

A Darwinian View of the Hygiene or “Old Friends” Hypothesis

When urban living reduced contacts of humans with microbes and worms, it increased our risk for chronic inflammatory disorders

Graham A. W. Rook

The notion that urban life is associated with increases in chronic inflammatory disorders traces back to the 19th century, when physicians in Europe noticed that allergies were rare among farmers. In sharp contrast, hay fever was regarded as the hallmark of prosperous, educated city sophisticates.

Several rigorous epidemiologic studies of more recent vintage lend support to the idea that growing up in a farming environment protects children against developing hay fever or other allergies. Further, in 1989 epidemiologist David Strachan of St. George’s University in London, United Kingdom, observed allergies as being less common in children with older siblings, espe-

cially boys, suggesting to him that microbial encounters might protect against allergic disorders.

Strachan’s and other studies led to the view that microorganisms and macro-organisms from mud, animals, and feces with which mammals coevolved play a critical role in immunoregulation and in inhibiting inappropriate immune responses to self, gut contents, and allergens. In describing this phenomenon, I prefer the term “old friends” to the more common “hygiene” hypothesis. The former term is broader, and implicates the effects of prenatal, neonatal, and adult exposures to such organisms as well as the crucial effects of microorganisms found in the gut, skin, lung, and the oral and nasal passages of the host. This area of clinical research is set to become a major branch of Darwinian medicine, with the potential for yielding new strategies for preventing and treating a widening variety of diseases.

Several Types of Disease Are Associated with Urban Living

Increases in inflammatory disorders that accompany the modern urban lifestyle are not confined to allergies. After the 1880s, for example, inflammatory bowel diseases began to appear in young adults from prosperous families in London. Later, physicians in many parts of the world began noticing lifestyle-related increases in autoimmune disorders. In 1966, for instance, Israeli investigators reported that multiple sclerosis (MS) was more common in families with advanced hygiene.

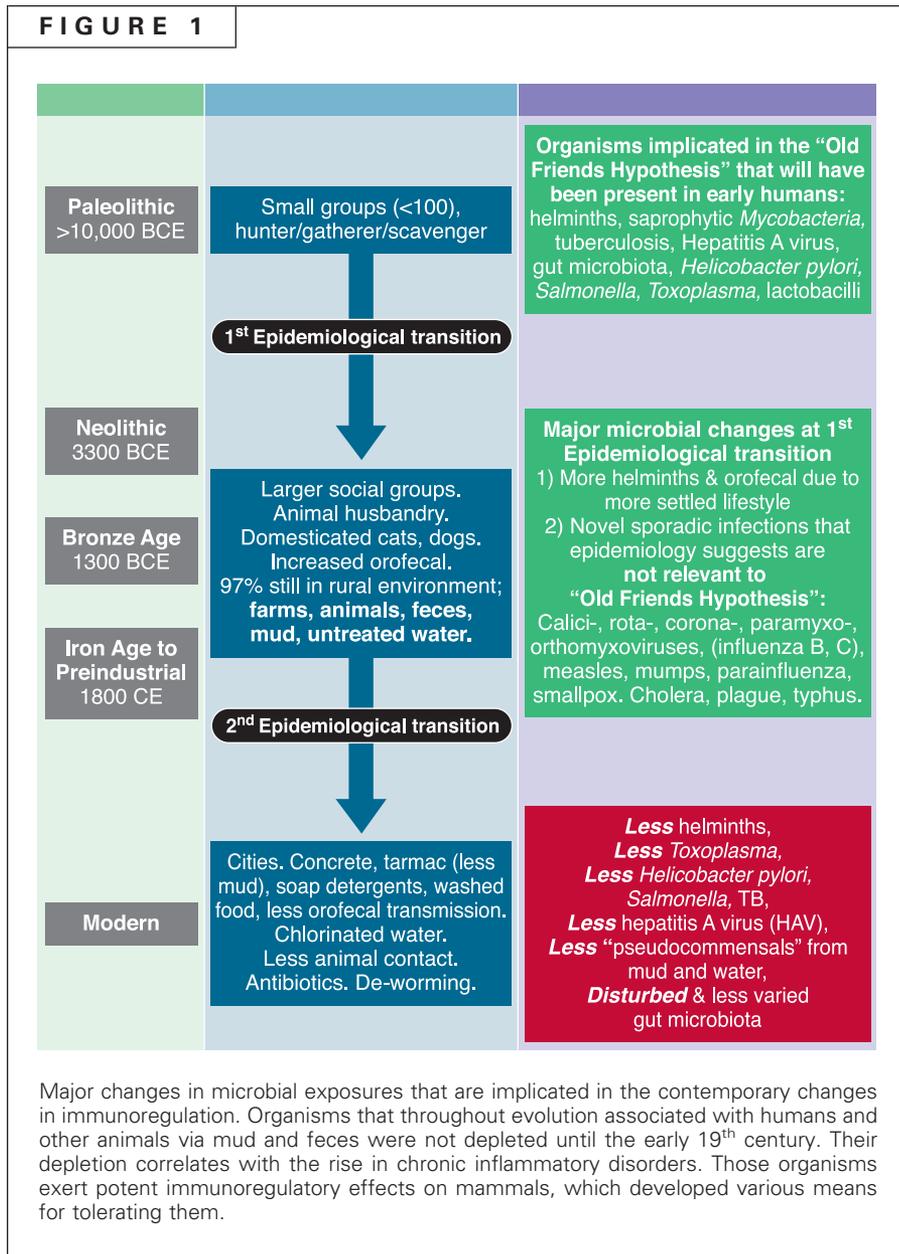
Summary

- Microorganisms and macroorganisms such as helminths from mud, animals, and feces play a critical role in driving immunoregulation.
- The term “old friends” is broader than “hygiene” to describe this hypothesis, and it implicates exposures to microbes and other organisms during critical phases of human development.
- Diseases and conditions of the modern era, including multiple sclerosis, type 1 diabetes, and allergies, involve disrupted immunoregulatory circuits, likely reflecting reduced exposures to “old friend” organisms with which humans coevolved.
- Several clinical trials are testing these concepts, determining whether renewed exposures to “old friend” organisms can help to combat these modern-era diseases.

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FIGURE 1



ease, whereas Karelians living in underdeveloped settlements just over the border in Russia do not. One big difference between these two populations is their different microbial exposures.

These Diseases also Tie into the Immune System

These differences in rates of allergy, IBD, autoimmunity, and type 1 diabetes are not merely the result of changing diagnostic criteria, as some investigators thought at first. Indeed, the continuing increases in their incidence during the last century led to careful studies that dispelled remaining doubt. Thus, we now recognize that those increases are very large, very real, and occur simultaneously across those disease groups.

Moreover, although these disease groups impinge on the immune system, allergies are mediated by Th2 lymphocytes, whereas autoimmunity is mediated by Th1/Th17 lymphocytes. Therefore, because these two types of disease are increasing in parallel, the problem is not likely to stem from an imbalance between these two effector arms of the immune system. The epidemiology instead suggests a fundamental defect at the level of immunoregulation affecting all arms of the immune response.

A broad defect in immunoregulation can lead to all these types of pathology because genetic defects in the transcription factor Foxp3, which controls the maturation and function of many regulatory cells, lead to a syndrome that includes features of allergy, autoimmunity, and enteropathy. We also now know that all these disease groups are accompanied by striking defects in immunoregulatory circuits, so an immunoregulatory hypothesis is probable. What links defective immunoregulation with urbanization and hygiene?

How Microbes in the Environment Can Affect Immunoregulation

Epidemiologists continue to search for and identify organisms that our urban lifestyle depletes

Other investigators noted that the incidence of type 1 diabetes (T1D) varies widely from one geographic region to another. Some of this variation is genetic, but much of it occurs within ethnic groups, and the rises are too recent and sudden to be explicable by genetics alone. There must be environmental factors.

For example, ethnic Karelians in Russia have very low levels of T1D, whereas the disease is six times more prevalent in Karelians living in nearby Finland. Thus, although genetically nearly identical and living at the same latitude, Karelians in modernized Finland suffer greatly from this dis-

Rook: From a Narrow Focus to a Broad Scientific View, and a Delight in Restoring Ruins

Early in his career, Graham Arthur William Rook overheard someone describing him as knowing “more and more about less and less.” It led him to worry that he was “drifting too far from any possibility of understanding anything in a physiological context,” he recalls. Thus, to his interests in tuberculosis and leprosy, Rook added interests in autoimmune diseases, endocrinology, glycosylation, and allergic disorders. That breadth “was crazy by modern standards,” he says. “This is *not* a good plan for a young scientist. But I could not work like that.”

Rook, Emeritus Professor of medical microbiology at University College London, still believes that too few scientists take the broad view. “I regard myself as ‘an integrative physiologist,’ trying to take a holistic and evolutionary view of biological science,” he says. “Curiously, the public is often ahead of the scientists in this respect. But it is easy to see why scientists tend to be so narrow. You have to be to do a Ph.D., or to write grant applications.”

It takes enormous energy to stay on top of neuroscience, endocrinology, microbiology, immunology, psychiatry, and oncology “to write the kinds of paper that I produce now,” Rook says. “And it goes without saying that it is *not* possible to do this without coauthors who are card-carrying specialists in the fields that are outside my own domain of infectious disease immunology. Now that, in theory, I am retired, I have the opportunity to do more of this

very time-consuming interdisciplinary thinking.”

Recently, he became interested in evolutionary psychology and sociobiology. “It is great fun trying to apply ideas from these disciplines to the development of human thought and culture,” he says. “As always I am arguing against the narrow view. So I have strayed a long way from my original territory. I hope to stray even further.”

Rook, 66, spent his research and teaching career at Middlesex Hospital Medical School in London, which transferred staff and services to University College Hospitals after closing in 2005. “After doing a basic science degree at the University of Cambridge, I studied clinical medicine despite the fact that I always intended to do research because I wanted to be sure that my work was relevant to real human problems,” he says. “That also explains why, after becoming a fully registered physician, I immediately abandoned clinical medicine.”

Rook was born in London, the middle of three sons, and moved as an infant to Cardiff, Wales, where his father worked as a dermatologist and wrote *Rook’s Textbook on Dermatology*, once the definitive textbook on that subject. The family moved to Cambridge when Rook was seven. “My bedroom was more laboratory than bedroom,” he recalls. “I used to use an electric heater as a resistance, and I acquired two amazing old rectifiers, huge primitive things, [and]

ran electrophoresis as a child with this kit . . . and experienced huge numbers of electric shocks, which really didn’t bother me. I also used this system for cooking sausages, by passing the current through them. It worked best with frankfurters. Too much fat in U.K. sausages led to more smoke and sparks than cooking.”

Rook studied basic natural sciences at the University of Cambridge, then clinical medicine at the University of London, and holds a series of degrees. He is married to Anne Demarquay (Anne Rook), a French artist. They have two sons, a physician and an artist, and four grandchildren.

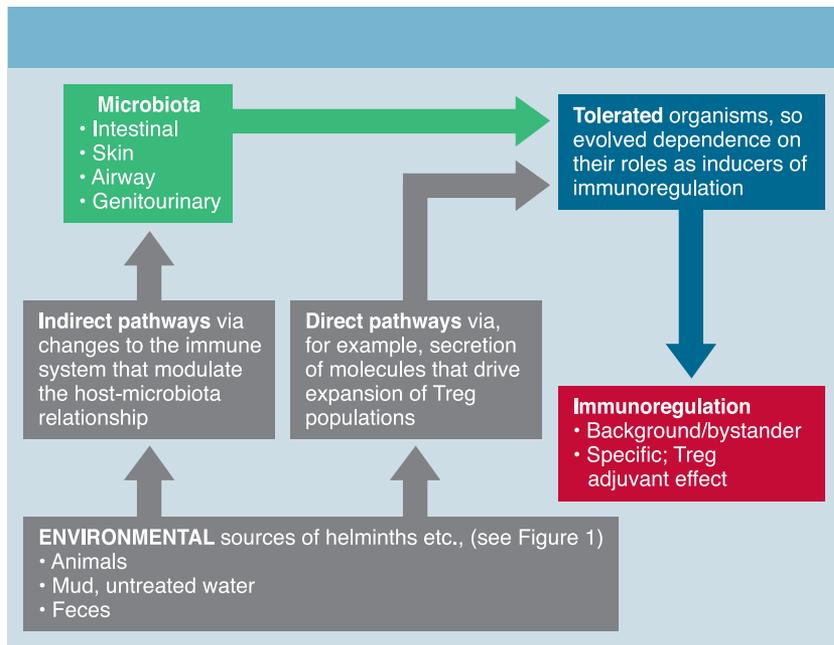
For more than three decades, he and his wife have been restoring a set of medieval ruins in southwestern France, where they live for part of the year. Initially, the place was a mess, with “trees growing inside the house, no roof, no drains, one cold water tap, one light bulb, one species of snake, rodents of three species, bats of three species, spiders, and insects of innumerable species,” Rook says. He did much of the restorative work, including wiring and plumbing. “It is all so much easier than science,” he says. “I spend a lot of time with a glass of red wine in my hand, staring at my handiwork and congratulating myself. I hope it will never be finished. What would I do then?”

Marlene Cimons

Marlene Cimons lives and writes in Bethesda, Md.



FIGURE 2



Two overlapping pathways of microbial immunoregulation. Although intestinal microbiota play a major role in driving Immunoregulation, non-commensal organisms also play a role, driving T-reg proliferation and changing the immune system by modulating host-microbiota relationships.

and whose loss correlates with increases in chronic inflammatory disorders. They report two crucial findings.

First, the common viral diseases of childhood do not protect against such disorders. Indeed, in some cases they help to induce chronic inflammatory disorders. This role as co-inducers is not so surprising because so many of these viral

diseases were relatively recently acquired from other animal host species during the Neolithic period (Fig. 1). Those diseases did not circulate widely among humans until towns with large populations developed, and European explorers and empire-builders introduced them into the American and Australian continents. Because these diseases came to humans so recently, the immune system did not reach a state of evolved dependence on those viruses.

Second, the organisms that appeared relevant to the hygiene hypothesis are those that coevolved with humans and other mammals. Prominent among these organisms are the helminths, which induce carrier states following fecal-oral transmission during the neonatal period, various microbiotas associated with the host gut, skin, and airways, and various environmental saprophytes encountered when hosts come into contact with mud and untreated water.

The host immune system tolerates all these organisms either because attempts to eliminate them lead to immunopathology, as is the case with helminths, or because the agents are so widely distributed, making contact with them nearly inevitable, as is the case with the hepatitis A virus and perhaps salmonella bacteria; because they are common and nonpathogenic contaminants of food and untreated water, as is the case with saprophytes; or because they are essential components of human physiology, as is the case with microbiota. Thus, it is easy to see how such organisms could have co-evolved with their mammalian hosts, taking on roles as inducers of immunoregulatory circuits (Fig. 2).

This example typifies what happens between separate species that develop evolved dependence on one another. To put it another way, instead of being encoded in the human genome, some of the genes for setting up immunoregulatory circuits are found within the genomes of organisms with which we coevolved. They include microorganisms and other multicellular organisms that live in places such as mud and feces. Such environments are depleted in towns that are covered with concrete or other solid materials. This

Table 1. Helminths used for treatment in animal models or clinical trials

Helminths used for treatment	Disorder treated
Animal models of disorder	
<i>Heligmosomoides polygyrus</i>	Allergy, T1D, colitis
<i>Schistosoma mansoni</i>	Allergy, T1D, EAE, colitis, arthritis
<i>Strongyloides stercoralis</i>	Allergy
<i>Fasciola hepatica</i>	EAE
<i>Trichinella spiralis</i>	T1D, EAE
<i>Hymenolepis diminuta</i>	Colitis, arthritis
Clinical trials (completed or in progress)	
<i>Trichuris suis</i>	Multiple sclerosis
<i>Trichuris suis</i>	Inflammatory bowel disease
<i>Necator americanus</i>	Asthma

“entrusting” of genes to other species for specialized activities is not unusual. For instance, humans depend on fruits and vegetables to obtain vitamin C, which other mammals make for themselves.

Helminths Can Develop an Immunoregulatory Hold over Hosts

Experiments with various mammalian species support this concept—namely, that microorganisms or multicellular organisms such as helminths can trigger the immunoregulatory potential of their hosts. For example, several helminth species, when tested in animals such as rodents, induce immunoregulatory circuits and thus can protect those host animals from developing a variety of conditions and diseases, including allergies, T1D, autoimmune encephalomyelitis (a model for MS), colitis, and arthritis (Table 1).

Clinical trials testing this concept in patients with some of these same conditions are either completed, in progress, or being planned (Table 1). Trials in MS have been encouraged by the clinical observation that among a group of MS patients, those who spontaneously developed chronic helminth infections experienced fewer exacerbations, or flare-ups, according to Jorge Correale of Raúl Carrea Institute for Neurological Research in Buenos Aires, Argentina, and his collaborators. Those patients also developed circulating myelin-recognizing regulatory T cells (T-reg) that secreted anti-inflammatory cytokines. In contrast, the disease progressed inexorably in those patients with MS who did not develop helminth infections.

The helminths apparently are acting as T-reg adjuvants (Fig. 2). Thus, they are driving not just the T cells that suppress responses to helminths, but also those that suppress an autoimmune response to human myelin, according to Correale.

That and other studies inspired a recent clinical trial in which patients with MS were deliberately infected with the helminth *Trichuris suis* rather than waiting for some of them to become infected by natural means. Although a Phase 1 safety study, and thus not intended to assess efficacy, the results were compatible with the view that such infections protect some MS patients against flare-ups. Further clinical trials are under way to evaluate whether infections with

helminths can protect individuals against this and other chronic inflammatory disorders.

Microbiota also Exert Partial Immunoregulatory Control over Hosts

Similarly, the microbiota of the gastrointestinal (GI) tract exerts partial immunoregulatory control over their mammalian hosts. Indeed, germ-free animals have poorly developed immune systems, and particularly poorly developed immunoregulatory circuits.

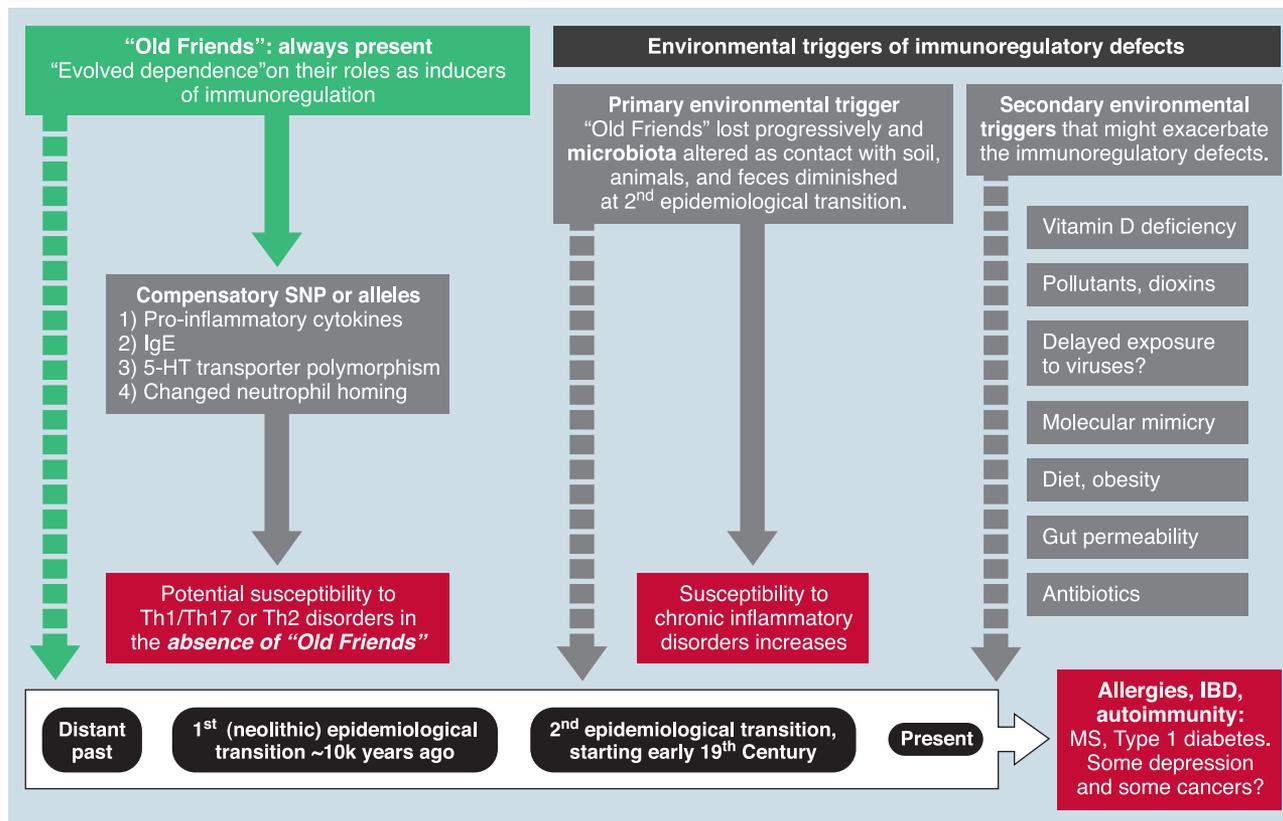
For example, unlike animals with a fully formed GI microbiota, germ-free animals do not develop tolerance to allergens when exposed to them orally. However, colonizing such germ-free mice with a defined intestinal flora, either a mixture of 46 *Clostridium* species or a single strain of *Bacteroides fragilis*, leads the T-reg cell population to expand, and consequently the animals develop increased resistance to several chronic inflammatory diseases.

The modern lifestyle alters the gut microbiota of humans. A recent study compared the bacterial microbiota of children in urban Europe to those from children living in a rural village in Burkina Faso in west Africa. The bacteria within these sets of microbiota were substantially different. Although there is no formal proof that the European microbiota is less immunoregulatory than the African microbiota, this difference appears likely.

Encounters with other “old friends,” such as helminths and bacterial pathogens, and differences in diet also can change the composition of the GI microbiota. Further, manipulating the immune system may change the gut microbiota. For example, a specific strain of pathogen-free, nonobese diabetic (NOD) mice, which spontaneously develop T1D, are protected from the autoimmune disease following knockout of a gene involved in innate immune responses, according to Li Wen at Yale University, Jeffrey Gordon at Washington University, and their collaborators at several institutions. However, this gene was not directly involved in the autoimmune response. Rather, that knockout led to changes in interactions between the immune system and the microbiota, and to subsequent changes in composition of that microbiota. This modified microbiota, rather than the gene knockout itself, was responsible for blocking development of T1D. Thus, it is important to



FIGURE 3



Genetics and the old friends. Heavy loads of immunoregulatory organisms such as helminths led to accumulation of single nucleotide polymorphisms (SNP) that increase the potential for inflammatory responses. This evolutionary balance is upset when the old friends are depleted by the modern lifestyle, making these SNPs risk factors for chronic inflammatory disorders. These effects are exacerbated by other environmental changes such as vitamin D deficiency and obesity.

note that helminth infections can modulate the microbiota, apparently with beneficial effects on immunoregulation.

More broadly, any modulation of the immune system, whether through infections by “old friends” such as helminths or from scientists knocking out genes that modulate the same microbiota, may lead to long-term health consequences that are mediated through changes in microbiota (Fig. 2). Similarly, some of the long-term immunological effects from dietary changes likely operate through changes within the microbiota.

Reconciling Competing Theories

The hygiene hypothesis and the emerging microbiota story fall along a continuum. Each de-

pends on the other, and the term “old friends hypothesis” can be used to describe them both.

This approach to linking these two lines of thought leads to the issue of competing hypotheses. The view that setting up appropriate immunoregulatory mechanisms requires interaction between the immune system and organisms with which the mammalian immune system has coevolved is well accepted. However, it is in danger of fragmenting, which is regrettable because the underlying principles are shared among supposedly competing hypotheses.

Although the hygiene hypothesis and more recent microbiota-based research fall along a continuum encapsulated within the old friends hypothesis, the hygiene hypothesis tends to split in other ways. For example, evidence that exposure to the microbiologically rich farming environment is most effective during the first few

years of life gave rise to the neonatal priming hypothesis. Further, because exposing pregnant women to this same environment apparently protects their children against subsequent allergic disorders, some investigators speak of the prenatal priming hypothesis.

Meanwhile, studies such as that of Correale or the effects on allergic manifestations of long-term elimination of helminths indicate that adults are also susceptible to immunomodulation by the “old friends,” and indeed the current spate of clinical trials is based on the view that this immunomodulation is clinically applicable. Instead of fragmenting these results into different hypotheses, it makes more sense to include them as examples of a more comprehensively framed old friends hypothesis that begins operating before birth and continues into adulthood.

Other Factors, Interaction with Genes

Although diet accounts for some differences in microbiota from children in Europe and Burkina Faso, it cannot explain the effects of the farming environment or the effects of helminths on MS patients in Argentina. Diet mainly changes the immunoregulatory microbiota.

Other factors that exacerbate the effects from loss of helminths or other old friends include delayed exposure to viruses that trigger autoimmunity when they are encountered later than the neonatal period, deficiencies in vitamin D₃, which plays a crucial role in immunoregulation, and exposure to pollutants such as diesel particulates and dioxins, which drive differentiation of Th17 cells (Fig. 3).

Humans living in regions with heavy loads of old friend organisms such as helminths tend to have higher levels of single-nucleotide polymorphisms (SNP) or other gene variants that partly compensate for this form of immunoregulation. Examples include genes encoding IgE, pro-inflammatory cytokines, STAT6 (a transcription factor involved in Th2 responses), and a truncated form of the serotonin transporter that has

a marked pro-inflammatory effect. When old friend organisms are withdrawn, these pro-inflammatory genetic variants become risk factors for chronic inflammatory disorders.

Broader Implications

We need to ask whether the same thinking is relevant to other inflammation-associated disorders that are increasing in the modern urban environment. Chronic inflammation can trigger cancer and also provide growth and angiogenic factors that enhance its growth and spread. Some types of cancer (for example, acute lymphatic leukemia of childhood, Hodgkin’s lymphoma, and colorectal and prostate cancer) show urbanization-related increases that are similar to those of the chronic inflammatory disorders discussed above.

Another important issue is major depressive disorder. In a large subset of patients this disorder is accompanied by raised levels of circulating pro-inflammatory cytokines even in the absence of any clinically apparent inflammatory disease, and we know that cytokines, when used clinically as therapeutic agents, can trigger depression. This increasingly important problem is more common in urban environments, and recent functional MRI studies have shown that a rural upbringing causes long-term changes to CNS function. There is accumulating evidence that anti-inflammatory strategies might be therapeutic.

Research to identify causes for diseases that were rare before the modern era might be rendered irrelevant if the old friends were still present. For example, the claim that Crohn’s disease is due to a genetic defect in the homing of neutrophils is difficult to reconcile with the fact that 100 years ago the disease barely existed. Perhaps recent environmental changes led that genotype to become a risk factor (Fig. 3). Similarly, other hypotheses attributing autoimmune diseases to molecular mimicry or viral infections might be secondary to underlying defects in immunoregulation.

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