

The Changing Microbial Environment and Chronic Inflammatory Disorders

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There is much to be gained from examining human diseases within the expanding framework of Darwinian medicine. This is particularly true of those conditions that change in frequency as populations develop from the human “environment of evolutionary adaptedness” to the living conditions of the rich industrialized countries. This development entails major changes in lifestyle, leading to reductions in contact with environmental microorganisms and helminths that have evolved a physiologic role as drivers of immunoregulatory circuits. It is suggested that a deficit in immunoregulation in rich countries is contributing not only to increases in the incidence of allergic disorders but also to increases in other chronic inflammatory conditions that are exacerbated by a failure to terminate inappropriate inflammatory responses. These include autoimmunity, neuroinflammatory disorders, atherosclerosis, depression associated with raised inflammatory cytokines, and some cancers.

In 1989, Strachan showed that in young adults, a history of hay fever was inversely related to the number of children in the family when the subject was 11-years old.¹ Further studies suggested that having many siblings, especially older ones, correlated with diminished risk of hay fever, and these findings were considered consistent with a protective influence of postnatal infection, which might be lost in the presence of modern hygiene.² So the “hygiene hypothesis” was born. The concept was initially vague and lacked mechanistic explanations, so in the 28 years since the original study, a multitude of different, often mutually exclusive, versions of this hypothesis have been considered. Often this has led to the “disproving” of hypotheses that few had intended to propose in the first place. However, during the last 9 years, an essentially new hypothesis has emerged, which we have preferred to designate “the old friends hypothesis.” This hypothesis might not be relevant to Strachan’s original findings, which remain unexplained, but it does have very broad importance for understanding the influence of changing patterns of microbial exposure on trends in human disease and is leading to encouraging clinical trials. Moreover, the old friends hypothesis belongs within the rapidly growing

framework of “evolutionary medicine,” which seeks to clarify our understanding of disease by considering our evolutionary history.

This review first outlines various “failed” versions of the hygiene hypothesis and then describes the old friends hypothesis and its implications not only for allergic disorders but also for other chronic inflammatory disorders, such as autoimmunity and inflammatory bowel disease (IBD). Finally, evidence is tentatively outlined suggesting that the concept might be relevant to other disorders in which proinflammatory cytokines play a major role, such as the metabolic syndrome, atherosclerosis, depression, and some types of neurodegeneration.

Failed Hypotheses

Childhood Infections

Strachan’s studies pointed to the possibility that the common infections of childhood might protect children from allergic disorders. Many allergologists found this view difficult to accept because allergies are rife in the inner cities of rich countries, where these infections are particularly common. More importantly, excellent studies have indicated that these infections do not protect children from allergies.³ Most strikingly, children in daycare centres do not have an increased risk of atopy if they wash more often and reduce their infection rate. Thus, prevention of common respiratory tract and enteric infections during early childhood does not change later allergic morbidity.⁴ It now seems likely that Strachan’s original findings were

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due to the decreasing incidence of hepatitis A virus (HAV) infection during the period in which the siblings studied were born.¹ HAV binds to lymphocytes via a receptor that modulates the development of T-cell subsets.⁵ Thus, it is part of the hygiene hypothesis but rather separate from the main theme of this review.

Domestic Hygiene

A second, partially overlapping view, largely created by journalists attracted to the word *hygiene*, was that home hygiene itself was to blame. Newspaper articles implied that we should avoid hygienic practices such as the use of bactericidal products, and interviewers tried to make scientists advise listeners to let their children live in squalor. Again, a detailed recent report rejected this simplistic concept.⁶ The history of the major changes in hygiene practices shows that they did not occur at the right times to correlate with increases in the incidence of allergies.⁶

T Helper 1/T Helper 2 Balance or Effector/Regulator Balance?

Although these hypotheses were failing, the suggested mechanism was overproduction of T helper 2 (Th)2 cells as a consequence of diminished infections and consequent diminished production of Th1 cells. According to this interpretation, the critical issue was Th1/Th2 balance, but this was never a strong hypothesis. First, Th1 cytokines such as interferon- γ (IFN- γ) are present in large quantities in both asthma⁷ and established atopic dermatitis.⁸ Second, profound defects in the interleukin (IL)-12 or IFN- γ (Th1) pathways do not lead to an increased incidence or severity of allergic disorders, implying that in humans Th1 is not a physiologic regulator of Th2 responses.⁹ Finally, the Th1/Th2 balance hypothesis has been untenable since as early as 1998,¹⁰ by which time, it had been well documented that there was a simultaneous increase in Th1-mediated chronic inflammatory diseases (type 1 diabetes, multiple sclerosis, inflammatory bowel disease),¹¹ occurring in the same countries as the increases in allergic disorders.¹² Moreover, individuals infected by helminths, which enhance Th2 responses, are paradoxically less likely to have allergic sensitization or allergic disorders, and treating the infection leads to increased allergic sensitization.¹³

These points all suggested that the critical problem was not Th1/Th2 balance but rather a broad and increasing failure in the rich developed countries of immunoregula-

tory mechanisms that should terminate inappropriate inflammatory responses, whether Th1 or Th2 and whether targeting allergens or self (autoimmunity) or gut contents (IBD). This is now the prevailing view.

Balance of Effector to Regulatory T Cells

In support of this concept, immunoregulation has been shown to be faulty in individuals suffering from allergic disorders¹⁴ and some autoimmune diseases,^{15,16} and probably in IBD too.^{17,18} A striking example in autoimmunity is a recent experiment of nature.¹⁹ Patients in Argentina suffering from multiple sclerosis were followed up for 4.6 years. It was found that those who developed parasite infections (which were not treated) had significantly fewer exacerbations than those who did not. Moreover, they also developed regulatory T cells (Tregs) (CD4⁺, CD25^{hi}, Foxp3⁺), which specifically responded to myelin basic protein. In other words, the presence of the parasite appeared to drive the development of Tregs that recognized the autoantigen and inhibited the disease process.

It is clear that a failure of immunoregulatory mechanisms can indeed lead to simultaneous increases in diverse types of pathology because genetic defects of Foxp3, a transcription factor that plays a crucial role in the development and function of Tregs, leads to a syndrome known as X-linked autoimmunity–allergic dysregulation syndrome (XLAAD), which includes aspects of allergy, autoimmunity, and enteropathy.²⁰

Old Friends Hypothesis

Rather than focusing on the common infections of childhood, the old friends hypothesis draws on epidemiologic studies demonstrating that protection from allergies is associated with living in developing countries and with the farming environment.^{21,22} These observations have been repeated many times in different environments and appear solid. Humans evolved in a hunter-gatherer environment, which is regarded as our environment of evolutionary adaptedness.²³ Much subsequent human evolution has been cultural rather than genetic. Nevertheless, the development of farming about 10,000 years ago led to a dramatic change in humans' microbial environment and to a further series of genetic adaptations. The intervening \approx 500 generations are sufficient to have allowed major changes in gene frequencies. For example, farmers needed to be able to digest the lactose in milk from domesticated animals, and the frequency of relevant

mutations in the gene encoding lactase has reached more than 90% in many populations.²⁴

Against this background, the old friends hypothesis suggests that the lack of appropriate levels of immunoregulatory pathways in rich northern countries is a consequence of diminished exposure to two categories of organism. First, harmless organisms associated with mud, untreated water, and fermenting vegetable matter were present throughout mammalian evolution but are greatly diminished in a world of concrete, treated water, and washed vegetables. These organisms include various *Lactobacillus* strains, saprophytic mycobacteria, other actinomycetes, and, no doubt, many other genera. Second, helminthic infections, always present in humans' progenitors but possibly increased in variety and load when animal husbandry began, are still common in developing countries but almost completely absent from rich ones.²⁵ The former needed to be tolerated because they were harmless but always present in large numbers in food and water. The helminthic parasites needed to be tolerated because, although not always harmless, once they were established in the host, any effort by the immune system to eliminate them was likely to

cause tissue damage. For instance, a futile effort to destroy *Brugia malayi* microfilariae resulted in lymphatic blockage and elephantiasis.²⁶

A cartoon of the pathway by which these organisms are currently thought to prime immunoregulation and mediate protection from allergies, autoimmunity, and IBD is shown in Figure 1. The host-parasite relationship evolved so that rather than provoking needless, damaging, aggressive immune responses, these organisms cause a pattern of maturation of dendritic cells (DCs) such that these drive Tregs rather than Th1 or Th2 effector cells.^{27,28} This, in turn, leads to two mechanisms that help control inappropriate inflammation. First, the constitutive presence of the old friends causes continuous background activation of the dendritic regulatory cells (DCregs) and of Tregs specific for the old friends themselves, resulting in constant background bystander suppression of inflammatory responses. Second, these DCregs inevitably sample self and gut contents and allergens and so induce Tregs specific for the illicit target antigens of the three groups of chronic inflammatory disorder. These inhibitory mechanisms are aborted when there are legitimate "danger" signals.²⁹

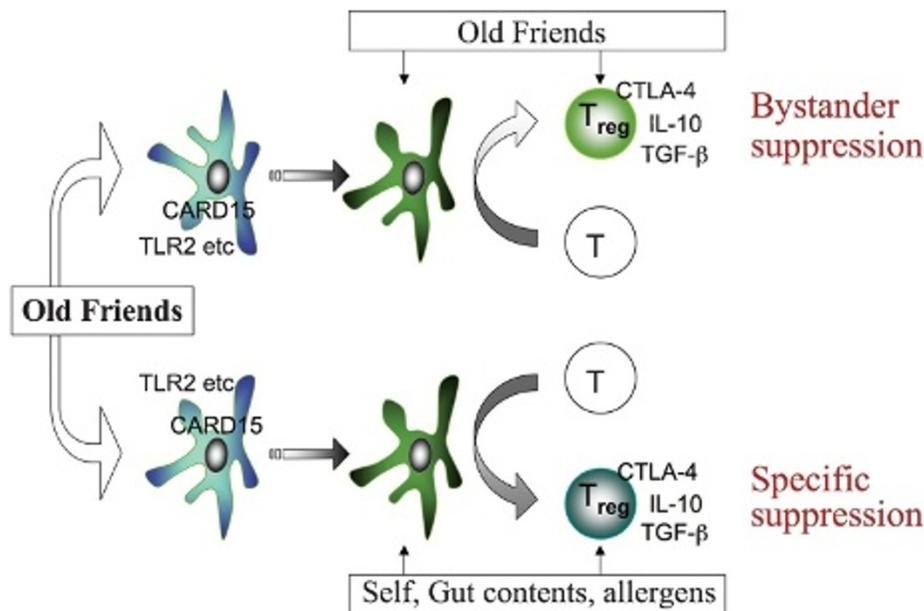


Figure 1. Organisms such as helminths and environmental saprophytes, which are part of mammalian evolutionary history ("old friends") and must be tolerated, are detected by pattern recognition receptors such as Toll-like receptor 2 (TLR2) and CARD15 (caspase recruitment domain family, member 15) on dendritic cells (DCs). The DCs mature into regulatory DCs that drive regulatory T cell (Treg) responses to the antigens of these organisms. The continuing presence of these antigens in the gut flora, in food, or resident as parasites such as microfilariae leads to continuous background release of regulatory cytokines from these Tregs, exerting bystander suppression of other responses, as shown in the upper arm of the figure. Meanwhile, the increased numbers of regulatory DCs lead to increased processing by such DCs of self-antigens, gut content antigens, and allergens, as shown in the lower arm of the figure. Therefore, the numbers of Tregs specifically triggered by these antigens is also increased, downregulating autoimmunity, inflammatory bowel disease, and allergies, respectively. CTLA-4 = cytotoxic T-lymphocyte antigen 4; IL-10 = interleukin-10; TGF- β = transforming growth factor β .

The validity of this hypothetical model is supported by clinical trials and experimental models in which exposure to microorganisms that were ubiquitous during mammalian evolutionary history but are currently “missing” from the environment in rich countries (or from animal units with specific pathogen-free facilities) will treat allergy,^{30–32} autoimmunity,³³ or intestinal inflammation.³⁴

We do not understand all of the ways in which DCregs and Tregs evoked by the old friends block or terminate inflammatory responses. However, we know that the release of the anti-inflammatory cytokines IL-10 and transforming growth factor (TGF)- β is often involved.^{30,31} The next sections summarize the evidence that a relative lack of Tregs and DCregs, leading secondarily to a decrease in immunoregulation and a decrease in these anti-inflammatory mediators, might be contributing to the increases in a wide variety of disorders in the developed countries (Figure 2).

IL-10-Dependent Disorders

In addition to the allergic disorders, autoimmunity, and IBD, all of which have been considered in detail in relation

to the old friends hypothesis in the past,^{11,25} several other disorders are also increasing and are likely to be exacerbated or made more common by a switch in the balance of inflammatory to anti-inflammatory mechanisms (see Figure 2).

Atherosclerosis

The metabolic syndrome, which involves abdominal obesity, hypertriglyceridemia, low high-density lipoprotein cholesterol, and insulin resistance, has risen to a prevalence of 41% in New York.³⁵ It has been observed recently that whereas women with uncomplicated obesity have increased serum levels of IL-10, those with the metabolic syndrome do not.³⁶ Could this imply less regulatory cell activity? The hypothesis is strengthened by considering atherosclerosis, which is a T cell-mediated inflammatory lesion in blood vessel walls and is considerably more common in patients with the metabolic syndrome.

Atherosclerotic plaques are inflammatory lesions driven mostly by Th1 cells.³⁷ Several independent groups have found that IL-10 and TGF- β have a downregulatory

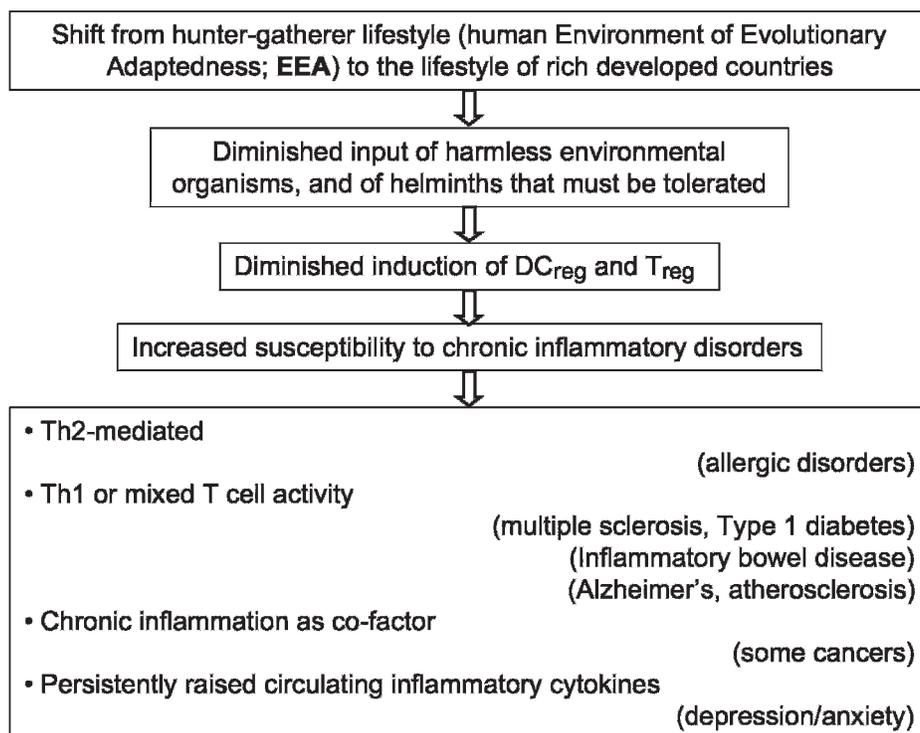


Figure 2. Human physiology was shaped by the hunter-gatherer way of life, which is regarded as the human “environment of evolutionary adaptedness,” although there have been further adaptations during the approximately 10,000 years (\approx 500 generations) since the introduction of farming and livestock. Most human evolution has been cultural and technological rather than genetic, and a gene-environment misfit may be occurring. Harmless organisms that were abundant in food and water (such as environmental actinomycetes) and helminths that had to be tolerated developed a role in the induction of immunoregulatory circuits. Without these, there may be a failure to terminate inappropriate inflammatory responses, leading to an increased susceptibility to chronic inflammatory disorders, the precise nature of which depends on the genetics and history of the individual. DCreg = dendritic regulatory cells; Th = T helper; Treg = regulatory T cells.

effect on the development of atherosclerotic plaques.³⁷ Atherosclerosis is exaggerated in IL-10-deficient mice.³⁸ By contrast, mice with transgenic T cells overexpressing IL-10 are protected from atherosclerosis,³⁹ and in several experimental models, transfer of Tregs will also inhibit atherosclerosis.⁴⁰ Infection with *Schistosoma mansoni* inhibits atherogenesis in mice, and although the authors attributed this to effects on lipid metabolism, induction of Tregs is likely.⁴¹ Further evidence was reviewed by Kuiper and colleagues.⁴²

There is similar evidence that IL-10 has a beneficial role in human atherosclerotic plaques,^{37,43–45} and serum levels of IL-10 are reduced in patients with unstable angina.⁴⁶ Interestingly, atherosclerotic lesions contain a very low percentage of Tregs as determined by direct immunohistochemistry.⁴⁷

Alzheimer Disease

The neurodegenerative disorders Alzheimer disease (AD) and Parkinson disease (PD) both appear to be mediated by inflammation.⁴⁸ This is apparent from both the pathology⁴⁸ and the epidemiology. Japanese American men in Honolulu were examined 25 years after a blood sample was taken to measure C-reactive protein (CRP), using very sensitive assays.⁴⁹ Raised CRP was associated with a higher incidence of AD and vascular dementia 25 years later. As mentioned earlier, the metabolic syndrome is itself associated with inflammatory cytokines, and a study in the United States yielded support for the hypothesis that the metabolic syndrome contributes to eventual cognitive impairment in the elderly, particularly in those with a high serum CRP and IL-6.⁵⁰ Other studies have yielded preliminary evidence that AD is associated with single nucleotide polymorphisms (SNPs) that lead to increased production of IL-6⁵¹ or tumor necrosis factor (TNF).⁵² Moreover, a large meta-analysis concluded that prolonged intake of nonsteroidal anti-inflammatory drugs can give some protection against AD.^{53,54}

There is also some evidence that the neurodegenerative conditions are more common in individuals with SNPs of their IL-10 genes that modulate production of this cytokine. For instance, in an Italian population, the presence of the -1082A allele was proposed as a genetic risk factor for AD.⁵⁵ Other studies found similar associations, although not always with the same allele or haplotype,^{51,56,57} and a study based in Germany found no associations at all,⁵⁸ so the matter remains unresolved.⁵⁴ The role of IL-10 is equally unclear in relation to PD. IL-10 SNPs were not related to sporadic PD in a Polish

population,⁵⁹ but a Swedish study that documented the -1082A/G SNP found that the age at onset of PD was delayed by 5 years in individuals with two G alleles compared with individuals with two A alleles.⁶⁰ Meanwhile, there is accumulating evidence that TGF- β might have anti-AD effects both because of its immunoregulatory and anti-inflammatory properties and because it enhances clearance of amyloid- β .⁵⁴

Depression and Anxiety

Some stress-related psychiatric conditions, particularly depression and anxiety, are associated with markers of ongoing inflammation, even in the absence of any accompanying inflammatory disorder.⁶¹ Thus, depressed subjects may have raised proinflammatory cytokines and evidence of an ongoing acute-phase response. Moreover, proinflammatory cytokines can induce depression, which is commonly seen in patients with cancer or hepatitis when they are treated with IL-2 or IFN- γ . In these patients, brain imaging shows a pattern similar to that which accompanies spontaneously occurring depression, and the depression can be treated with paroxetine, a serotonin reuptake inhibitor antidepressant.⁶² Similarly, there is evidence that depression can be associated with polymorphisms that lead to the overproduction of proinflammatory cytokines,⁶³ whereas in sharp contrast, treatments that neutralize these cytokines can alleviate depression.⁶⁴ Therefore, some psychiatric disorders in developed countries might be attributable to failure of immunoregulatory circuits to terminate ongoing inflammatory responses, leading to prolonged “sickness behaviour” and mood changes. This view is further supported by the fact that depression is associated with low expression of TGF- β and IL-10 relative to expression of proinflammatory cytokines.^{65,66} Moreover, antidepressants increase secretion of IL-10.⁶⁷

Recently, unexpected improvements in mood were observed during clinical trials with an immunomodulatory vaccine that induces Tregs. This led to an investigation of this material in a mouse model and to the discovery that it activates a specific group of brain serotonergic neurons involved in the pathophysiology of mood disorders and exerts a fluoxetine hydrochloride (Prozac)-like effect in an industry standard test for antidepressant activity.⁶⁸ This area has now been reviewed in depth.⁶⁹

Cancer

Chronic inflammatory lesions, such as those induced by chronic infections (viruses, *Chlamydia*, or bacteria),

asbestos, or chronic exposure to smoke or alcohol, are all associated with increased cancer risk.⁷⁰ This is partly because inflammatory mediators are involved in control of cell replication, angiogenesis, and cell migration and also drive increased levels of reactive oxygen intermediates that can cause deoxyribonucleic acid (DNA) damage.⁷⁰ Many of these functions of inflammation are regulated by the transcription factor nuclear factor κ (NF- κ B), and manipulating the activity of NF- κ B has profound effects on tumorigenesis.⁷¹ Interestingly, TNF- $\alpha^{-/-}$ or TNF receptor 1^{-/-} mice are more resistant to chemically induced carcinogenesis.^{70,72} Similarly, several SNPs of chemokines and cytokines are associated with malignancy.⁷⁰ Conversely, anti-inflammatory drugs may be protective. Regular input of nonsteroidal anti-inflammatory drugs, such as aspirin, that inhibit cyclooxygenase-2 (the inducible form of prostaglandin H synthase) is associated with reduced risk of colorectal cancer.⁷³ Esophageal cancer provides a vivid example of the role of inflammation; reflux of gastric acid, alcohol, and tobacco all predispose individuals to esophageal cancer.⁷⁴

Clearly, this is a complex issue where cancer is concerned. It is reasonable to suggest that failing immunoregulation might allow chronic inflammation and so predispose individuals to carcinogenesis. On the other hand, excessively effective immunoregulation might impede the immune system's attempts to destroy cancer cells once the cancer is established.⁷⁵ Nevertheless, a proinflammatory haplotype of SNPs in IL-6, IL-10, and TNF- α is associated with a poor prognosis in gastroesophageal malignancy,⁷⁶ and it is clear that the inflammatory response can provide mediators that assist the growth and spread of the cancer.⁷⁰ Thus, diminished background immunoregulation in rich countries, as explained by the old friends hypothesis, might explain some of the increase in certain cancers.

Conclusions

The original hygiene hypothesis gave rise to several daughter hypotheses, all of which lacked epidemiologic and historical credibility, as outlined above. This review attempts to show how a broader view, focusing on major changes in lifestyle that accompany the shift from hunter-gatherer to industrialized society, passing via herding and farming, can lead to a hypothesis that falls within Darwinian medicine and has considerable explanatory power. It is clear that multiple environmental changes must contribute to changing patterns of disease, and this brief overview does not intend to imply that diminished input of Treg-inducing organisms

such as helminths and harmless environmental saprophytes is the only factor. There are numerous other ways in which harmless microorganisms or infections can influence health. For example, changes in bowel flora secondary to dietary changes will play a role, and obesity itself increases inflammatory cytokine levels. Similarly, urbanization led to increased incidences of diseases such as tuberculosis, and selection of genetic variants conferring resistance to such infections might lead to increased susceptibility to autoimmunity. This review therefore covers only one subset of the relevant mechanisms and emphasizes our increasing awareness of humans' place within the evolutionary framework of the biosphere. This must be considered in the light of humans' unique ability to change their environment by means of technology, with little accompanying adaptive genetic change. Therefore, it is logical to explore the possibility of a gene-environment misfit, leading to increased susceptibility to chronic inflammatory disorders. The list of disorders discussed here in this context is illustrative, and many others might need to be added.

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