

T_{H1}/T_{H2} lymphocytes: Doubt some more

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The concept of T helper lymphocyte heterogeneity with T_{H1} and T_{H2} subclasses, which can be distinguished on the basis of their cytokine repertoire, has been a useful paradigm in studies on the immunology of allergic disorders.¹ T_{H1} cells are generally defined by their synthesis of IL-2, interferon (IFN)- γ , and tumor necrosis factor- β and are involved in cellular immunity; whereas T_{H2} cells are reported to produce IL-4, IL-5, IL-9, IL-10, and IL-13 and are involved in humoral immune responses and allergy. This paradigm has led to the concept that T helper lymphocyte responses to allergens are T_{H2} -like in allergic subjects and T_{H1} -like in nonallergic subjects, and as such, immunotherapy should work by converting T_{H2} into T_{H1} responses.

Although appealing in its simplicity, the true nature of cytokine responses to antigens is much more complex than can be explained by this T_{H1}/T_{H2} dichotomy. In most immune responses, T cells do not develop distinct T_{H1}/T_{H2} cytokine profiles, but rather many different cytokine combinations. Restricted cytokine profiles are primarily observed after the generation of antigen-specific clones but are implied by in vivo and ex vivo pathology. However, cloning is an artifactual process, and the result does not necessarily reflect the physiologic response to the antigen. The repeated pulse stimulation with antigen, the physical chemical nature and dose of the antigen, the choice of antigen-presenting cell, the requirement for cytokine growth factors to maintain T-cell viability, and many other variables will all influence T helper differentiation. There are additional caveats concerning this differentiation in vitro of T_{H1}/T_{H2} lymphocytes. No firm differentiation markers exist that distinguish a T_{H1} from a T_{H2} cell, and these subgroups may not represent distinct subpopulations. The situation in human beings is additionally confused by significant limitations concerning the clear distinction between the cytokine profiles of T_{H1} and T_{H2} cells. In human beings, both subsets may make IL-2, IL-10, and IL-13 in addition

Abbreviation used

IFN: Interferon

tion to their shared production of granulocyte-macrophage colony-stimulating factor and IL-3.

Despite these concerns, a role for T_{H2} -like cells in the pathophysiology of allergic disorders is convincingly demonstrated by numerous observations. The presence in vivo of malignant, functionally active T_{H2} lymphocyte clones in patients with Sézary syndrome or other lymphoproliferative disorders has been associated with elevated IgE and eosinophilia.^{2,3} Bronchial biopsy specimens from patients with allergic asthma⁴ and skin test challenge sites from atopic patients⁵ are characterized by T helper lymphocytes displaying a T_{H2} -like cytokine profile. However, although there may be a reduced presence of the T_{H1} cytokines, allergic disorders are also characterized by the presence of IFN- γ . IFN- γ can readily be identified both as transcripts and protein in bronchoalveolar lavage fluid and biopsy specimens⁴ (D. Broide, Personal communication), in nasal lavage fluid in which it may be one of the more prevalent cytokines,⁵ and in the supernatant of allergen-specific T helper lymphocyte cell lines.^{6,7} Through its ability to activate accessory cell function, stimulate IL-1, tumor necrosis factor- α , and IL-6 release, induce endothelial cell adhesion molecule expression, and support the recruitment and activation of inflammatory cells, it is likely that IFN- γ is a proinflammatory cytokine that exacerbates the severity of allergic inflammation.

The pattern of cytokine response to allergens observed in nonallergic individuals is even more complex. Normal individuals are exposed to the same concentrations of allergens as patients with allergy living in the same environment. Remaining healthy requires active systems, which prevent the development of inflammation. It is frequently stated that the immune response to allergens in nonallergic subjects is characterized by T_{H1} -like lymphocyte responses.⁸⁻¹⁰ Numerous published observations support this hypothesis, including the generation of allergen-specific T_{H1} -like clones from nonallergic subjects, which secrete IFN- γ but not IL-4 or IL-5. However, functional T_{H1} responses will also stimulate the recruitment and activation of mononuclear phagocytes. T_{H1} -like lymphocytes are readily identified in the cellular immune responses to purified protein derivative and other immune responses characterized by cellular

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immunity and granuloma formation. However, the normal state is *not* characterized by delayed-type hypersensitivity-like cellular inflammatory responses to allergens. Normal subjects remain healthy because they are able to inhale allergens without experiencing inflammation. Therefore the demonstration of T_{H1} responses either represents an *in vitro* artifact of the cloning process or, if present *in vivo*, must be present in a milieu that actively prevents cellular inflammation from developing. *In vitro* studies that remove T helper lymphocytes from their natural surroundings may artificially promote the differentiation of T_{H1} -like cells. For example, the use of activated antigen-presenting cells, biologically implausible concentrations of the allergens, or adjuvants such as contaminating endotoxin in the culture media or allergen extract may all permit the *in vitro* differentiation of T_{H1} cells.

The absence of inflammation in normal subjects is primarily maintained by influences that promote the development of tolerance. Immune responses to allergens may develop in nonatopic subjects, but despite identical antigen doses, these responses are generally of a lower order of magnitude than those observed in allergic subjects. Thus nonallergic subjects demonstrate decreased allergen-induced T-cell proliferation and lower allergen-specific IgG antibody responses in comparison with patients with allergy.¹¹ One influence contributing to immune nonresponsiveness is diminished accessory cell function. In contrast to the asthmatic lung, the resident antigen-presenting cell population of the healthy lung is unable to present allergen to T helper lymphocytes and cannot stimulate cellular activation and proliferation.¹² The cytokine milieu of the nonasthmatic respiratory tract may also help mitigate proinflammatory responses. The normal respiratory tract is characterized by elevated concentrations of IL-10,¹³ which may be derived from airway macrophages and respiratory epithelial cells.¹⁴ This antiinflammatory cytokine contributes to the downregulation of accessory cells and inhibits cytokine release.¹⁵ In the gastrointestinal tract the normal nonresponsive state is maintained by a different antiinflammatory cytokine, transforming growth factor- β , which may similarly prevent inflammation in the respiratory tract.¹⁶

In this issue of the *Journal of Allergy and Clinical Immunology*, Hamid et al.¹⁷ report on the presence and modulation of IL-12 in the immune response to allergens and modulation of IL-12 with immunotherapy. IL-12 is the cytokine primarily responsible for the differentiation of naive undifferentiated lymphocytes into T_{H1} -like cells.¹⁸⁻²¹ IL-12 also augments release of T_{H1} cytokines^{22, 23} and inhibits IL-4 synthesis by T_{H2} T cells.^{24, 25} Although IFN- γ is required as a secondary signal to induce the T_{H1} phenotype, induction of IL-12 early in the immune response is the primary signal for T_{H1} induction.

One can imagine an increasingly complex picture in which the immune response to allergens in nonallergic subjects is characterized by a state of immune nonre-

sponsiveness but also by the presence of IL-12-mediated induction of T_{H1} -like responses. Additional factors may include transforming growth factor- β and IL-10, which prevent the development of cellular inflammation. It is conceivable that a low-grade T_{H1} response to allergens with IFN- γ secretion is needed to help control development of the T_{H2} response. However, additional triggers that enhance IL-12 production will drive the immune response toward cellular inflammation such as that observed with *Mycobacterium tuberculosis*, sarcoidosis, and the interstitial lung diseases. In contrast, a milieu characterized by the presence of IL-4 and other factors directs immune responses into T_{H2} -driven allergic disorders.

How then does immunotherapy fit into the delicate balance between tolerance and T_{H1} or T_{H2} response? Immunotherapy reduces IL-4 production through the induction of tolerance by T_{H2} lymphocytes. Published data establish the ability of high-dose immunogenic peptides to render their specific T helper lymphocytes nonresponsive.²⁶⁻²⁸ These studies have been extended to allergen immunotherapy, which has been shown to make allergen-specific T cells unresponsive with decreased proliferation and IL-4 production after exposure to allergen.²⁹⁻³³ The article by Hamid et al.¹⁷ reports the enhanced production of IL-12 after 4 years of grass immunotherapy. Induction of IL-12 after a course of immunotherapy may have limited benefits through the capacity of IL-12 to decrease IL-4 production by T_{H2} lymphocytes and to reduce T_{H2} differentiation. The increased presence of IL-12 is consistent with published observations that T_{H1} -like cells are induced by immunotherapy.³³⁻³⁵ However, the belief that IL-12 and IFN- γ might be the basis for the beneficial effects of immunotherapy is tempered by three observations: (1) the asthmatic lung and allergic nose already contain IFN- γ , which clearly is not effectively lessening inflammation; (2) if it were useful for IFN- γ to be induced by immunotherapy, then therapeutic trials with IFN- γ would have shown clinical benefit, which they have not; and (3) IL-12 and IFN- γ do not behave like "magic bullets," which manage to inhibit IL-4 synthesis, IgE production, and T_{H2} activity without simultaneously activating accessory cell function, inducing adhesion molecule expression, and stimulating neutrophils and eosinophils. Immunotherapy may therefore restore the state observed in nonallergic subjects with mixed populations of tolerized nonresponsive T helper lymphocytes and activated T_{H1} lymphocytes. However, if true, this induction of IL-12/ T_{H1} responses—as opposed to the induction of tolerance—is not a desirable result and must be limited and controlled because excessive IL-12 production would be expected to induce cellular inflammation. It is therefore relevant that immunotherapy is also associated with upregulation of the antiinflammatory cytokines IL-10 and transforming growth factor- β .^{5, 36, 37}

Overall, immunotherapy may promote immune tolerance by subtly regulating the cytokine milieu, rather than by redirecting inflammation from a T_{H2} to a T_{H1} pattern

of response. The T_{H1}/T_{H2} theory of tolerance in immunotherapy implies that there are two waves of infiltrating cells that respond to either the allergen or the immunotherapy (i.e., an initial infiltrate of T_{H2} cells responding to the allergen and a subsequent wave of T_{H1} cells migrating in to reverse the response in a secondary wave of treatment). The data for this do not exist in spite of in situ hybridization studies, such as that by Hamid et al.¹⁷ What these findings seen on immunohistochemistry and in situ hybridization do suggest is a changing cytokine milieu that may permit outgrowth of a T_{H1} - or T_{H2} -like pattern of cells. Furthermore, it is entirely possible that cells other than T cells may be affected by or may contribute to this change in cytokine gene expression initiated by immunotherapy. Hence, tolerance may be generated in cells other than T cells, and the changed cytokine milieu may affect signaling and selection of cytokine gene usage in mast cells, basophils, or eosinophils as well.

A final question to ask is whether IL-12 therapy itself should be recommended for patients with allergy. IL-12-mediated downregulation of T_{H2} responses and the induction of T_{H1} -like cells may be beneficial. It is equally plausible, however, that this intensely proinflammatory cytokine may worsen the inflammation of the respiratory tract. By analogy, trials of IFN- γ in asthma proved to be disappointing, and IL-12 may only recapitulate these results. The more we learn about cytokines, the more complex their spectrum of action becomes. Each cytokine mediates a range of biologic activities, which may be either proinflammatory or antiinflammatory, depending on the concentration, responding cell type, and timing within the immune response. Attempts to reduce each cytokine to a limited function, such as has been attempted with the T_{H1}/T_{H2} paradigm, do not reflect the pleiotropic nature of these proteins and although appealing in their simplicity, ignore the true complexities of immune regulation.

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