

Asthma: an epidemic of dysregulated immunity

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The remarkable increase in asthma prevalence that has occurred over the last two decades is thought to be caused by changes in the environment due to improved hygiene and fewer childhood infections. However, the specific infections that limit T helper type 2 (T_H2)-biased inflammation and asthma are not fully known. Infectious organisms, including commensal bacteria in the gastrointestinal tract and hepatitis A virus, may normally induce the development of regulatory T (T_R) cells and protective immunity that limit airway inflammation and promote tolerance to respiratory allergens. In the absence of such infections, T_H2 cells—which are developmentally related to T_R cells—develop instead and coordinate the development of asthmatic inflammation.

Asthma is an immunological disease that has increased dramatically in prevalence over the past two decades. In industrialized countries, the incidence of asthma has nearly doubled since 1980; in the United States and other industrialized countries, one in five to ten individuals is affected. As a result, asthma has reached epidemic proportions, and current health care expenditure for asthma in industrialized countries is enormous.

Asthma is caused by environmental factors, such as allergen exposure and infection, in genetically predisposed individuals. It is estimated that at least a dozen polymorphic genes regulate asthma, controlling the inflammatory response, immunoglobulin E (IgE), cytokine and chemokine production¹ as well as airway function and airway remodeling^{2,3}. Although the genetic composition of the populations in industrialized countries has not changed significantly in the last 20 years, the environment in industrialized cultures has changed dramatically in a broad sense, in ways that appear to directly exaggerate the environmental effects on asthma pathogenesis. However, the specific conditions in industrialized societies that are responsible for driving development of the T helper type 2 (T_H2)-biased immune responses and the overproduction of cytokines such as interleukin 4 (IL-4), IL-5, IL-9 and IL-13, which characterize asthma, are not yet clear. Nevertheless, we know that a dysregulated T_H2 immune response is readily induced in the setting of the modern environment and that this dysregulated T_H2 response overwhelms the protective mechanisms that normally prevent the development of asthma. The inappropriate T_H2 response causes pulmonary inflammation, airway eosinophilia, mucus hypersecretion and airway

hyperreactivity (AHR) to a variety of specific and nonspecific stimuli that result in the symptoms of asthma.

The Hygiene Hypothesis

The hypothesis that has received the greatest attention in explaining the increase in asthma prevalence is the Hygiene Hypothesis (Fig. 1)⁴. This hypothesis suggests that improved hygiene in industrialized societies, with improved public health measures and the use of vaccines and antibiotics, has reduced the incidence of infections that would normally stimulate the immune system in some way that mitigates against asthma⁵. The data that support this theory are imprecise, but they include several epidemiological studies showing an inverse relationship between family size and the risk of developing asthma⁶ as well as studies showing that early placement in day care settings, and presumed exposure to infectious agents, appears to protect against the development of asthma⁷. Other epidemiological studies that provide support for this hypothesis include observations demonstrating that exposure to farm animals and raw milk early in life reduces the likelihood of developing asthma, allergic rhinitis and atopic sensitization⁸. Several investigators suggest that exposure to bacterial endotoxin may be important in producing this protective effect, and others have shown that extensive exposure to cats or dogs and their epidermal allergens may replicate the protective effects of farm animal exposure⁹. Additional studies demonstrate that early childhood exposure to antibiotics, which may alter gastrointestinal flora and exposure to intestinal endotoxin, is associated with an increased incidence of allergy¹⁰. However, the specific infectious pathogens or the specific mechanisms that are responsible for these effects have not been identified, and thus, the link between asthma pathogenesis and infections remains nebulous.

Specific pathogens and the Hygiene Hypothesis

Infections with several pathogens—for example, *Mycobacteria tuberculosis* or respiratory viruses—may enhance T_H1 responses and limit T_H2-driven responses and, therefore, may be important in asthma. Thus, skin test reactivity to tuberculosis in children in Japan inversely correlated with the likelihood of having asthma, suggesting that exposure and response to *M. tuberculosis* inhibits the development of asthma^{11,12}. However, this observation could be explained more directly by the fact that individuals who develop asthma and atopy genetically may have less robust cell-mediated immune responses to most antigens, including *M. tuberculosis*¹³, but have increased humoral (IgE-mediated) responses to allergens and helminths¹⁴.

A link between infection with specific respiratory viruses and asthma has been recognized for decades: respiratory viruses precipitate acute airway obstruction and wheezing in patients with asthma¹⁵. Although the Hygiene Hypothesis suggests that a reduced incidence of such infections may result in increased asthma prevalence, there are few data to suggest that infections with common respiratory viral pathogens are less frequent

today than in the past. In addition, infection with respiratory syncytial virus (RSV) in young children appears to increase the risk of developing asthma, although this risk disappears by the age of 13 years¹⁶. Therefore, frequent respiratory viral infections appear to exacerbate, rather than prevent, the development of asthma, although it is possible that globally, multiple infections may in some way promote maturation of the immune system towards protection against asthma.

In contrast to respiratory viral infection, gastrointestinal exposure to bacteria and bacterial products may have a significant effect on maturation of the immune system and indeed protect against the development of asthma. Increased incidence of allergy is associated with reduced prevalence of colonization of the gastrointestinal tract in children with bifidobacteria and lactobacillus strains, two Gram-positive commensal bacteria¹⁷. In addition, exposure of infants to lactobacillus in the neonatal period appears to protect against the development of atopy¹⁸. The effect of gastrointestinal bacteria on the developing immune system may be mediated through Toll-like receptors (TLRs)—for example, TLR2, TLR4 and TLR9—which may inhibit the development of T_H2-biased immune responses. “Improved” hygiene may eliminate exposure, but it may also eliminate the protective effects of these commensal gastrointestinal bacteria. The specific TLRs that may protect against asthma are not clear, although several investigators suggest that TLR4 and exposure to bacterial lipopolysaccharide (LPS)—based on epidemiological studies of farm environments as discussed above—may be protective against asthma¹⁹. However, examination of the specific effects of LPS and TLR4 on T cell differentiation and cytokine production indicates that LPS may in fact exacerbate, rather than limit, T_H2 cell development²⁰.

HAV and the *Tim1* asthma susceptibility gene

In addition to gastrointestinal bacteria, several other gastrointestinal pathogens appear to have potent effects on protection against the development of asthma. For example, evidence of infection with hepatitis A virus (HAV) is strongly associated with protection against the development of asthma²¹. Infection with other gastrointestinal pathogens, for example, *Helicobacter pylori* and *Toxoplasma gondii*, may protect against the development of asthma, although to a lesser extent²². However, because HAV, *H. pylori* and *T. gondii* are not respiratory pathogens and because HAV is transmitted through fecal-oral routes, clinical investigators have assumed that infection with these agents is merely a marker of poor hygiene and that other infectious pathogens, possibly involving the respiratory tract⁷, are more directly involved in protection against asthma.

The mechanisms by which HAV infection prevents the development of asthma may be more direct than previously suspected. The positional

cloning of a newly identified asthma susceptibility gene, *Tim1*, provides evidence for a direct role of HAV in the prevention of atopic disease²³. The human homolog of *Tim1*, which lies at human chromosome 5q33.2, a region that has been repeatedly linked to asthma, codes for the cellular receptor for HAV (hHAVcr-1)²³. In addition, in mice, the *Tim1* gene product is expressed on T cells and appears to regulate the production of IL-4 in T cells by affecting CD4⁺ T cell differentiation, the development of T_H2 cells and the development of AHR²³. Polymorphisms in *Tim1* are associated with enhanced development of allergen-induced AHR in some strains of mice (for example, BALB/c), whereas other polymorphisms protect against the development of AHR (for example, in DBA/2 mice). By interacting with HAV, human TIM-1 may directly alter the T_H cell balance of the infected individual. HAV persists in lymphoid tissues for weeks to months, and HAV infection or interaction with certain lymphocyte subsets may have significant effects on T_H2 differentiation and

T_H2 cell survival. Because *Tim1* is associated with the development of T_H2-biased immune responses and may be selectively expressed on T_H2 cells, infection with HAV may selectively eliminate allergen-specific T_H2 cells by clonal deletion and, thus, specifically protect against the development of atopy. Alternatively, HAV may alter T cell development and enhance the development of immune responses that protect against asthma. Therefore, HAV infection may have direct and specific effects on T_H2 responses and AHR. The specific mechanisms, however, by which *Tim1* alters T cell function and by which HAV limits T_H2 cell development require further elucidation and are currently being studied.

Even in the absence of HAV infection, *Tim1* may contribute to asthma susceptibility by affecting T_H2 responses. Genetic variants of *Tim1* may either enhance or prevent T_H2-dependent inflammation in asthma and contrasts with another asthma susceptibility gene, *ADAM33*³². This gene codes for a metalloprotease, which may regulate the response of the respiratory epithelium to damage and stress, rather than regulate immunological components of asthma. Epithelial and mesenchymal cell interactions (epithelial-mesenchymal trophic unit) coordinate the response of the respiratory epithelium to damage after repeated injury from the environment. Asthma-inducing alleles of *ADAM33* may amplify these repair mechanisms, leading to greater inflammation, airway remodeling and to the development of asthma. The development of asthma, therefore, depends on immunological as well as intrinsic airway function abnormalities, which may explain why T_H2-allergic responses are necessary but not sufficient for the development of asthma.

Do T_H1 responses protect against asthma?

Until recently, specific pathogens that were suspected of limiting the development of asthma were those that could induce T_H1 responses.

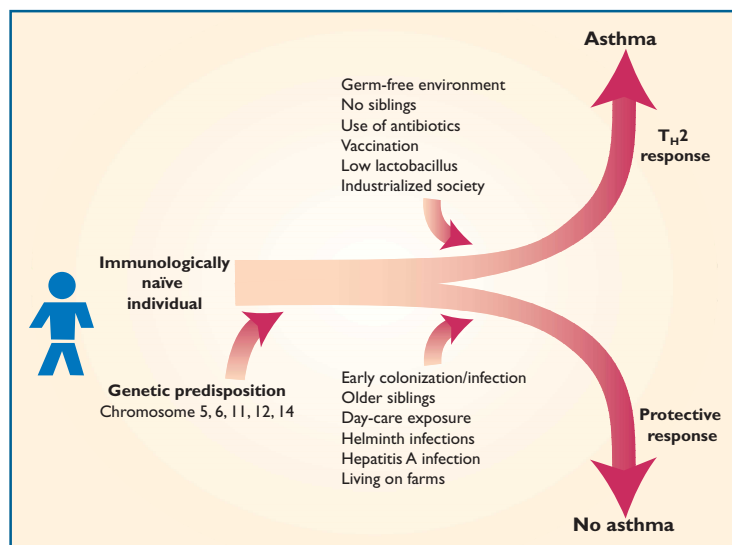


Figure 1. Asthma is a complex genetic trait caused by environmental factors in genetically predisposed individuals. Environmental effects, particularly in young children, can enhance the development of T_H2 responses and asthma. On the other hand, certain environmental effects enhance protective responses and limit the development of asthma.

This is because the primary mechanism thought to protect against asthma involved T_H1 cells, as predicted by the T_H1 - T_H2 paradigm. T_H1 responses were thought to protect against allergic disease by dampening the activity of T_H2 responses, as has been shown in models of parasite infection²⁴. In accordance with this idea, infection in infants has been proposed to stimulate the immune system nonspecifically to mature and convert the predominantly T_H2 bias of infants towards a T_H1 bias, by increasing the production of IFN- γ ²⁵. According to this hypothesis, however, in atopic infants this conversion towards T_H1 fails to occur, perhaps because of infrequent infection. The T_H2 bias therefore persists, resulting in asthma and allergy in the child.

The evidence that supports the hypothesis that T_H1 cells render salutary effects in allergic disease and asthma is indirect. T_H1 cells inhibit the proliferation and, therefore, the development of T_H2 cells²⁶, and interferon- γ (IFN- γ) inhibits IgE synthesis and eosinophilia^{27,28}. In reality, however, T_H1 cells may exacerbate asthma and allergy, as human asthma is associated with the production of IFN- γ that appears to contribute to disease pathogenesis^{29,30}. Allergen-specific T_H1 cells, when adoptively transferred into naïve recipients, migrate to the lungs but fail to counterbalance T_H2 cell-induced AHR. Instead, allergen-specific T_H1 cells caused severe airway inflammation³¹. Thus, although T_H2 cells play a critical role in the pathogenesis of asthma, the binary T_H1 - T_H2 paradigm—where T_H1 cells balance T_H2 cells—cannot explain all the immunological processes that occur in asthma. These processes in asthma may be much more complex than is predicted by the T_H1 - T_H2 paradigm^{32–34}, and “unhygienic” environments may protect against asthma by inducing additional non- T_H1 - T_H2 immunological regulatory mechanisms. This is consistent with the fact that T_H1 -mediated autoimmune diseases—such as type 1 diabetes and inflammatory bowel disease—are also increasing in prevalence in industrialized cultures over the past 20 years. If improved hygiene in these cultures reduced T_H1 responses, then the prevalence of autoimmune diseases should decrease rather than increase, suggesting that mechanisms other than pure T_H1 responses must be involved in protection against asthma.

T cell tolerance and asthma

One immune mechanism that may protect against and regulate the development of asthma and could be significantly affected by changes in the environment in industrialized cultures is immune tolerance induced by mucosal (respiratory and gastrointestinal) exposure to antigen. In support of this possibility, peripheral $CD4^+$ T cell tolerance, induced by respiratory exposure to allergen, prevents the development of T_H2 -biased responses and allergen-induced AHR^{35–37}. In humans, the degree of allergen exposure appears to affect the induction of tolerance, with high exposure inducing greater tolerance⁹. The mechanisms by which respiratory antigens induce T cell tolerance include T cell clonal deletion, anergy or active suppression mediated by regulatory cells secreting IL-10 or transforming growth factor- β (TGF- β), as is thought to occur in oral tolerance^{38–40}. The development of respiratory tolerance is initiated by uptake of antigen in the lungs by immature dendritic cells (DCs)⁴¹ that continuously sample the antigenic contents in the lung. Within 24 h after respiratory exposure to ovalbumin (OVA), pulmonary DCs migrate to the draining bronchial lymph nodes, where they mature, transiently produce IL-10 and express high amounts of B7-1 and B7-2 costimulatory molecules⁴². Even in the absence of inflammation, DCs migrate from the lungs to the bronchial lymph nodes. In the lymph nodes, DCs induce an initial phase of allergen-specific T cell activation, proliferation and expansion, followed by depletion of these T cells from the lymphoid organs, although a stable population of these cells survive but remain refractory (functionally

disabled) to antigenic rechallenge³⁶. The establishment of a tolerant state in allergen-specific T cells prevents the development of airway inflammation and airway AHR. Antigen-specific B cells also play a role in this process because in the absence of B cells, for example in JHD mice, respiratory tolerance cannot be induced⁴³.

Allergen-specific $CD4^+$ regulatory T (T_R) cells mediate, in part, the T cell tolerance that inhibits airway inflammation and AHR⁴⁴. These antigen-specific $CD4^+$ T_R cells produce IL-10, can potently inhibit the proliferation of OVA-specific T cells, inhibit the development of AHR when transferred to allergen-sensitized recipient mice and can be generated by activating $CD4^+$ T cells with IL-10-producing bronchial lymph node DCs from tolerized mice⁴⁴. Pulmonary DCs from tolerized mice express high amounts of costimulatory molecules, including inducible costimulatory molecule ligand (ICOSL) and possess a low capacity for phagocytosis, all of which are characteristics of mature DCs⁴². The production of IL-10 by these DCs plays a major role in the generation of T_R cells, as IL-10 monoclonal antibody (mAb) blocks this process⁴⁴. The *in vivo* and *in vitro* inhibitory capacity of the T_R effector cells on AHR and inflammation can be abrogated by neutralization of IL-10 with an IL-10 mAb or by interrupting ICOS-ICOSL interactions with an ICOSL mAb. In addition, interference with the ICOS-ICOSL signaling pathway not only abrogates the inhibitory capacity of T_R effector cells, but also blocks the development of IL-10 production and the induction of T cell tolerance after intranasal exposure to antigen⁴⁴. Thus, T_R cells may play an important role in mediating respiratory tolerance and protection against asthma *via* specific pathways that involve IL-10, ICOS-ICOSL costimulatory interactions and B7-2, which preferentially costimulates IL-10 production (Fig. 2)^{45,46}.

Similar T_R cells (T_R1 cells) have been generated by other groups by the addition of large quantities of exogenous IL-10 to cultures of T cells or with immunosuppressive drugs^{47,48}. In other systems, immature DCs expressing low amounts of costimulatory molecules induced T cell tolerance and the development of regulatory cells, often when the DCs were derived after extensive propagation *in vitro* in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF)⁴⁹. These T_R -like cells develop in the absence of costimulation and, therefore, may be related to anergic cells^{50,51}. In addition, a variety of other mechanisms may be important for the development of other previously described regulatory cells—such as T helper type 3 (T_H3)³⁸, $CD4^+CD45RB^{lo}CD25^{+52,53}$ or T_R1 cells⁴⁷—or with CTLA-4-mediated suppression⁵⁴. Heat-killed *Mycobacterium vaccae* can indeed induce $CD4^+CD45RB^{lo}$ T cells that protect against airway inflammation⁵⁵. However, although TGF- β -producing T cells may inhibit AHR⁴⁰, TGF- β -producing T_H3 cells may be more important in the gastrointestinal tract than in the lungs⁵⁶, and CTLA-4-mediated suppression may not occur in the respiratory tract because inhibition of CTLA-4 signaling does not inhibit respiratory tolerance induction³⁶. Nevertheless, T_R cells producing anti-inflammatory cytokines such as IL-10 are more likely than pro-inflammatory T_H1 cells to be involved in inhibiting airway inflammation in asthma.

The role of IL-10 in limiting AHR and inflammation has been controversial. IL-10 has been considered to be an essential T_H2 cytokine²⁴, particularly because IL-10 inhibits T_H1 cytokine production by inhibiting IL-12 synthesis⁵⁷, and IL-10^{-/-} mice develop poor T_H2 responses and resist the development of AHR⁵⁸. However, IL-10 may have several roles in asthma, not only by playing a critical function in initiating the development of T_H2 -polarized responses, but also by playing an important regulatory role late during immune responses by down-modulating T_H2 -driven inflammation^{58–61}. Administration of IL-10 inhibits AHR and T_R cells that produce IL-10 prevent the development of AHR, even

in allergen-sensitized mice. This suggests that T_R cells producing IL-10 normally develop during respiratory exposure to allergen and protect against allergic asthma⁴⁴.

ICOS and ICOSL in asthma and tolerance

The ICOS-ICOSL pathway is important in the activation and function of effector T_H2 cells, and induces CD28-independent T cell proliferation and cytokine production^{45,62,63}. This promotes preferential IL-10 production, along with the production of IL-4, IL-5 and GM-CSF^{64,65} but little IL-2^{45,62,63}. Mice deficient in ICOS show major reductions in IgE production⁴⁵, T_H2 cytokine production and in the development of AHR, which indicates a key role for ICOS in the development of allergic respiratory inflammatory responses⁶⁶. In addition to its role in amplifying the development of T_H2 responses, ICOS costimulation pathways are critically important in immune regulation and tolerance⁴⁴. ICOS costimulates the induction of T_R cells that inhibit the function of antigen-specific T cells and development of AHR⁴⁴. Although the role played by ICOS in immune regulation was not expected, in retrospect, is not surprising because ICOS costimulation induces large quantities of T cell IL-10 production^{45,67} and ICOS^{-/-} mice appear to be prone to the development of experimental allergic encephalomyelitis^{68,69}.

The idea that ICOS-ICOSL interactions costimulate the development of both T_H2 -driven inflammation and T_R cell-mediated tolerance suggests that these distinct processes are related. Both T_H2 and T_R cells are associated with respiratory mucosal responses, require costimulation through CD28 and ICOS for induction and produce both IL-4 and IL-10, although in different quantities. T_H2 cells produce IL-4, IL-13 and IL-10 primarily, whereas T_R cells produce IL-10 primarily and low amounts of IL-4⁴⁷, but not IL-13⁴⁴. In addition, the functions of T_R and T_H2 cells in a mouse model of asthma were clearly distinct because T_R cells, but not T_H2 cells, blocked the development of AHR⁴⁴. Pulmonary DCs from mice exposed to intranasal OVA induced T cell production of both IL-4 and IL-10 initially, but with subsequent stimulation, IL-4 production waned, whereas production of IL-10 was maintained. The specific signals that preferentially induce development of T_R cells rather than T_H2 cells are not entirely clear, but may involve IL-10 production by DCs. In the presence of IL-10-producing DCs (or in the presence of exogenously derived IL-10), T_R cells develop⁴⁷. In contrast, in the absence of IL-10, for example, when antigen is presented by DCs from IL-10^{-/-} mice or from mice with allergic asthma⁴², T_R cells fail to develop. The development of T_H2 cells and of allergic diseases may represent an aberration of T_R cell development, possibly due to inadequate production of IL-10 (Fig. 2)⁷⁰. Thus, T_H2 cells in allergic asthma may develop as a consequence of limited IL-10 and enhanced IL-4 and IL-13 production and from the failure to develop allergen-specific T_R cells or “modified T_H2 cells”, rather than a failure to develop T_H1 cells. However, further study of the

signals that differentially affect T_H2 cell and T_R cell development are required to fully understand the development of tolerance and its relationship to the pathogenesis of allergic asthma.

Loss of tolerance and the Hygiene Hypothesis

Based on the above studies, we propose that the natural environment in the past (prior to widespread industrialization) maintained the respiratory and gastrointestinal mucosal systems in a state that favored the development of T cell tolerance to nonreplicating antigens encountered at mucosal surfaces, for example, in food and inhaled material. The mechanisms at mucosal surfaces that favor the development of tolerance are not clear, but may involve epithelial cells, DCs, intraepithelial B cells and other cells in the mucosa and also involve the production of IL-10 and possibly TGF- β by DCs and by antigen-specific T_R cells^{37,71,72}. The T_R cells that develop at these mucosal sites inhibit inflammatory responses in the lung and gut, inhibiting both the development of T_H2 -biased inflammation—for example, in allergic disease—preventing both respiratory diseases, such as allergic rhinitis, and gastrointestinal diseases, such as food allergy. Such mechanisms could also inhibit T_H1 -biased inflammation, for example, in autoimmune diseases such as diabetes

mellicus, multiple sclerosis and inflammatory bowel disease. T_H1 cells may, in some instances, be involved in inhibiting the development of T_H2 cells, but this form of inhibition most likely occurs primarily in lymphoid organs such as the lymph nodes, where the proinflammatory effects of T_H1 cells cause little tissue damage. In contrast, the major anti-inflammatory effector mechanisms in the mucosa involve T_R cells, although cells producing IFN- γ and IL-10 together (for example, in CD4, CD8 cells or $\gamma\delta$ T cells) may exert anti-inflammatory effects in the mucosa as well⁷³. Finally, deletion of antigen-specific T cells, *via* activation-induced cell death, may also be involved as a mechanism to control inflammation in these organs.

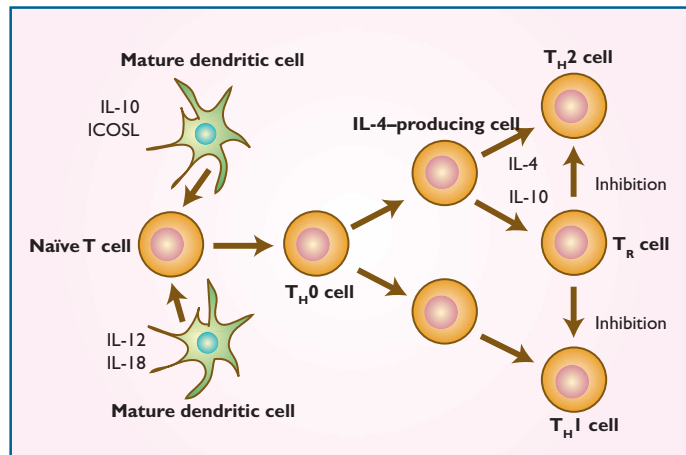


Figure 2. DCs direct the development of T_H1 , T_H2 and T_R cells. DCs producing IL-12 and IL-18 enhance the development of T_H1 cells. On the other hand, DCs expressing ICOSL enhance the development of IL-4-producing cells. The IL-4-producing cells differentiate into either T_H2 cells, which produce high concentrations of IL-4 and IL-13, or T_R cells, which do not produce IL-4 or IL-13 but do produce high concentrations of IL-10. T_R cells appear to inhibit the function of both T_H1 and T_H2 cells.

The establishment of these tolerance mechanisms at mucosal surfaces may require the presence of commensal bacteria in the gastrointestinal and upper respiratory tracts⁷⁴⁻⁷⁷. Immune tolerance induced by mucosal exposure to antigen does not seem to occur in germ-free animals^{76,77}, which indicates that normal microbial flora in the intestine, and perhaps in the upper respiratory tract, have significant effects on tolerance to allergens. Commensal bacteria may enhance production of IL-10 through mechanisms that involve the innate immune system and receptors such as nucleotide-binding oligomerization domain 2 (NOD2, which is encoded by *CARD15*).

CARD15 is a member of the NOD family of proteins, which are involved in the regulation of programmed cell death and host defense against pathogens⁷⁸. *CARD15* expression is restricted to monocytes and, when activated by LPS from a bacterium, it induces nuclear translocation of NF- κ B in a manner that appears to down-modulate inflammation. Alterations in NOD2 function due to polymorphisms in

CARD15 are associated with Crohn's disease, an inflammatory bowel disease^{79,80}. The anti-inflammatory effects induced in the gastrointestinal tract by NOD2 may be related to the enteric "immunosuppressive" effects observed with nonvirulent *Salmonella* strains, which block NF- κ B activation by inhibiting I κ B degradation⁸¹. Infection with high numbers of helminths may also favor the development of tolerance to non-replicating environmental antigens by enhancing IL-10 production^{82,83}.

Therefore, some microorganisms may reduce inflammation at mucosal surfaces. Changes in the environment in industrialized societies limit exposure to these types of organisms and thereby diminish the normal tolerance-inducing state of mucosal surfaces, resulting in enhanced mucosal inflammation. These changes prevent the development of allergen-specific T_R cells in some individuals, resulting in an aberrant form of T_R cells developing in atopic individuals—T_{H2} cells—which induce the development of allergy. The responsible environmental changes may include frequent use of antibiotics or changes in diet⁸⁴, which alter the normal gastrointestinal flora, resulting in reduced IL-10 and TGF- β production by epithelial cells, DCs and B cells in the mucosa and causing increased development of T_{H2} cells and allergy.

Immune-based therapies for asthma

If the significant increase in asthma is due to lack of tolerance to environmental allergens, then treatments that induce tolerance to these agents should be effective in treating and potentially curing asthma. Although the currently available pharmacotherapies for controlling the symptoms of asthma (such as inhaled corticosteroids and leukotriene antagonists) and the several that are being developed (IgE mAb, IL-5 mAb and IL-4 mAb) are effective, none of these therapies can cure established asthma. For example, corticosteroids are very effective at controlling symptoms, but are associated with side-effects and lose their beneficial effects soon after they are discontinued. Furthermore, therapies such as corticosteroids, although controlling the pathogenic T_{H2} responses, may limit protective tolerance responses.

Therapies that may enhance the tolerance process include the administration of probiotics, such as *Lactobacillus*, which improves and possibly prevents the development of atopic diseases¹⁸. Precisely how probiotics function is not clear, but may involve activation of the mucosal innate immune system—for example, NOD2 pathways—in a way that reduces inflammation. Oral allergen immunotherapy, which reduces symptoms of allergy⁸⁵, may also utilize mucosal tolerance mechanisms. This allergen-specific therapy is a variant of conventional allergen immunotherapy, in which allergen is given in increasing amounts subcutaneously. Conventional allergen immunotherapy not only controls symptoms, but also reverses established disease and prevents progression from allergic rhinitis towards asthma⁸⁶. Immunotherapy induces allergen-specific immune deviation and allergen-specific tolerance, which are associated with a reduction in IL-4 production⁸⁷, increased IgG4 and, when bee venom is used as the antigen for bee venom allergy, increased IL-10 production⁶⁰. This therapy is curative, particularly in children⁸⁶ and in patients with bee venom allergy, and the beneficial effects are maintained for years after therapy has been discontinued⁸⁸. However, the major problem with conventional allergen immunotherapy that decreases its appeal is that it is inefficient, requiring over 100 shots for successful completion. Therefore, modification of allergen immunotherapy, for example with the use of allergens conjugated with CpG motifs that bind to TLR9, are currently being studied and appear to be a promising form of allergen immunotherapy⁸⁹. Perhaps even more effective therapies might be developed with specific subsets of allergen-pulsed DCs that induce tolerance or T_R cells. Such therapies, however, await a more complete understanding of the immunobiology

of tolerance and allergic disease and the mechanisms by which HAV prevents the development of asthma.

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