OPINION

The germless theory of allergic disease: revisiting the hygiene hypothesis

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Rising rates of allergic disease accompany the healthier benefits of a contemporary westernized lifestyle, such as low infant mortality. It is likely that these twinned phenomena are causally related. The hygiene hypothesis states that allergy and increased longevity are both consequences of reducing infectious stressors during early childhood. Mechanistic explanations for the hygiene hypothesis have typically invoked the T-helper-type 1/2 (T_H1/T_H2) model. Here, we discuss why we favour a broader 'counter-regulatory' model — one that might also explain the increasing incidence of autoimmune disease in westernized countries.

The westernized lifestyle that is spreading around the globe seems to be carrying unwanted allergic baggage. The prevalence of diseases such as allergic ASTHMA, atopic dermatitis, and hay fever has increased markedly in the developed world over the past 40 years. The documentation is best for asthma. In the United States alone, from 1980 to 1994 the prevalence of asthma increased by 75%[1]. In the developing world, the low baseline prevalence of allergic disease has not changed appreciably over the same period. Although these disorders clearly have a heritable component, the rapidity of this epidemiological shift indicates that the search for a mechanism needs to focus on the environment (FIG. 1). The aspects of the westernized lifestyle that are responsible have not been clearly defined; changes in air pollution, indoor exposure to ALLERGENS and 'general living standards' have all been suspected. Although there is an association between certain types of air pollution (for example, particulates and diesel exhaust) and asthma exacerbation, overall air quality has improved throughout this epidemic of ALLERGY. Indeed, a careful comparison of asthma prevalence in former East Germany (low asthma prevalence, high levels of air pollution) and West Germany (the reverse) failed to support an important role for pollution[2]. Furthermore, although exposure to indoor allergens does increase with indolence and the provision of energy-efficient housing, a strong correlation between the intensity of exposure to such allergens and the development of allergic disease has yet to be found[3].

The hypothesis that has gained the most attention is derived from the observation of an inverse relationship between the risk of hay fever and family size[4]. This 'hygiene hypothesis' argues that early childhood infections inhibit the tendency to develop allergic disease. As a consequence, children with westernized lifestyles, protected as they are from the lethal infectious burdens of early life that are common in the developing world, suffer an increased risk of developing allergic disease. From an evolutionary perspective, the

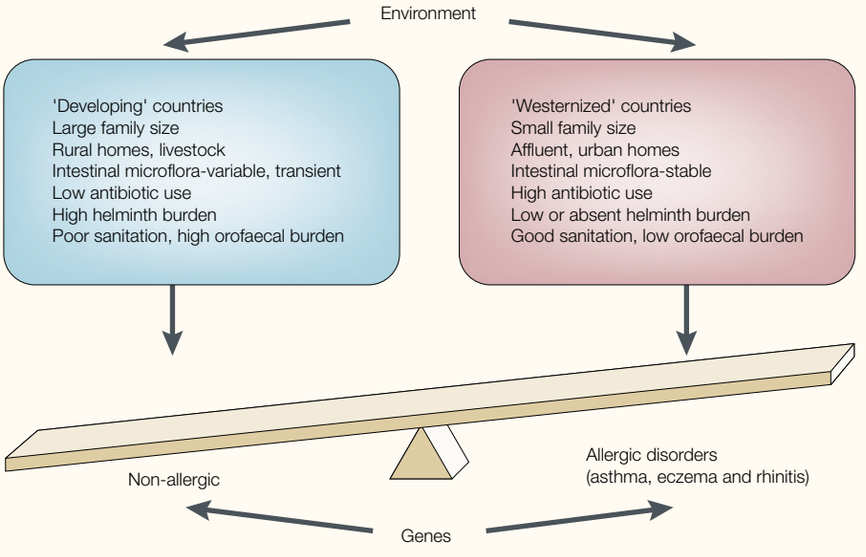

Figure 1 | **Allergy: genes and environment.** Increases in allergy prevalence have occurred primarily in 'westernized' societies over the past few decades. Although both genetic and environmental factors influence the aetiology of asthma, changes in the genetic make-up of stable populations does not occur in this time frame. The recent rise in the prevalence of allergies and asthma is therefore thought to be primarily due to changes that have taken place in the environment in developed countries as a result of modernization. A comparison of environmental conditions in developed and underdeveloped countries has provided insight into possible causes.

hygiene hypothesis indicates that the twin consequences of reducing the infectious stressors that have guided the development of the immune system during early childhood for millennia are increased longevity and allergy.

Indirect measures of infection

The infectious burden borne by infants in the developing world clearly outweighs that in the developed world. Problems of sanitation, access to drinkable water, adequate nutrition and crowding all contribute to the infectious challenge to early childhood in much of the world. However, direct comparisons between developed and developing countries are complicated by a plethora of confounding variables. Careful epidemiological studies have addressed some of these issues in single countries. The initial observation of an inverse correlation between the number of older siblings (a presumed surrogate for intensity of early childhood disease exposure) and the prevalence of allergic disease has been confirmed in studies across the developed world[5,6]. Similar effects have been noted for early day-care attendance[7]. Another line of inquiry has documented that childhood residence on a farm with livestock is associated with protection from allergic disease. Again, this effect is observed across the developed world[8,9]. Finally, studies in several countries have shown a correlation between low parental socio-economic status and a reduced prevalence of allergic disease[10]. Needless to say, the explosion of asthma cases in the inner cities of the United States[11] provides a puzzling apparent counterpoint to these latter observations. Although the general implication of these studies is that protection from allergy seems to be associated with an increase in microbial exposure, the link has remained fairly indirect[12].

> "This 'hygiene hypothesis' argues that early childhood infections inhibit the tendency to develop allergic disease"

Pathogens and commensal organisms

Although European studies that characterize the relationship between childhood infectious syndromes and allergy have provided contradictory answers[13,14], a defined role for several specific pathogens has been sought. A cross-sectional study of Italian military recruits found a significant, inverse relationship between the presence of antibodies to hepatitis A and various measures of ATOPY, including skin sensitization to AEROALLERGENS, serum immunoglobulin E levels and a lifetime diagnosis of respiratory allergy[6]. In all likelihood, however, such evidence of previous infection with hepatitis A merely provides evidence of a risk of exposure to faecal–oral pathogens[15]. In Japanese schoolchildren, an inverse association was found between DELAYED-TYPE HYPERSENSITIVITY responses to tuberculin (almost certainly a measure of infection with *Mycobacterium tuberculosis*) and indices of atopy[16]. Moving to the tropics, and to defined infectious exposures, Aaby and co-workers[17] documented that vaccination with *M. bovis* BCG (Bacille Calmette–Guérin) was associated with a reduction in the prevalence of allergy in Guinea-Bissau. Notably, the earlier the vaccination, the greater the protection. However, such protection was not observed in a similar retrospective study of BCG vaccination in Swedish children with a family history of allergy[18].

Recent studies and reviews of the hygiene hypothesis have paid particular attention to measles. The initial observations derived from a historical cohort study carried out in Guinea-Bissau, in which an inverse relationship was found between a history of survival of measles (and lack of measles vaccination) and the risk of skin-prick positivity to house dust mite[19]. The effect was large, but so too was measles-related mortality in the study (25%). The likelihood that these findings were based on mortality-driven selection bias has been bolstered by several studies in Europe, where measles-related mortality is low (0.1–0.3%): either no effect, or a positive association between natural measles and allergy was found[20,21]. So, measles might not deserve the prominent role it has received in the literature on the hygiene hypothesis. Furthermore, as noted below, discussion of measles in this context has generally proceeded from a misunderstanding of measles immunology. Data on other respiratory viruses indicate that if the hygiene hypothesis has validity, not all infections, or sites of infection, are created equal. Most respiratory virus infections are clearly not protective, and might even be positively associated with the development of allergic disease[15,22,23].

The complex interface with the microbial world provided by the gastrointestinal tract might well be important to the interrelationship between infection and allergy. The endogenous flora of the gut provides a wealth of stimuli for the developing immune system. There seem to be important differences, both quantitative and qualitative, in early childhood patterns of bacterial colonization between the developed and developing world. Comparison of Swedish and Pakistani infants indicates that intestinal colonization with aerobic Gram-negative bacteria tends to occur later in westernized than developing countries. Once colonized, infants in developed countries tend to carry enterobacterial strains for months, whereas infants in developing countries are more often colonized serially with several enterobacterial strains[24,25].

Glossary

AEROALLERGEN
Airborne allergens; important in allergic asthma.

ALLERGEN
An environmental antigen that typcially elicits allergic responses in susceptible individuals.

ALLERGY
Clinically evident reactions to ubiquitous allergens reflecting acquired immune responses that are marked, phenotypically, by the presence of allergen-specific IgE, along with mast cell and eosinophil recruitment and/or activation. $CD4^+$ T cells that produce a T_H2 profile of cytokines (IL-4, IL-5 and IL-13) are thought to be central to the development of allergic responses.

ASTHMA
A chronic disease of the lung, marked by airway hyper-responsiveness and inflammation. The most common form of the disease, allergic asthma, results from inappropriate immune responses to common allergens in genetically susceptible individuals. Allergic asthma is characterized by infiltration of the airway wall with mast cells, lymphocytes and eosinophils. $CD4^+$ T cells producing T_H2 cytokines are thought to have a pivotal role in orchestrating the recruitment and activation of these effector cells of the allergic response.

ATOPY
The propensity for developing allergic diseases, such as asthma, atopic dermatitis, food allergy or hay fever, defined operationally by elevations in serum levels of IgE reactive with allergens or by skin-test reactivity to allergens.

DELAYED-TYPE HYPERSENSITIVITY
(DTH). A T-cell-mediated immune response marked by monocyte/macrophage infiltration and activation. DTH skin tests have classically been used for the diagnosis of infection with intracellular pathogens such as *M. tuberculosis*, and as a measure of the vigour of the cellular immune system. Classical DTH responses to intracellular pathogens are thought to depend on $CD4^+$ T cells producing a T_H1 profile of cytokines (IFN-γ and TNF-β).

PROBIOTIC
Viable bacteria used therapeutically or prophylactically to colonize the intestine for the purpose of modifying the intestinal microflora in ways presumed to be beneficial to the host.

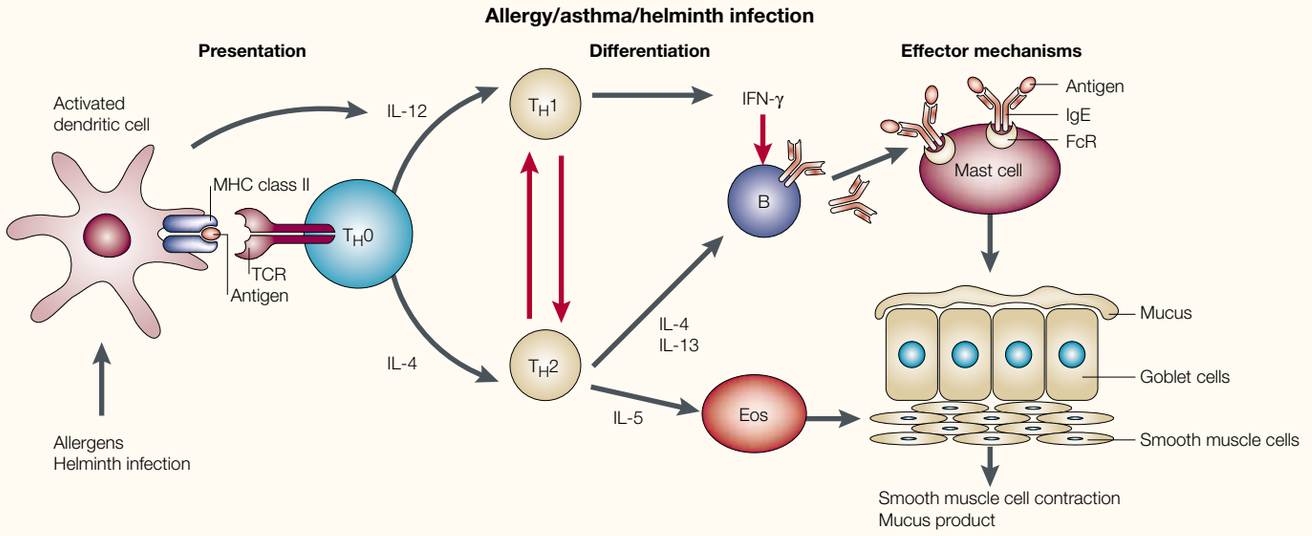

Figure 2 | **Regulators and effectors of T_H2 responses.** Stimulation of allergen-specific T cells by allergen-derived peptides, presented by dendritic cells in the context of class II MHC molecules, results in differentiation of CD4+ T cells into T_H2 cytokine-producing cells. Similar processes lead to the differentiation of helminth antigen-specific T_H2 cells. T_H2 cells produce interleukin (IL)-4, IL-13 and IL-5, which coordinately regulate the allergic response. IL-4 directs the differentiation of T cells towards a T_H2 cytokine profile and acts as a growth factor for the expression of these cells. IL-4 and IL-13 regulate the synthesis of IgE by B cells. IL-5 regulates the differentiation and egress of eosinophils from the bone marrow into the blood. B, B cell; Eos, eosinophil; FcR, Fc receptor; IFN-γ, interferon-γ; MHC class II, major histocompatibility complex class II; TCR, T-cell receptor; T_H, helper T cell.

Although this might seem unexceptional, marked differences in intestinal flora have also been found between allergic and non-allergic children in Europe[26,27]. For example, allergic 2-year-olds seem to be colonized less often with lactobacilli in Sweden and Estonia than non-allergic controls[28], something of particular interest given that recent studies have indicated possible preventive and therapeutic efficacy for *Lactobacillus* administration in allergic disease[29,30]. Finally, antibiotic use during the first 2 years of life is associated with a pronounced, dose-dependent increase in the risk of developing allergic disease[31,32]. Although the direction of the causal arrows is unclear in these studies (antibiotics are, of course, given for presumptive bacterial infection), it should be noted that the intestinal microflora is markedly altered by such therapy.

Helminths and the T_H2 paradox

The extent of intimacy with worms is a prominent environmental difference between the developed and developing populations of the world. After the initial observations of Godfrey and colleagues[33], many groups have documented a striking inverse correlation between the presence and extent of infestation with helminths and the presence of allergy. Lynch and colleagues[34] were able to limit confounding socio-economic variables in an interventional study carried out in Venezuela among heavily parasitized children. They found that prolonged treatment with anti-helminthics led to elevations in skin-test positivity and levels of serum IgE specific for environmental allergens. By contrast, placebo treatment was associated with an increase in parasitism, and a decrease in skin-test positivity and levels of serum IgE specific for such antigens[34].

As with most recent speculation about the hygiene hypothesis, parasitic studies have mainly been discussed in the context of the T-helper-type 1/2 (T_H1/T_H2) model of functional subsets of CD4+ T cells defined phenotypically by the production of polarized sets of cytokines (FIG. 2). T_H1 cells that produce interferon-γ (IFN-γ) and tumour-necrosis factor-β (TNF-β) are important in macrophage activation, phagocytic responses and the development of cell-mediated immunity. T_H2 cells produce cytokines, such as interleukin (IL)-4, IL-5, IL-10 and IL-13, and are important in the inhibition of macrophage activation, and the development of IgE responses and eosinophilia. The cytokine milieu during T-cell priming is important in driving CD4+ T-cell polarization. IL-12 is indispensable for the development of most T_H1 responses, and IL-4 has a similar role for T_H2 responses. The presence of these cytokine phenotypes in a variety of diseases has provided considerable descriptive power and theoretical insight into immunopathogenesis. It should be noted that other biologically plausible subsets of CD4+ T cells have been described, and also that similar phenotypically polarized populations are found among immune effector cells other than T cells. Allergy has provided the type specimen (an exemplar kept in museums as a reference for identification and description) for T_H2 responses. The cardinal phenotypic features of allergy are associated with the production of T_H2 cytokines: IL-4 and IL-13 are central to the regulation of IgE class switching, whereas IL-5 is a necessary factor for the differentiation, recruitment and activation of eosinophils.

The immune system of neonatal mice and humans is thought to have a T_H2 bias[35,36]. Studies of human infants indicate that this T_H2 skew gradually diminishes during the first 2 years of life in non-allergic individuals[36,37], a time course that correlates well with that of the ontogeny of IL-12 productive capacity in early life[38]. In allergic infants, the reverse occurs, with the strength of neonatal T_H2 responses increasing over a similar period[36,37]. In the search for a plausible immunological foundation for the hygiene hypothesis, speculation has therefore focused on the possibility that the developing immune system needs a T_H1 stimulus from the environment to avoid the development of allergic disease. Many bacterial infections, including mycobacteria, can provide just such a stimulus. Indeed, BCG infection ameliorates experimental allergic asthma in mouse models[39], and administration of killed *Mycobacterium vaccae* can ameliorate the

severity of atopic dermatitis in children[40]. Some viruses also leave a T_H1 imprint on the immune system. (Measles virus is not among them, however; measles leaves a profound type 2 cytokine bias associated with suppression of IL-12 production in its wake[41,42].)

Within this framework, however, the fact that parasitism with helminths protects from allergy is paradoxical. Helminth infection, like allergy, is typically associated with T_H2-polarized responses marked by upregulation of IgE production and eosinophilia in both mice and men. Helminth-related protection from allergic disease, observed in mouse models[43] as well as in humans, is therefore clearly not due to the remedial provision of an absent T_H1 stimulus. (Indeed, the provision of just such a T_H1 stimulus does not necessarily downregulate allergic phenomena in mice or men[44,45]). Ingenious mechanistic hypotheses for helminth-related protection from allergy have been proposed. It has been suggested that exuberant, parasite-induced production of IgE suppresses allergen-specific IgE production or causes the saturation of cell-surface IgE receptors, diluting out allergen-specific IgE effects[33]. Careful *in vivo* and *ex vivo* studies in heavily parasitized populations have provided little support for such hypotheses[46,47].

Benefits of counter-regulation

A recently published study[47] of schistosome-parasitized children in Gabon, suggests an entirely plausible mechanism for helminth-related protection from allergy, and provides a potentially unifying explanation for many of the phenomena that have fallen under the purview of the hygiene hypothesis. In this landmark work, van den Biggelaar and colleagues found evidence that parasite-driven IL-10 production is associated with a lower risk of developing skin-test reactivity to aeroallergens[47]. Previous studies had implicated IL-10 as an inhibitory regulator of various parasite-specific and -unrelated immune responses during chronic helminthiasis[48,49]. However, the van den Biggelaar study provided the first convincing mechanistic link between worms and protection from allergy[50]. And perhaps much more.

Although first discovered as a T_H2 cytokine, IL-10 is made by a wide variety of cells. Most bacteria, Gram-negative and Gram-positive, directly induce IL-10 secretion by monocytes, macrophages and dendritic cells. Other immune cells, including regulatory $CD4^+$ T cells, $CD8^+$ T cells, B cells, natural killer (NK) T cells and neutrophils, are also biologically significant producers. IL-10 provides, in many ways, an all-purpose 'anti-danger' signal. The immunoregulatory effects of IL-10 include: inhibition of macrophage activation, antimicrobial effector functions, co-stimulatory activity and production of proinflammatory cytokines; upregulation of the anti-inflammatory IL-1 receptor antagonist; inhibition of dendritic cell differentiation; suppression of inflammatory chemokine production; inhibition of matrix metalloproteinase production; downregulation of leukocyte adhesion molecule expression; suppression of T-cell proliferation; inhibition of T_H1 cytokine production; and the induction of antigen-specific anergy in $CD4^+$ T cells (for a review on IL-10, see REF. 51). It should be noted that, in addition to inhibitory effects, IL-10 has some stimulatory functions as well, including effects on thymocytes, B cells and mast cells. Furthermore, high doses of exogenous IL-10, and transgenic overexpression of IL-10 in sensitive organ parenchyma, can clearly exacerbate inflammatory processes[52,53]. However, the principal biological role of IL-10 seems to be a homeostatic one. IL-10 is important in dampening down most immune responses, whether such responses result from activation of the innate or the adaptive immune system. This is clearly shown in mice with a genetic deletion in *IL10*. These mice are highly susceptible to acute inflammatory processes, such as endotoxaemia[54]. They are also remarkably prone to profound immune polarization, both T_H1 and T_H2, and to the devastating pathological processes that result[55–57].

> "In terms of the hygiene hypothesis, chronic (or serial) infection or colonization with various microbial agents … leads to upregulation of IL-10 production, secondarily suppressing an underlying predisposition to allergy."

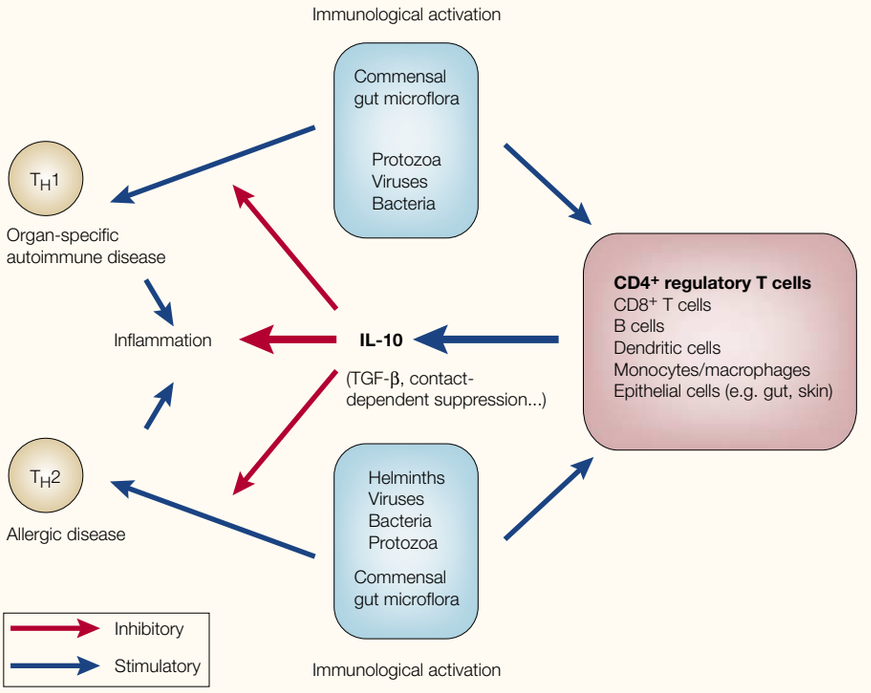

Figure 3 | **Schematic view of the counter-regulation hypothesis.** Produced by cells of both innate and adaptive immune systems, interleukin-10 (IL-10) has a key homeostatic role in the control of inflammatory processes. Most exposures that lead to immune activation leave IL-10 in their wake. Chronic (or serial) infection or colonization with various microbial agents leads to upregulation of IL-10 production, secondarily suppressing underlying allergic and autoimmune diatheses. Immunological 'negotiations' in the intestinal tract may be of vital importance to this process of feedback inhibition. The decreased infectious and antigenic pressure of the westernized lifestyle weakens this important counter-regulatory input to the developing immune system, leading to an increased incidence of allergic and organ-specific autoimmune diseases. Note that IL-10 functions in this scheme as a marker of counter-regulatory processes, but various complementary and redundant mechanisms are likely to be involved. TGF-β, transforming growth factor-β.

In essence, IL-10 has a prominent counter-regulatory role across the immune system. In terms of the hygiene hypothesis, chronic (or serial) infection or colonization with various microbial agents (whether a T_H1 stimulus, a T_H2 stimulus or neither) leads to upregulation of IL-10 production, secondarily suppressing an underlying predisposition to allergy (FIG. 3). The decreased infectious and antigenic pressure of the westernized lifestyle has deprived us of this important counter-regulatory feedback.

IL-10, allergy and immune pressure

IL-10 production has been characterized in the lungs and gut mucosae of individuals with allergic asthma and healthy controls[58,59]. Steady-state levels of IL-10 messenger RNA are increased in patients with allergic asthma, a likely reflection both of the presence of a chronic inflammatory response in such patients and of the lack of appropriate inflammatory control groups. Again, not surprisingly, studies have shown that direct pulmonary challenge with aeroallergens (a powerful immune stimulus) leads to secondary upregulation of lung expression of IL-10 mRNA in asthmatics[59,60]. Notably, however, direct measurement of IL-10 protein levels in the bronchoalveolar lavage fluid has shown markedly lower IL-10 production in the lungs of patients with allergic asthma, even compared with normal control subjects[60]. Furthermore, a generalized defect in IL-10 production seems to be present in patients with allergic asthma. Peripheral blood cells from asthmatics produce markedly less IL-10 in response to endotoxin stimulation than control subjects[60], and an *IL10* promoter polymorphism, more common in asthmatic individuals, has recently been described[61]. Studies in mouse models confirm a role for IL-10 in suppressing airway inflammation and cytokine production[62,63], although paradoxical effects on airway hyper-responsiveness have been found in knockouts[64].

Chronic tissue parasitism with helminths leads to an increase in IL-10 production, mainly by T cells. Most bacteria are also powerful inducers of IL-10 from antigen-presenting cells *in vitro*. It is therefore not surprising that there are clear data on *in vivo* upregulation of IL-10 during infection with bacterial agents that have been implicated in the hygiene hypothesis, including *M. tuberculosis* and BCG[65,66]. As for the diarrhoeal diseases that are so important in early childhood mortality in the developing world, both viral (for example, rotavirus) and bacterial (for example, *Shigella*) diarrhoeal diseases lead to IL-10 production[67,68].

The gut might well be a privileged environment for counter-regulation. The gastrointestinal tract, the immunological homeostasis of which is dependent on maintaining non-responsiveness to the confusion of harmless microflora and food antigens that bathe its mucosal surfaces, is normally an IL-10-dominant environment. In addition to strong IL-10 production by resident T cells, dendritic cells and macrophages, intestinal epithelial cells themselves make IL-10 (REFS 69–71). Notably, *IL10* knockout mice develop spontaneous inflammatory bowel disease in response to the gut microflora[72]. In a T-cell-dependent process such as allergy, the regulatory effects of IL-10 probably occur both during the induction phase as well as during the effector phase of the response. Recent studies indicate that priming for allergy to aeroallergens may occur in the gut[73]. It is thus notable that oral tolerance is difficult to achieve in germ-free animals[74], that administration of endotoxin together with food antigens increases the tolerizing effect of feeding in mouse models[75], and that house-dust endotoxin concentrations have been shown to be inversely correlated with allergen sensitization in infants at high risk for developing asthma[76]. Interestingly, *Lactobacillus* administration to children with atopic dermatitis, a strategy shown to have some preventative and therapeutic efficacy, induces IL-10 upregulation *in vivo*[77].

> "…counter-regulation would be expected to inhibit the likelihood of developing both T_H2-mediated allergic diseases and T_H1-mediated autoimmune diseases."

If counter-regulation provides a tenable mechanistic foundation for the hygiene hypothesis, it is not surprising that European studies of specific pathogens or overall numbers of infectious episodes have yielded unpromising data. The efficacy of such counter-regulation is probably a function of the integration of the effects of multiple immunological exposures, leaving different amounts of IL-10 in their wake. The focus on defined pathogenic episodes might obscure other relevant exposures. Serial or chronic immunological negotiations with various commensal and pathogenic microbes in the intestinal tract might be of particular importance[78].

It should be noted that IL-10 is unlikely to carry out this homeostatic, counter-regulatory role alone. Other mediators such as transforming growth factor-β (TGF-β) are likely to be important as well. Furthermore, the role of IL-10 might be indirect, at least in part. Recent work on $CD4^+CD25^+$ T-regulatory (T_R) cells (reviewed in REF. 79) is instructive. T_R cells produce IL-10 *in vitro* and *in vivo*. *In vitro*, the suppressive effects of T_R cells are contact dependent and soluble cytokine independent. However, IL-10 has been implicated in *in vivo* models of T_R-cell-mediated suppression. IL-10 might therefore be important for the generation of such cells, but not necessarily for their suppressive activity.

Further implications

Although appealing, this counter-regulation theory of protection from allergic disease (FIG. 3) will require further experimental testing. Pressing mechanistic questions, addressable in mouse models, include the cell types (for example, T_R cells)[80], anatomical locations (for example, gut mucosae) and exposures (for example, serial colonization or infection with orofaecal microbes) that are most important for the development of efficient counter-regulation. If valid, however, the hypothesis might provide an explanation for phenomena from outside the world of allergy. The hygiene hypothesis, in its T_H1/T_H2 formulation, has been plagued by a paradox over and above the one provided by the protective role of helminths. Together with increasing rates of allergic T_H2-mediated disease, the contemporary Western lifestyle is also beset by an increase in the prevalence of T_H1-mediated (or IL-12-mediated) organ-specific autoimmune diseases[81]. In an echo of earlier work on allergy, epidemiological studies of Type I diabetes have implicated similar surrogates for early childhood infectious exposure in protection from diabetes: crowding, family size and birth order, socio-economics, and day-care attendance[82–84]. Although it is difficult to incorporate these findings into a 'missing T_H1' hypothesis, strong immunological counter-regulation would be expected to inhibit the likelihood of developing both T_H2-mediated allergic diseases and T_H1-mediated autoimmune diseases. From a T_H1 perspective, the counter-regulatory hypothesis may well explain the difficulty of generating organ-specific autoimmune disease in 'dirty' animal colonies.

It should not need saying that the current epidemic of allergic disease is a small price to pay for the marked suppression in infant mortality provided by measures such as improved sanitation, access to drinkable water and vaccination. Allergic disease is also a relatively small price to pay for our current principled avoidance of the cancer-inducing effects of sunlight (ultraviolet radiation being a powerful inducer of IL-10 from the skin[85]). Careful science is likely to lead to the devising of non-lethal ways for amplifying the counter-regulatory environment of the developing immune system. Novel vaccine adjuvants, PROBIOTICS and a greater tolerance for dirt all come to mind. But it is clearly incumbent at this point to quote H. L. Mencken: "There is always an easy solution to every human problem — neat, plausible, and wrong."[86]

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