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Updates on cells and cytokines

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T_{H1} and T_{H2} CD4⁺ cells in human allergic diseases

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CD4⁺ T cells are heterogeneous and can be subdivided into at least two subtypes: T_{H1} cells, which are important in activating macrophages and in inducing cell-mediated immunity, and T_{H2} cells, which are important in humoral immunity and allergic disease. T_{H1} and T_{H2} cells derive from common precursor cells, which secrete only IL-2, through a series of differentiation steps driven by exposure to antigen. CD4⁺ T cells differentiate first into T_{H0} cells, producing an unrestricted cytokine profile; and if antigenic stimulation continues in a chronic and intense manner, the CD4⁺ T cells then differentiate into polarized T_{H1} or T_{H2} cells. T_{H1} and T_{H2} cells with highly restricted profiles are observed mainly in situations of intense or prolonged stimulation, and other types of effector T cells, producing a variety of other combinations of cytokines, such as IL-4 with interferon- γ (IFN- γ), can be observed in other situations. Therefore T_{H1} and T_{H2} cells, distinguished by their cytokine profiles and by their capacities to respond to IL-12 and IFN- γ , represent ends of a spectrum of cytokine profiles (Fig. 1). Heterogeneity among CD4⁺ T

Abbreviations used

APC: Antigen-presenting cell
IFN- γ : Interferon- γ
TGF- β : Transforming growth factor- β

cells has become widely accepted because it explains *immune deviation*, that is, the inverse relationship between humoral and cell-mediated immunity, which has been observed clinically and experimentally for decades. CD4⁺ T-cell heterogeneity also explains the development of allergic disease as the result of an overproduction of T_{H2} cytokines (IL-3, IL-4, IL-5, IL-10, and IL-13) by allergen-specific CD4⁺ T_{H2} cells. T_{H2} cells represent an inappropriate or detrimental adaptive response to environmental antigens and amplify and prolong allergic inflammation and late-phase reactions, which are associated with airway hyperreactivity, the hallmark of asthma and allergic rhinitis.

The specific cytokine profile that develops in CD4⁺ T cells in both allergic and nonallergic diseases is influenced by the antigen type, dose, antigen-presenting cell (APC) type, cytokine microenvironment, costimulatory signals received by the T cell, and host or genetic factors. Thus the cytokine profiles and the function of CD4⁺ T cells are not predetermined but depend on how the cells are stimulated with antigen. T_{H1} cytokines inhibit the production of T_{H2} cytokines, whereas T_{H2} cytokines inhibit the production of T_{H1} cytokines, thus accentuating the production of polarized cytokine profiles during immune responses. Once CD4⁺ T cells fully differentiate into *effector* T_{H1} and T_{H2} cells (in sites such as the lungs

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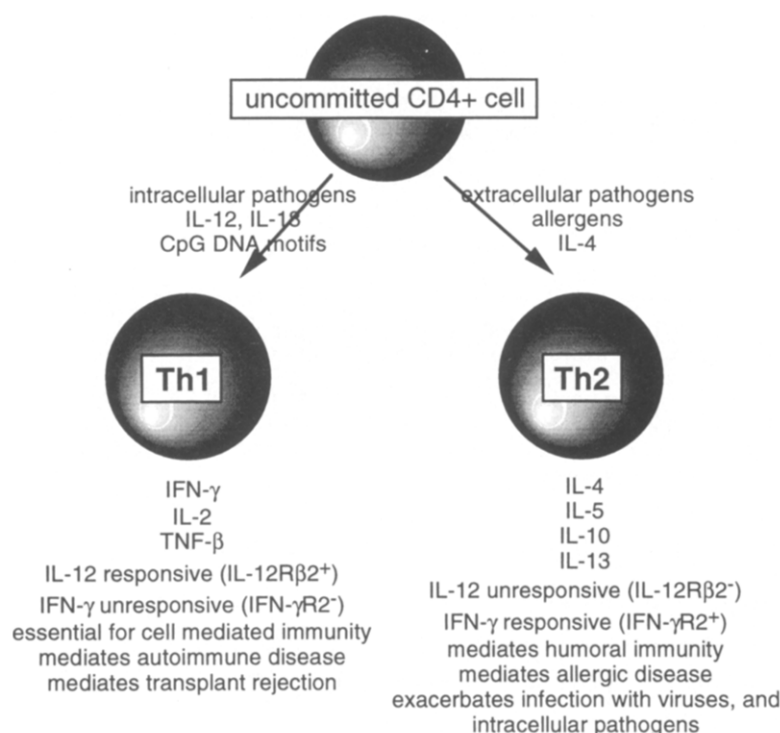


FIG. 1. T_{H1} and T_{H2} effector cells can be distinguished by their cytokine profiles and by their capacities to respond to IL-12 and IFN- γ .

or ophthalmic mucosa), the cytokine profiles become relatively fixed.

Like effector T_{H1} or T_{H2} cells, memory $CD4^+$ T-cell populations generated *in vivo* under polarizing conditions tend, on restimulation, to produce the same cytokine profile established previously.¹ However, the cytokine profiles of *resting* memory T-cell populations can be altered. Thus allergen-specific T_{H2} memory cells will produce T_{H1} cytokines when activated in the presence of IL-12, a potent IFN- γ -inducing protein.² IL-12, however, when given in large doses *in vivo*, paradoxically enhances IL-4 synthesis, possibly by generating a rebound increase in IL-10 production. The reversibility of cytokine production in memory $CD4^+$ T cells, however, is lost after repeated stimulation, as the more malleable resting memory T cells differentiate into end-stage effector cells¹ in which T_{H2} effector cells lose IL-12 receptor function (Fig. 1).³ Nevertheless, the idea that the cytokine profiles of resting memory T-cell populations are relatively flexible and can be "reprogrammed" is an important concept, which has significant implications for therapy of allergic and autoimmune diseases, as will be discussed later.

T_{H1} AND T_{H2} COMPLEXITY

Although the Th1 and Th2 paradigm is extremely appealing in its simplicity, a number of observations suggest that immune regulation is far more complex than initially suggested by the T_{H1}/T_{H2} model. For example, T_{H2} cells, identified mainly by the capacity to

produce IL-4 but not IFN- γ , may be further subdivided. The amount of cytokines produced by T_{H2} cells (e.g., IL-5, IL-10, or transforming growth factor- β [TGF- β]) may vary considerably and change the specific functions of T_{H2} cells. This heterogeneity in cytokine production among T_{H2} cells may explain preferential isotype production during humoral immune responses (e.g., enhanced IgG₄ or IgE during some responses and enhanced IgA or IgG₂ in other responses). In addition, there is growing evidence that at least some T_{H2} cells can function as regulatory cells to suppress immunity (e.g., to suppress autoimmune inflammatory responses such as encephalitis or diabetes mellitus) (Fig. 2). Because some T_{H2} cytokines, such as TGF- β , have potent inhibitory properties, it is possible that "regulatory/suppressor" T_{H2} cells differ from conventional "helper" T_{H2} cells that enhance humoral immunity in that "regulatory/suppressor" T_{H2} cells produce much greater levels of TGF- β , which greatly inhibits both cell-mediated and humoral immunity. Furthermore, it is possible that anergized/unresponsive T cells, which may be capable of secreting IL-4 but not IL-2, may also be variants of T_{H2} cells. Allergic rhinitis and asthma then may represent a pathologic aberration of mucosal tolerance in which T cells that should normally develop into "regulatory/suppressor" T_{H2} cells (or into T_{H1} cells) instead develop into "helper" T_{H2} cells that initiate and intensify humoral immunity and allergic inflammation.

Studies of mucosal immunity and oral tolerance indicate that antigen-specific regulatory $CD4^+$ T_H cells,

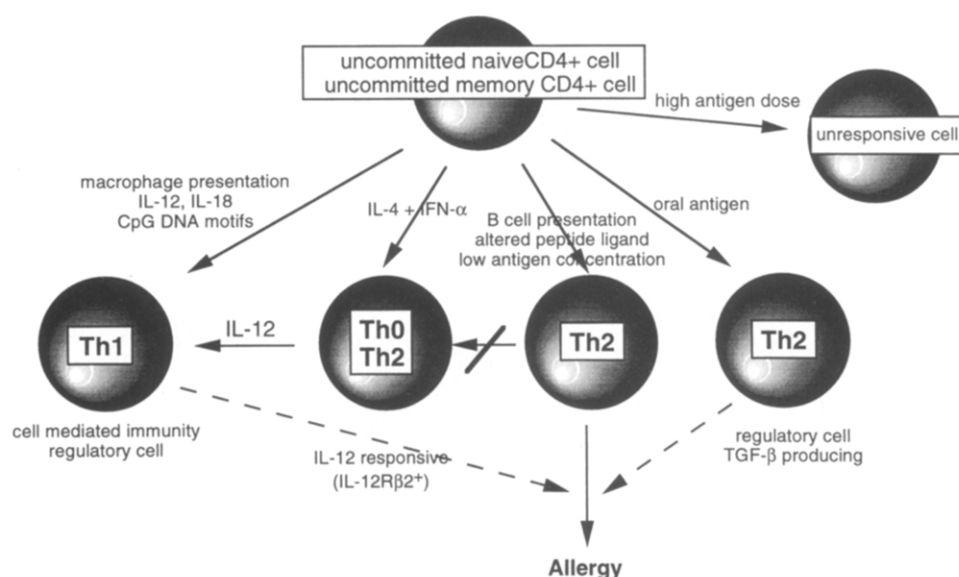


FIG. 2. Alternative pathways for uncommitted CD4⁺ T cells. These pathways include several different types of IL-4-producing (T_{H2} and T_{H0} cells). Dashed lines indicate negative/inhibitory effects.

producing IL-4 and TGF- β , are induced by the oral administration of low doses of antigen, whereas oral administration of high doses of antigen induces antigen-specific unresponsiveness, tolerance, or deletion. "Regulatory" T_{H2} cells (a.k.a. Th3 cells) must be distinguished from "helper" T_{H2} cells, because therapeutic measures to induce regulatory responses (or tolerance) may generate "helper" T_{H2} cells instead. In one nonhuman primate autoimmunity model of multiple sclerosis, antigen immunotherapy induced "helper" rather than "regulatory" CD4⁺ T cells, resulting in late severe neurologic exacerbation caused by autoantibody production.⁴ Similarly, oral administration of autoantigen induced autoantigen-specific cytotoxic CD8⁺ T cells that resulted in autoimmune diabetes mellitus.⁵ These observations together clearly demonstrate that the T_{H1}/T_{H2} paradigm is exceedingly complex and that different forms of T_{H2} (and T_{H1}) cells may exist.

ALTERING THE CYTOKINE PROFILES OF CD4⁺ T-CELL SUBSETS

On the basis of our current understanding that overproduction of T_{H2} cytokines in patients with allergy causes allergic disease, conversion of allergen-specific T_{H2} into T_{H1} cells is thought to be desirable in such patients (Fig. 3). T_{H1} cells are assumed to protect against the development of allergic symptoms, particularly because IFN- γ has inhibitory effects on IgE synthesis and because allergen-specific T_{H1} cells can be found in nonallergic individuals. However, inasmuch as vigorous T_{H1} responses (e.g., cell-mediated or delayed-type hypersensitivity responses) are associated with significant tissue destruction, overzealous induction of T_{H1}-dominated responses at mucosal sites in patients with allergy (i.e., conversion of T_{H2} to T_{H1} cells in the respiratory or

gastrointestinal tract) could result in significant tissue injury. Nonetheless, experimental therapy for allergic disease is currently focused on manipulating the cytokines produced by T_H cells.

Conventional allergen immunotherapy effectively ameliorates allergic rhinitis by increasing the development of allergen-specific T_{H1} cells and decreasing the development of T_{H2} cells,⁶ by utilizing relatively high antigen doses, which favors the involvement of macrophages/monocytes rather than B cells as APCs. Antigen presentation by B cells favors the induction of IL-4 rather than IFN- γ in responding T cells, possibly by enhancing costimulation of T cells through the CD40 ligand.⁷ The outcome of immunotherapy is reversal of the dominant allergen-specific T_H phenotype in clinically improved patients, as a result of the conversion of individual T_{H2} cells into T_{H1} cells or the selective expansion of antigen-specific T_{H1} cells.

Conventional allergen immunotherapy may be improved by use of complementary DNA for the allergen proteins. Injected allergen cDNA is taken up by somatic cells and slowly translated into protein, resulting in a reduced likelihood of anaphylaxis and in a T_{H1} response in naïve mice. The effectiveness of cDNA in inducing T_{H1} responses is dependent on the presence of unmethylated cytosine/guanosine DNA motifs in associated DNA, which act as adjuvants by inducing IL-12 and IL-18, greatly enhancing IFN- γ production and cellular immunity. Injection of allergen cDNA during ongoing protein-induced T_{H2} responses can convert T_{H2} responses into T_{H1} responses,^{8,9} indicating that cDNA immunization may be an effective method for the treatment of patients with allergies or asthma.

Another method for treating excess production of T_{H2} cytokines involves tolerizing, inducing unresponsiveness,

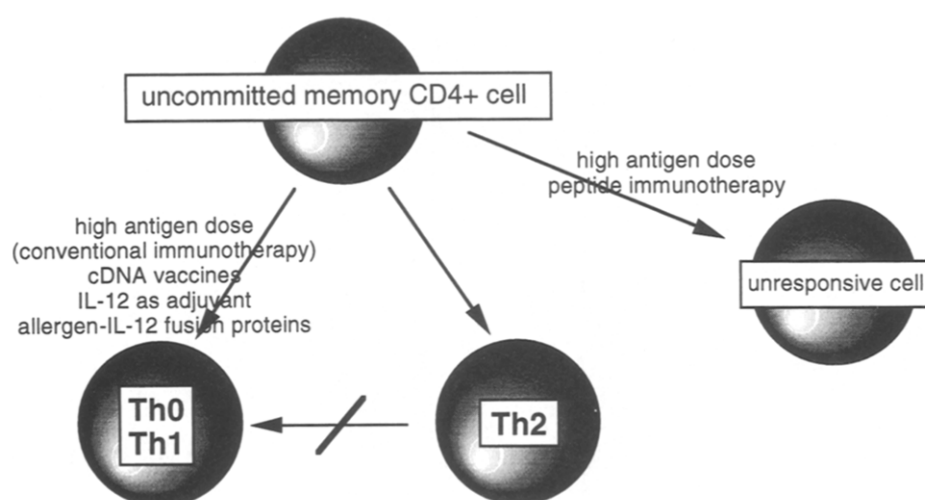


FIG. 3. Therapies for T_{H2} -mediated disease. Conventional immunotherapy (high antigen concentration), tolerization with high antigen dose or with peptide immunotherapy, cDNA allergen vaccines, IL-12 as adjuvant, or allergen-IL-12 fusion protein immunotherapy.

or inducing apoptosis in antigen-specific T cells. Tolerization can be achieved by oral administration of high doses of soluble antigen without adjuvant or administration of high doses of *peptide fragments* of allergens. Both strategies are in various stages of clinical testing for the treatment of autoimmune inflammatory diseases (rheumatoid arthritis with collagen, multiple sclerosis with myelin basic protein, diabetes mellitus with insulin) and treatment of allergy (allergen given orally or allergen peptides given subcutaneously). In allergic disease, immunodominant peptides for dust mite antigen (Der p 1), cat allergen (Fel d 1), and other allergens have been identified; and studies in mice have demonstrated that T-cell tolerance can be induced in both naïve and primed $CD4^+$ T cells in vivo with such immunodominant peptides. Human studies with allergen peptides have demonstrated effectiveness in reducing allergy symptoms in patients, although the widespread application of this mode of therapy is dependent on identification of immunodominant peptides that are applicable to the major histocompatibility complex of all treated individuals.

An additional method for treating allergic disease relies on IL-12 or IL-18 as adjuvant, because both IL-12 and IL-18 are potent inducers of IFN- γ .^{10,11} For example, IL-12 can function as an effective treatment for cutaneous leishmaniasis by inducing a protective T_{H1} immune response, if given early during infection or when used as an adjuvant in a vaccine against *Leishmania major*, by directing the development of leishmania-specific $CD4^+$ T_{H1} cells or inducing a switch from a T_{H2} to a T_{H1} response. In a mouse model of asthma, administration of IL-12 inhibited antigen-induced airway hyperresponsiveness, decreased IL-4 and IL-5, and increased IFN- γ expression in the lungs.¹² Although in this model an ongoing T_{H2} -dominated pulmonary response was not present before administration of IL-12, these

studies indicate that local administration of IL-12 may provide effective immunotherapy for the treatment of pulmonary allergic disorders such as atopic asthma.

A fourth strategy with which to tilt the T-cell response toward a T_{H1} phenotype relies on IL-12 covalently linked by genetic means to antigen, which functions effectively to reverse a T_{H2} -dominated immune response in vivo in an antigen-specific fashion.¹³ Like conventional immunotherapy with allergen, antigen-IL-12 fusion proteins can inhibit IL-4 and enhance IFN- γ synthesis in memory $CD4^+$ T cells in an antigen-specific fashion. A most surprising characteristic of IL-12, when covalently linked to antigen, was that the effects of IL-12 appeared to be confined to antigen-specific cells, whereas free recombinant IL-12 induced a great increase in antigen-nonspecific IFN- γ production. In addition, the ovalbumin-IL-12 fusion protein was much more effective than free recombinant IL-12 in reducing antigen-specific IL-4 synthesis and reducing antigen-specific IgE synthesis.

GENETICS OF CYTOKINE REGULATION AS IT RELATES TO ATOPIC DISEASE

It is likely that allergic individuals are predisposed to the production of T_{H2} cytokines not only against allergens but also in response to many encountered antigens (e.g., infectious agents or self antigens). Patients with allergy should therefore be susceptible to infection with intracellular organisms, because antigen-specific cells producing T_{H2} instead of T_{H1} cytokines would protect against autoimmune disease but would impede protective immunity against intracellular pathogens. Such an inverse relationship between atopic disorders and the development of delayed-type hypersensitivity against *Mycobacterium tuberculosis* was in fact observed in a large population of children.¹⁴ The inverse relationship, however, may be the result of rather than the cause of *M. tuberculosis* infection. Conversely, a predisposition toward a specific cytokine profile

in response to most antigens predicts that patients with autoimmune inflammatory diseases should be protected from the development of allergy, because allergen-specific T cells producing T_{H1} cytokines in these individuals would protect against allergy. Indeed, patients with multiple sclerosis, a disease caused by excess production of T_{H1} cytokines in myelin-reactive T cells, are unlikely to have allergic disease, as defined by allergy symptom scores and positive allergen-specific IgE test results, when compared with control subjects with noninflammatory neuroconvulsive disorders.¹⁵

Specific genes that regulate the allergic diathesis are being pursued by many laboratories. Although examined for many years, genes of the major histocompatibility complex have not been found to be linked to allergy. The major histocompatibility complex might control immune responsiveness to single antigens (e.g., insulin or penicillin); however, each individual with allergy becomes allergic to a large number of different mucosally encountered allergens (inhaled environmental allergens, foods, or latex), suggesting that genes that broadly regulate the form of immunity are defective in atopic individuals. The gene for the β subunit of the high-affinity IgE receptor (Fc ϵ R1 β) on chromosome 11 has been linked to allergic disease,¹⁶ but this finding has been controversial. More recently, Marsh et al.¹⁷ have found that *total* serum IgE concentration but not *specific* IgE antibody concentration is linked to microsatellite markers on chromosome 5q31.1, a region that includes genes for IL-4, IL-5, and IL-13. This region has also been linked to bronchial hyperreactivity, which is consistent with previous studies associating elevated IgE with the risk for developing asthma.¹⁷

Genetic predisposition toward production of a predominant Th2 cytokine profile in response to antigen/allergen may be explained by immunologic defects at several different levels. For example, defects intrinsic to T cells may affect cytokine production, when T cells in patients with allergy preferentially develop the T_{H2} phenotype, possibly because of a more rapid loss by T_{H2} cells of the second chain of the IL-12 receptor (IL-12R β 2) in T_{H2} -predisposed individuals than in T_{H1} -predisposed individuals. Alternatively, genetic predisposition toward production of a predominant T_{H2} cytokine profile may be caused by defects intrinsic to APCs, for example, by the increased production of IL-10 or a reduced production of IL-12 in APCs. Because IL-10, by inhibiting production of IL-12, inhibits the synthesis of IFN- γ and enhances IL-4 synthesis, overproduction of IL-10 by APCs from atopic individuals might divert the immune response toward T_{H2} rather than T_{H1} responses. Limitation in the production of IL-12 has not yet been demonstrated in human beings, although in a mouse model we have demonstrated that APCs from BALB/c mice (T_{H2} -predisposed) produce substantially less IL-12 than those from C57BL/6 and DBA/2 mice (T_{H1} -predisposed).¹⁸

In summary, the T_{H1}/T_{H2} paradigm has helped to greatly improve our understanding of allergic disease

and asthma. The model is not yet fully developed, and many of the nuances regarding Th subsets remain to be determined. Future studies are needed to: (1) determine the precise immunologic mechanisms that critically regulate cytokine synthesis in CD4⁺ T cells, (2) identify genes that predispose atopic individuals toward the overproduction of T_{H2} cells and those that prevent nonallergic individuals from producing T_{H2} cells, and (3) determine the precise role of CD4⁺ T_{H1} cells and CD4⁺ T_{H2} or CD8⁺ regulatory/suppressor cells in the prevention and containment of human allergic disease. From these studies, novel strategies to control allergic disease and asthma will be developed to revolutionize therapy for these common disorders.

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