

Molecular mechanisms in allergy and clinical immunology

Series editors: William T. Shearer, MD, PhD, Lanny J. Rosenwasser, MD, and Bruce S. Bochner, MD

Regulatory T cells control the development of allergic disease and asthma

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The role of T_H2 cells in the pathogenesis of allergy and asthma has been well described. However, the immunologic mechanisms that downmodulate and protect against the development of these disorders are poorly characterized. A spectrum of $CD4^+$ T cells, including T_H1 cells, T_H3 cells, regulatory T cells, $CD25^+$ T cells, and natural killer T cells might play a critical role in regulating these diseases and are discussed in this review. (J Allergy Clin Immunol 2003;112:480-7.)

Key words: *T regulatory cells, T_H2 , asthma, allergy*

Since 1986, the T_H1/T_H2 paradigm has dominated our understanding of the pathophysiology of asthma and allergic disease. However, it has become apparent in recent years that other immunologic principles are important in explaining the regulation of asthma and allergy and that new paradigms encompassing a more diverse set of cell types must be developed to explain the immunologic events that regulate these disorders. In this article we will review the spectrum of CD4 T cells that critically regulate the development of asthma and allergy.

T_H1/T_H2 PARADIGM

The findings of Mosmann and Coffman¹ in 1986 that T_H1 and T_H2 cells cross-regulate each other and control immune deviation and the form of immune responses rapidly transformed the understanding of allergic disease and asthma. Prior investigations of allergic disease focused on allergen-specific IgE, mast cells, and immediate type 1 immune responses. After the delineation of the T-cell subsets, it became clear that T_H2 cells produc-

Abbreviations used

AHR: Airway hyperreactivity
DC: Dendritic cell
HAV: Hepatitis A virus
NK: Natural killer
TCR: T-cell receptor

ing IL-4, which enhanced IgE synthesis; IL-5, which increased eosinophil growth and differentiation; IL-9, which enhanced mast cell differentiation; and IL-13, which increased mucus production and induced airway hyperreactivity (AHR), a cardinal feature of asthma, controlled and amplified the allergic inflammatory response.²⁻⁴ Because T_H1 cells cross-regulated T_H2 cells in some systems, particularly in infectious disease models, it was assumed that T_H1 cells had a beneficial role in allergic disease and asthma by downmodulating the effects of T_H2 cells.⁵

The idea that T_H1 cells had a salutary role in allergic disease was consistent initially with several findings. Allergen-specific T cells generated from nonallergic individuals were shown to express a T_H1 cytokine profile.⁶ In addition, allergen immunotherapy for allergic disease was shown to convert T_H2 cytokine profiles into T_H1 cytokine profiles, which correlated with symptomatic improvement in both human subjects⁷⁻⁹ and animal models of disease.¹⁰⁻¹³ Furthermore, administration of IL-12,¹⁴ which greatly enhances IFN- γ production, or IL-18,^{15,16} which enhances the effects of IL-12, also caused a reduction in allergen-induced AHR. Finally, individuals predisposed toward the production of T_H1 cytokines (eg, patients with multiple sclerosis or rheumatoid arthritis) have a reduced likelihood of having allergic disease.^{17,18}

However, as a greater understanding of T_H1 cells and T_H1 responses developed, it became clear that T_H1 cells were pro-inflammatory and that the development of a T_H1 -associated inflammatory response was unlikely to downmodulate asthma and allergic disease.^{19,20} In addition, IFN- γ is present at sites of allergic inflammation and is thought to contribute to disease. Furthermore, transfer of allergen-specific T_H1 cells into the lungs of mice, although not directly inducing AHR, resulted in

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Supported by National Institutes of Health Public Health Service Grants RO1 AI26322, RO1 HL62348, and RO1 HL69507 and a grant from the American Lung Association of California.

Received for publication April 24, 2003; revised June 18, 2003; accepted for publication June 19, 2003.

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0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.1717

enhanced, rather than reduced, inflammation in the airways. Finally, it became clear that the increase in prevalence of atopic diseases over the past 20 years has been accompanied by an increase in the prevalence of autoimmune, T_H1 -driven diseases. This suggested that the environmental changes that have resulted in increased asthma prevalence cannot be due to solely a reduction in T_H1 (anti-atopy) regulatory elements, because T_H1 -driven diseases have also increased during this period. These observations therefore suggest that forms of immunity, other than pure T_H1 responses, must be effective in regulating the development of asthma and allergic diseases.

REGULATORY T CELLS

Over the past 5 years, a great deal of interest has focused on regulatory T cells that appear to control the development of autoimmune disease and transplant rejection and that might also play a critical role in controlling the expression of asthma and allergy. The term *regulatory T cell* refers to cells that actively control or suppress the function of other cells, generally in an inhibitory fashion. The specific mechanisms by which the regulatory cells function or the specific characteristics of these cells are still being investigated. These cells have been difficult to study, primarily because of the difficulty in culturing them in vitro. For many years, different laboratories have found evidence of lymphocytes that suppress immune responses, but these laboratories have usually used disparate protocols to generate these cells, making interpretation of these observations difficult. However, the 5 most promising recent candidates of CD4 regulatory/suppressor T cells are T_H3 , T_R1 , T_R , $CD4^+CD25^+$, and natural killer (NK) T cells (Table I).

T_H3 cells were described by Chen et al²¹ as CD4 T cells induced by oral antigen in the mesenteric lymph nodes that produce TGF- β with various amounts of IL-4 and IL-10. These T cells suppressed the development of experimental autoimmune encephalomyelitis, suggesting that T cells producing TGF- β or IL-10 have regulatory or suppressive properties that inhibit the effects of pathogenic autoantigen-specific T cells.

$TR1$ cells were described by Groux et al,²² who showed that chronic activation of both human and murine $CD4^+$ T cells in the presence of IL-10 gave rise to $CD4^+$ T cells with low proliferative capacity that produced high levels of IL-10, low levels of IL-2, and no IL-4. These antigen-specific T cells suppressed the proliferation of $CD4^+$ T cells in response to antigen and prevented experimental colitis induced in SCID mice. This suggested that $CD4^+$ T_R1 cells induced with IL-10 could regulate antigen-specific immune responses and inhibit the function of pathologic autoantigen-specific T cells in vivo. The relationship between T_H3 and T_R1 cells is not clear, except for the fact that T_H3 cells produce detectable levels of TGF- β whereas T_R1 cells do not.

$CD4^+CD25^+$ cells were described by Sakaguchi et al²³ as a minor fraction (approximately 10%) of CD4 T cells that play a critical role in the prevention of organ-

specific autoimmunity and allograft rejection and maintenance of self-tolerance. These cells constitutively express the IL-2 receptor α chain, are similar to those identified as $CD45RB^{low}$,²⁴ and are produced by the thymus as a functionally mature T-cell subpopulation. The study of these regulatory cells might be easier in the future, now that improved markers have been identified, including the glucocorticoid-induced TNF receptor²⁵ and a transcription factor, *Foxp3*, that appears to be specific for $CD25^+$ regulatory T cells.²⁶ Depletion of $CD25^+$ cells results in the development of autoimmune diseases such as thyroiditis, gastritis, type 1 diabetes, and inflammatory bowel disease, and is clinically seen in patients with X-linked autoimmunity-allergic dysregulation (XLAAD; also known as *immune dysregulation, polyendocrinopathy enteropathy X-linked syndrome* [IPEX])²⁷ and in scurfy mice.²⁸ In some systems it appears that second signals (nonspecific proliferation, T-cell receptor [TCR] activation, or inflammation) are needed to induce autoimmunity in the absence of $CD25^+$ regulatory T cells, as occurs when populations of $CD25^+$ -depleted T cells are transferred into lymphopenic environments.²⁹ In tumor models, depletion of $CD25^+$ cells enhances some forms of tumor immunity, indicating that these cells function to suppress a wide range of immune responses. $CD25^+$ cells suppress immune responses through direct cell-cell contact in a process that is dependent on signaling via CTLA-4, as well as secretion of TGF- β . The antigen specificity of $CD25^+$ T cells is not well understood, but studies in TCR transgenic mice suggest that these cells undergo thymic selection and require a TCR with high affinity for self-peptides in a process that is distinct from positive selection and deletion.³⁰ However, Barthlott et al³¹ showed that monoclonal TCR transgenic T-cell populations lacking characteristic markers ascribed to regulatory cells can function as regulatory T cells, especially when they expand rapidly in lymphopenic environments, indicating that inhibition of autoreactive T cells can occur simply by T cells competing for expansion during homeostatic and antigenic stimulation. Importantly, the suppressive effects of $CD25^+$ regulatory cells can be blocked by the presence of IL-6, which is often produced by dendritic cells (DCs) activated through Toll-like receptor pathways,³² suggesting that the inhibitory effects of regulatory T cells are inhibited during infection.

NKT cells comprise a population of T cells that express cell surface markers characteristic of both NK cells and conventional T cells and that have been shown to exert strong regulatory activity in autoimmune diseases such as diabetes mellitus,³³ experimental autoimmune encephalomyelitis,³⁴ and oxazolone-induced colitis,³⁵ as well as in tumor immunity.³⁶ NKT cells are either $CD4^+$ or double negative (lacking CD4 and CD8). NKT cells express a limited TCR repertoire and express an invariant V α 14-J α 281 TCR in mice and a V α 24-J α 15 TCR in human beings.³⁷ Through this TCR, NKT cells recognize glycolipid antigens presented by the nonpolymorphic MHC class I-like protein CD1d. Most impor-

tantly, when activated, NKT cells rapidly produce large quantities of IL-4 and IFN- γ . This rapid production of cytokines is thought to allow NKT cells to exert strong regulatory activity, and recent studies suggest that NKT cells exert potent activity in controlling the development of asthma, as discussed below.

REGULATORY T CELLS IN ASTHMA AND ALLERGIC DISEASE

Regulatory T cells have been studied primarily in the context of tolerance to self-antigens. However, tolerance is also important in the peripheral immune system in terms of immune responses to antigens encountered in the respiratory and intestinal tracts. Normally, these sites are continuously exposed to a wide variety of environmental, nonreplicating, nonpathogenic antigens, which normally induce tolerance or hyporesponsiveness rather than active immunity. Thus, for example, food allergen in the intestinal tract or inhaled allergens in the respiratory tract generally do not induce productive immune responses. Consistent with this, exposure to cats or dogs in the first year of life has recently been shown to reduce the subsequent risk of allergic sensitization to multiple allergens.^{38,39} Other studies have shown that children growing up on farms, particularly those with direct contact with animals, were less likely to be allergic than children growing up in urban environments,⁴⁰⁻⁴² possibly because of respiratory exposure to endotoxin.⁴³ In these cases, tolerance to environmental allergens encountered at mucosal surfaces, such as the lungs or intestinal tract, could be mediated by the development of regulatory T cells or, alternatively, by clonal deletion or anergy of allergen-specific cells. Moreover, because patients who are thought to specifically lack CD25⁺ regulatory T cells (patients with XLAAD/IPEX disease) develop severe eczema, elevated IgE levels, eosinophilia, and food allergy, it is very likely that some form of regulatory T cells control the development of allergic disease and asthma.

In support of a role for regulatory T cells in maintaining tolerance to inhaled antigens, studies have shown that T cells engineered to secrete TGF- β , in contrast to IFN- γ -secreting T_H1 cells, could very effectively reduce airway inflammation and AHR.⁴⁴ T cells secreting TGF- β , generated by transducing T cells with a retrovirus containing the cDNA for latent TGF- β , when adoptively transferred, greatly reduced airway inflammation and AHR induced by T_H2 cells. This inhibitory effect was dependent on TGF- β , because neutralization of TGF- β with an anti-TGF- β mAb completely abolished the effect. Furthermore, blockade of TGF- β signaling in mature T cells enhanced airway inflammation and AHR, suggesting that regulation of T cells by TGF- β negatively regulates inflammatory responses in the lungs.⁴⁵

Inflammation in asthma can be inhibited not only by TGF- β -secreting cells but also by IL-10-secreting cells. Thus T cells engineered to secrete IL-10 with a retrovirus or adenovirus containing the cDNA for IL-10 greatly reduced the development of AHR and inflammation.^{46,47}

Adoptive transfer of IL-10-secreting T cells greatly inhibited AHR and inflammation induced by transferred T_H2 cells.⁴⁶ The inhibitory effect was due to IL-10, because neutralization of IL-10 with an anti-IL-10 mAb completely abolished the effect. These observations indicate that cells other than T_H1 cells are very effective in downmodulating pulmonary inflammation in asthma and that responses that involve TGF- β or IL-10 are very effective in downregulating asthma. These studies support the idea that immune tolerance, which is associated with production of TGF- β and IL-10,⁴⁸ might be important in regulating asthma.

IMMUNE TOLERANCE

Immune tolerance can be established by respiratory or oral exposure to the allergen.⁴⁹⁻⁵² Early studies provided evidence that CD8⁺ T cells expressing $\gamma\delta$ TCR were involved in the respiratory tolerance process,^{53,54} although this finding is now controversial.^{55,56} Intranasal delivery of a single immunodominant peptide from allergen can induce tolerance to the whole protein in a process called *linked suppression*,^{57,58} but this process appears to induce the development of functional inactivation of antigen-specific CD4⁺ T cells, a form of anergy, rather than induction of regulatory T cells. In contrast, respiratory administration of whole allergens on 3 consecutive days appears to induce the development of T-cell hyporesponsiveness, associated with the development of T_R cells. Production of ovalbumin (OVA)-specific IgE in these mice was reduced, although production of OVA-specific IgG1 and IgG2a was increased, indicating that immune deviation or split tolerance was induced.⁵⁰ Most importantly, however, the induction of such respiratory tolerance or immune deviation to OVA greatly inhibited the subsequent development of AHR and airway inflammation.⁵⁹

The protective response afforded by tolerance induced by exposure to OVA, an intranasal allergen, involved the initial activation of OVA-specific T cells, which expressed activation markers (eg, CD69, CD25, and CD44), reduced levels of CD62 ligand, and rapidly expanded in number. DCs were also involved in this tolerance process, as DCs isolated from the lymph nodes of mice that were exposed to intranasal OVA expressed high levels of B7-1, B7-2, and CD40, as well as high levels of ICOS ligand.⁶⁰ These DCs also had a low capacity to endocytose FITC-labeled dextran, another marker of mature DCs, indicating that these DCs behaved as mature DCs rather than immature DCs, which previously were thought to be involved in the development of tolerance.⁶¹⁻⁶³ Moreover, the mature DCs from tolerized mice transiently produced IL-10, as detected by RT-PCR or intracellular cytokine staining, and induced OVA-specific T cells to proliferate and produce IL-4 and IL-10 but not IFN- γ or TGF- β . On further stimulation of the OVA-specific T cells with DCs, IL-4 synthesis waned but IL-10 synthesis persisted.

To study the function of the IL-10-producing T cells, which express a cytokine profile reminiscent of regulato-

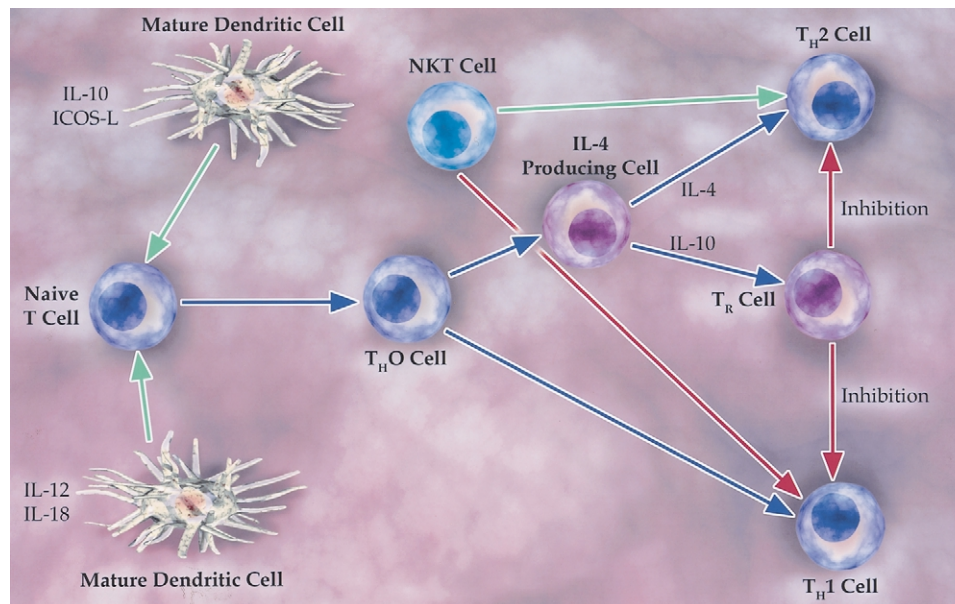


FIG 1. T_R and NKT cells regulate the differentiation (blue arrows) and function of T_H1 cells and T_H2 cells. DCs producing IL-12 and IL-18 enhance (green arrows) T_H1 cell development, whereas DCs expressing ICOS ligand and producing IL-10 enhance the development of T_H2 and T_R cells. T_R cells and T_H2 cells develop from a common precursor cell producing IL-4, but as T_R cells differentiate, they lose the capacity to produce IL-4 but retain the capacity to produce IL-10. T_R cells inhibit (red arrows) the function of both T_H1 and T_H2 cells. NKT cells, which produce large quantities of IFN- γ and IL-4, have critical effects on T_H1/T_H2 cell differentiation and are required for the development of allergen-induced AHR.

ry T cells, we adoptively transferred these T cells into mice that had been previously primed by immunization with OVA in adjuvant. Transfer of such cells greatly reduced the development of AHR and airway inflammation. The inhibitory effects of the T_R cells were mediated in part by an inhibitory effect on allergen-specific T cells and were dependent on the production of IL-10, because neutralization of the IL-10 with an anti-IL-10 mAb reversed the inhibitory effect of the T_R cells. Moreover, the inhibitory function of the T_R cells depended on the ICOS–ICOS ligand costimulatory pathway. Previous studies have linked the ICOS–ICOS ligand pathway with the development of T_H2 responses and humoral immunity.^{64–67} However, these studies suggest that the ICOS–ICOS ligand pathway might also regulate the development and function of T_R cells.

These studies suggest that T_R cells secreting IL-10 have a direct inhibitory effect on the function of T_H2 cells (Fig 1) and might also inhibit the function of T_H1 cells.²² The fact that (a) both T_R cells and T_H2 cells require ICOS–ICOS ligand costimulation to develop, (b) they produce both IL-4 and IL-10 (although in different quantities), and (c) they are associated with mucosal responses suggests that these 2 forms of immunity are related. However, T_H2 cells, but not T_R cells, produce IL-13 and induce AHR. We propose that normal exposure to respiratory allergen should result in the development of tolerance and T_R cells and that T_H2 cells develop as an aberration of T_R cell development, possibly as a result of inadequate production of IL-10 or enhanced IL-4 and IL-

13 production resulting in the development of allergy and asthma. Further studies are needed to understand all of the signals that differentially affect T_H2 and T_R cell development and the relationship of tolerance to the pathogenesis of allergic disease and asthma.

NKT CELLS AND ASTHMA

Another cell type that can affect the differentiation of T_H2 cells is NKT cells. NKT cells rapidly produce large quantities of cytokines on activation after the recognition of self-glycolipid antigens. These self-glycolipid antigens in the respiratory tract can be exposed or expressed when exogenous allergens enter the lungs, which are then recognized by NKT cells. NKT cells are present in the lungs and produce high levels of IL-4 and IL-13, which appear to regulate the development of AHR.⁶⁸

Thus in the absence of NKT cells, for example, in NKT cell-deficient $CD1d^{-/-}$ mice, allergen-induced AHR fails to develop in response to allergens, such as OVA, BSA, or *Aspergillus* species. The failure of AHR to develop in $CD1d^{-/-}$ mice was not due to an intrinsic inability of $CD1d^{-/-}$ mice to have AHR, because administration of recombinant IL-13, which directly affects airway smooth muscle and mucus glands, induced severe AHR. In addition, another NKT cell-deficient mouse strain, $J\alpha 281^{-/-}$, which lacks the invariant TCR of NKT cells and therefore lacks NKT cells, also did not develop allergen-induced AHR and airway inflammation. The failure of AHR to develop was due specifically to the

TABLE I. Different types of regulatory T cells

Regulatory cell	Cytokines produced	Cell surface markers	Comments
T _H 1	IFN- γ , IL-2	CD4	T _H 1 cells can inhibit the development of T _H 2 cells but not the function of T _H 2 effector cells. T _H 1 cells are pro-inflammatory and cause end-organ tissue damage.
T _H 3	TGF- β	CD4	T _H 3 cells appear to develop during oral tolerance and production of TGF- β . T _H 3 cells have been shown to inhibit EAE.
T _R 1	IL-10	CD4	T _R 1 cells develop during culture with IL-10 and produce IL-10. T _R 1 cells have been shown to inhibit experimental colitis and T _H 2 responses.
T _R	IL-10	CD4	T _R cells develop during culture with DCs producing IL-10 and possibly during respiratory tolerance. T _R cells have been shown to inhibit the development of AHR.
CD25 ⁺	IL-10, TGF- β	CD4, CD25, GITR, <i>Foxp3</i>	CD25 ⁺ cells are naturally occurring regulatory cells that are involved in preventing autoimmune disease. CD25 ⁺ cells have also been shown to inhibit T _H 2 responses but not AHR.
NKT	IL-4, IL-13, IFN- γ , restricted TCR repertoire	CD4 or double negative (neither CD4 or CD8)	NKT cells are naturally occurring cells that rapidly produce large quantities of cytokines. NKT cells regulate the development of autoimmune disease and appear to exacerbate the development of asthma in mice.

EAE, Experimental autoimmune encephalomyelitis; GITR, glucocorticoid-induced TNF receptor.

absence of NKT cells, because reconstitution of J α 281^{-/-} mice by adoptive transfer of NKT cells isolated from wild-type BALB/c mice with CD1d tetramers loaded with α -galactosyl ceramide fully reconstituted the development of AHR. Production of IL-4 and IL-13 by the NKT cells was required for reconstitution to occur; this is because NKT cells from IL-4/IL-13 double-knockout mice were unable to reconstitute AHR, whereas NKT cells from IL-4 knockout or IL-13 knockout mice partially reconstituted AHR and NKT cells from IFN- γ knockout mice fully reconstituted AHR.⁶⁸

The precise mechanisms by which NKT cells induce AHR are not clear. Previous studies have indicated that T_H2 responses can occur in NKT cell-deficient mice.^{69,70} However, T_H2 responses alone are not sufficient for the development of AHR (ie, not all individuals with allergic rhinitis develop asthma), indicating that NKT cells must regulate additional elements in the lower respiratory tract that are required for the development of AHR. We suggest that NKT cells in the lung become activated by antigens encountered in the lung and provide a prerequisite for the development of AHR, perhaps by localizing the T_H2 response to the lungs or “licensing” T_H2 cells that enter the pulmonary compartment to become competent to induce AHR. Thus IL-4 and IL-13 produced by NKT cells potentiate the development and action of T_H2-biased T cells in the lungs, which drive the development of asthma.

WHICH REGULATORY T CELL CONTROLS ASTHMA AND ALLERGY?

Although it is likely that tolerance and regulatory T cells are involved in controlling the development of asthma and allergic disease, it is not yet clear which of the various regulatory T cells—T_H3, T_R1, T_R, CD25⁺, and

NKT cells—are most important in controlling the development of these disorders. It is possible that T_H3 cells are primarily involved in regulating gastrointestinal disease whereas T_R cells are more involved in respiratory disease.⁷¹ This is because DCs from the respiratory tract produce IL-10, which is required for T_R cell development, whereas DCs from the gastrointestinal tract produce both IL-10 and TGF- β , which might be required for the development of T_H3 cells.⁶⁰ T_R1 cells have been shown to inhibit T_H2 responses as well as T_H1 responses,⁷² and T_R cells have been shown to regulate the development of T_H2 responses and AHR.⁷³ CD25⁺ cells have been shown to inhibit the development of airway eosinophilia⁷⁴ but do not appear to inhibit the development of AHR.⁷⁵ NKT cells appear to be essential for the induction of AHR in mice, but their importance in human asthma has not yet been studied.

TOLERANCE, THE HYGIENE HYPOTHESES, AND *TIM*1

The role of regulatory T cells in asthma and allergic disease is also consistent with the hygiene hypothesis, in that the natural environment in the past might have maintained the respiratory and gastrointestinal mucosal systems in a state that favored the development of tolerance to antigens encountered at mucosal surfaces. Germ-free animals do not develop tolerance,^{76,77} indicating that normal microbial flora in the intestine and perhaps in the upper respiratory tract stimulate the immune system in ways that enhance tolerance development. Commensal bacteria, which might be reduced or altered in westernized cultures, might normally enhance the production of IL-10 and tolerance through mechanisms that involve the innate immune system and receptors such as nucleotide-binding oligomerization domain 2 (which is encoded by

CARD15). In addition, tolerance might be enhanced by other gastrointestinal microorganisms, such as hepatitis A virus (HAV), which binds to *TIM1*, a recently described asthma susceptibility gene.⁷⁸

Infection with HAV has been associated in several studies with protection against the development of asthma and atopy in human subjects in both European and US populations.^{79,80} Before 1970, the incidence of HAV infection was nearly 100% in western countries on the basis of serologic data⁸¹ and might have resulted in protection against the development of atopy. HAV might provide protection by binding to *TIM1*, which is the HAV receptor⁸² expressed on T cells,⁷⁸ directly altering T-cell differentiation and limiting the development of T_H2 cells and atopy. This mechanism is consistent with studies in mice demonstrating that T-cell immunoglobulin-domain and mucin-domain-containing molecules regulate the development of T_H1/T_H2 differentiation and allergen-induced AHR. Because the incidence of HAV infection has been greatly reduced in western societies with improved hygiene (HAV now infects only about 10% to 30% of all individuals on the basis of serologic data), we suggest that the HAV protective effect has been greatly reduced, resulting in a great increase in the prevalence of atopic diseases and thus providing a molecular mechanism for the hygiene hypothesis.⁸³ Further studies are required to establish this possibility and to precisely determine how HAV might enhance protective immunity against asthma and allergy.

SUMMARY

Our understanding of the mechanisms that protect against the development of allergic disease and asthma is relatively limited. T_H1 responses, which exhibit pro-inflammatory rather than anti-inflammatory activity, are unlikely to provide the primary mechanism of protection against allergy and asthma. Rather, regulatory T cells, in the form of T_H3 cells, T_R cells, or CD25⁺ T cells, are more likely to be involved in actively suppressing the development of allergy and asthma and providing anti-inflammatory responses and protective immunity. Therapies that enhance the development of regulatory T cells and inhibit the function of NKT cells in patients with allergic disease and asthma might be useful in the prevention and treatment of these disorders.

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