

[90] Hornig M, Weissenbock H, Horscroft N, Lipkin WI. An infection-based model of neurodevelopmental damage. Proc Natl Acad Sci USA 1999;96:12102–7.

[91] Nelson KB, Willoughby RE. Infection, inflammation and the risk of cerebral palsy. Curr Opin Neurol 2000;13:133–9.

[92] Rantakallio P, Jones P, Moring J, Von Wendt L. Association between central nervous system infections during childhood and adult onset schizophrenia and other psychoses: a 28-year follow-up. Int J Epidemiol 1997;26:837–43.

[93] Shi L, Fatemi SH, Sidwell RW, Patterson PH. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J Neurosci 2003;23:297–302.

[94] Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 2005;57:67–81.

[95] Bilbo SD. Early-life infection is a vulnerability factor for aging-related glial alterations and cognitive decline. Neurobiol Learn Mem 2010;94:57–64.

[96] Godbout JP, Johnson RW. Age and neuroinflammation: a lifetime of psychoneuroimmune consequences. Immunol Allergy Clin N Am 2009;29:321–37.

[97] Lynch MA. Age-related neuroinflammatory changes negatively impact on neuronal function. Front Aging Neurosci 2010;1:6.

[98] Lee HK, Min SS, Gallagher M, Kirkwood A. NMDA receptor-independent long-term depression correlates with successful aging in rats. Nat Neurosci 2005;8:1657–9.

[99] Nicholson DA, Yoshida R, Berry RW, Gallagher M, Geinisman Y. Reduction in size of perforated postsynaptic densities in hippocampal axospinous synapses and age-related spatial learning impairments. J Neurosci 2004;24:7648–53.

[100] Lafferty KD. Microbiology. Interacting parasites. Science (New York, NY) 2010;330:187–8.

[101] Lesher A, Li B, Whitt P, Newton N, Devalapalli AP, Shieh K, et al. Increased IL-4 production and attenuated proliferative and proinflammatory responses of splenocytes from wild-caught rats (Rattus Norvegicus). Immunol Cell Biol 2006;84:374–82.

[102] Abolins SR, Pocock MJO, Hafalla JCR, Riley EM, Viney ME. Measures of immune function of wild mice, Mus musculus. Mol Ecol 2011;20:881–92.

[103] McInnes IB, Leung BP, Harnett M, Gracie JA, Liew FY, Harnett W. A novel therapeutic approach targeting articular inflammation using the filarial nematode-derived phosphorylcholine-containing glycoprotein ES-62. J Immunol 2003;171:2127–33.

[104] Johnston MJG, Wang A, Catarino MED, Ball L, Phan VC, MacDonald JA, et al. Extracts of the rat tapeworm, Hymenolepis diminuta, suppress macrophage activation in vitro and alleviate chemically induced colitis in mice. Infect Immun 2010;78:1364–75.

[105] Zandman-Goddard G, Shoenfeld Y. Parasite infection and autoimmunity. Lupus 2009;18:1144–8.

[106] Harnett W, Harnett MM. Helminth-derived immunomodulators: can understanding the worm produce the pill? Nat Rev Immunol 2010;10:278–84.

[107] Warren KS, Mahmoud AA. Algorithms in the diagnosis and management of exotic diseases. XIV. Tapeworms. J Infect Dis 1976;134:108–12.

[108] Feary J, Venn A, Brown A, Hooi D, Falcone FH, Mortimer K, et al. Safety of hookworm infection in individuals with measurable airway responsiveness: a randomized placebo-controlled feasibility study. Clin Exp Allergy: J Br Soc Allergy Clin Immunol 2009;39:1060–8.

[109] Brooker S. Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers – a review. Int J Parasitol 2010;40:1137–44.