MINIREVIEW

Th1 and Th2 CD4+ Cells in the Pathogenesis of Allergic Diseases (44109)

DALE T. UMETSU[1] AND ROSEMARIE H. DEKRUYFF
Division of Immunology and Trransplantation Biology, Department of Pediatrics, Stanford University, Stanford, California 94305-5119

Abstract. **Our understanding of the molecular and genetic etiologies of allergic disorders, which affect 20%–30% of the general population, has greatly improved over the past several years. Previously, research focused on examination of immediate hypersensitivity reactions, initiated by the cross-linking of IgE molecules on the surface of mast cells/basophils, resulting in the release of a host of mediators, which cause symptoms typified by acute anaphylaxis. Although there has been substantial progress in understanding the molecular biology of mast cell and basophil activation and of the regulation of IgE synthesis, recent studies have shifted attention to the cellular and molecular mechanisms that cause a broader allergic inflammatory response and underlie the more chronic and severe symptoms of allergy and asthma. In this report, we will review a substantial body of recent experimental work that has provided the basis for our new understanding of the allergic inflammatory response and the pathogenesis of allergic diseases. We will describe the recent progress in defining the immunological basis for allergic disease, and how subsets of helper CD4+ T cells secreting a specific array of cytokines (Th2 cytokines) regulate/cause allergic inflammation. We will review the cell biology of Th2 cells, the role of Th2 cells in allergic disease, and biological, genetic, and therapeutic mechanisms that influence the differentiation of CD4+ T cells and enhance or suppress cytokine synthesis in Th2 cells. These mechanisms control the expression of allergic diseases, which occur in some but not all individuals following environmental exposure to allergens.** [P.S.E.B.M. 1997, Vol 215]

The Allergic Inflammatory Response

The allergic inflammatory response that occurs in individuals with asthma, allergic rhinitis, or atopic dermatitis is characterized by the presence of mast cells, as well as other cell types, such as basophils, eosinophils, monocytes, T cells, and B cells (1). Allergic reactions often begin with an immediate-phase response (due to mast cell degranulation) typically lasting 20–30 min. Although immediate phase responses can be clinically dramatic, as seen in anaphylaxis, an immediate phase reaction by itself represents only the beginning of the complex inflammatory reaction that results in chronic symptoms of asthma or allergic rhinitis. On antigen challenge, the immediate phase response is followed 4–6 hours later by a late-phase reaction, with more profound tissue swelling, accompanied by an influx of inflammatory cells causing symptoms such as bronchospasm and nasal congestion. This late-phase reaction is much more severe clinically in terms of bronchospasm and tissue swelling, persists for much longer time periods than the immediate-phase reactions, and is associated with bronchial hyperreactivity, the hallmark of asthma, and

This work was supported by Grants RO1AI26322, RO1AI24571, RO1AI37219, and KO7AI01026 from the U.S. Public Health Services, National Institutes of Health.
[1] To whom request for reprints should be addressed at Division of Immunology and Transplantation Biology, Department of Pediatrics, Stanford University, Stanford, CA 94305-5119.

[P.S.E.B.M. 1997, Vol 215]

0037-9727/97/2151-0011$10.50/0

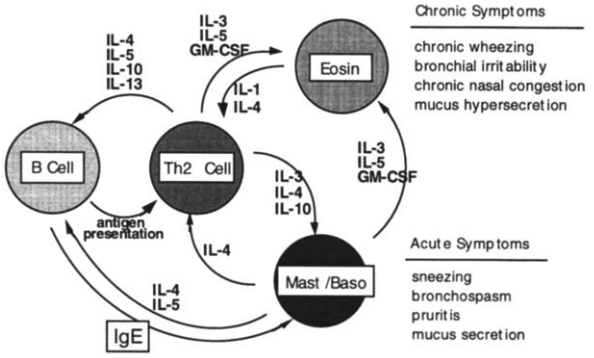

Figure 1. CD4+ Th2 lymphocytes play a central role in regulating the allergic inflammatory response by producing cytokines that affect B cells, eosinophils, mast cells, and basophils. These cells, all of which produce cytokines, interact with each other in a network that amplifies and prolongs allergic inflammation. Although mast cells/basophils are involved in the acute symptoms of allergy, other cells, particularly eosinophils, induce the more chronic symptoms of allergic disease.

nasal hyperreactivity, a prominent component of nasal symptomatology. Mast cell degranulation during the immediate-phase response initiates an increase in vascular permeability but, more importantly, initiates the expression by vascular endothelial cells of specific adhesion molecules such as VCAM-1 or secretion of chemokines such as RANTES and ectoxin (2, 3), resulting in the selective recruitment of eosinophils, monocytes, and T and B lymphocytes (4), which amplify and prolong the allergic inflammatory response.

Role of CD4+ T Cells in Allergic Disease

The CD4+ T cell is now believed to play a critical role in the allergic inflammatory response by enhancing the recruitment, growth, and differentiation of all other cell types involved in the response (5) (Fig. 1). It performs this function by secreting several cytokines, including interleukin-4 (IL-4) and IL-13, which enhance the induction of IgE synthesis in B cells, mast cell growth, and the recruitment of lymphocytes, mast cells, and basophils to sites of inflammation. In addition, CD4+ T cells produce IL-5, which enhances the growth and differentiation of eosinophils and B cells, and IL-10, which enhances the growth and differentiation of mast cells and inhibits the production of γ-interferon (IFN-γ). The combination of IL-4, IL-5, IL-10, and IL-13 is produced by a subset of CD4+ T cells called Th2 cells, which are found in increased abundance in allergic individuals.

Th1 and Th2 Helper Cell Subsets

CD4+ T cells can be subdivided into subsets based upon their cytokine profiles and function. The concept that CD4+ T cells can be divided into subsets developed through the analysis of T-cell clones, which were derived after repeated *in vitro* stimulation of CD4+ T cells (6, 7). This model has been widely accepted as an important immunological mechanism that regulates immune responses. The initial analysis in mice provided evidence for the existence of two very distinct CD4+ T-cell types, Th1 cells, which secreted cytokines important in the activation of macrophages (IFN-γ, IL-2, tumor necrosis factor–β [TNF-β]) and in inducing cell mediated immunity, and Th2 cells, which secreted cytokines important in humoral immunity and allergic disease (IL-4, IL-5, and IL-10) (8). The concept of heterogeneity among CD4+ helper T cells was very useful since it could explain the apparent inverse relationship between humoral and cell-mediated immunity which was observed clinically and experimentally for decades (9). Th1 cytokines inhibit the production of Th2 cytokines, while Th2 cytokines inhibit the production of Th1 cytokines, thus accentuating the production of polarized cytokine profiles during immune responses. A balance, however, in the production of these opposing cytokine sets is critical, since overproduction of Th1 cytokines is thought to result also in autoimmune inflammatory diseases, such as multiple sclerosis and diabetes mellitus, whereas overproduction of Th2 cytokines results in allergic inflammatory disease, such as asthma and allergic rhinitis, or ineffective immunity to intracellular pathogens (Table I). In this model, dissemination of infection is not due to a lack of immunity but rather due to the development of T cells secreting an inappropriate cytokine profile. With regard to allergy, disease is caused by CD4+ T cells inappropriately secreting Th2 cytokines, whereas nonallergic individuals remain asymptomatic because they develop T cells secreting Th1 cytokines, which in-

Table I. Diseases Caused by T Cells Producing Inappropriate Profiles of Cytokines

Diseases caused by the overproduction of Th2 cytokines
Infectious diseases—exacerbation of infection with
Leishmania major
Mycobacterium leprae
Candida albicans
Toxoplasma gondi
Respiratory syncytial virus
Human immunodeficiency virus
Allergic disorders
Asthma
Allergic rhinitis
Atopic dermatitis
Vernal conjunctivitis
Diseases caused by the overproduction of Th1 cytokines
Autommune inflammatory disease
Experimental allergic encephalomyelitis, multiple sclerosis
Insulin-dependent diabetes mellitus
Experimental autoimmune uveoretinitis
Crohn's disease (inflammatory bowel disease)
Autoimmune thyroid disease
Allograft rejection

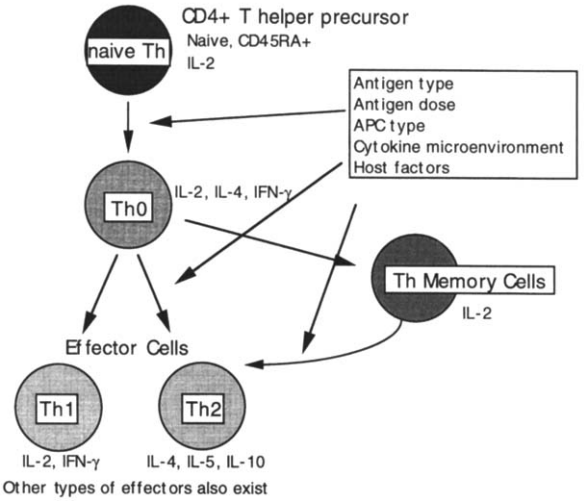

Figure 2. T helper cell subsets arise from a single naïve precursor cell type through a series of differentiation steps driven by exposure to antigen. These differentiation steps can be affected by several parameters, including antigen type, antigen concentration, antigen presenting cell (APC) type, and the cytokine microenvironment, as well as by host factors. Although only Th1 and Th2 effector cells are shown, other effector cell types exist producing additional cytokine profiles.

hibit IgE synthesis and mast cell and eosinophil differentiation.

The Th1/Th2 Paradigm Is Complex

Although the Th1 and Th2 paradigm is extremely appealing in its simplicity, there are a number of observations that suggest that immune regulation is far more complex than the Th1/Th2 model initially suggested. Th1 and Th2 cells derive from a common precursor cell, which secretes only IL-2, and differentiate through a series of steps driven by exposure to antigen (Fig. 2). This differentiation is influenced by a number of factors, including the antigen type, antigen dose, antigen presenting cell type, the cytokine microenvironment, as well as by host factors. It appears that Th1 and Th2 cells develop primarily under conditions of chronic and intense antigen exposure, and represent end-stage effector cells. Analysis of a larger number of murine and human T cell clones indicate that in addition to Th1 and Th2 cells, T cells with a variety of other combinations of cytokines exist, including Th0 cells, which secrete both Th1 and Th2 cytokines (7, 10).

The Th1/Th2 paradigm is also complicated by additional observations that indicate that production of Th2 cytokines is quickly downregulated *in vivo*. For example, Th2 cells actively producing IL-4, IL-5, or IL-10 are not easily found in allergic patients, unless the T cells are obtained from sites of active immune responses (e.g., the lung or ophthalmic mucosa) (11). T cells in the peripheral blood are in a relatively resting state and fail to produce Th2 cytokines immediately on stimulation (12). However, the capacity of memory cells to produce Th2 profiles persists *in vivo*, but Th2 profiles are observed only after induction of "differentiation" of the resting populations by reexposure to antigen, and not immediately on activation (13–15). Therefore, Th2 cells producing large quantities cytokines (i.e., effector T cells) are found only during ongoing immune responses at sites of antigen exposure, and are distinct from resting memory $CD4^+$ T cells, which develop when active antigenic stimulation has subsided. This is an important distinction, as discussed below, when examining whether such cells are irreversibly polarized or are still able to be "reprogrammed" by, for example, immunotherapies.

Different Types of Th2 Cells

Since Th1 and Th2 cells have no surface markers that define them (except possibly for CD30 [16]), Th2 cells have been generally identified simply by the capacity to produce IL-4 and not IFN-γ. Other cytokines may be produced by the Th2 cells, and the relative amounts of each of these cytokines (e.g., IL-5, IL-10, or transforming growth factor–β [TGFβ]) may change the specific functions of Th2 cells. This further heterogeneity among Th2 cells may explain variations in humoral immune responses (e.g., enhanced IgG1 versus IgG2, versus IgE or IgA responses). In addition, there is growing evidence that at least some Th2 cells can function as regulatory cells to suppress immune responses or suppress autoimmune diseases such as autoimmune encephalitis and diabetes mellitus (17, 18). Since the Th2 cytokines IL-4 and IL-10 have inhibitory properties, it is possible that regulatory/suppressor Th2 cells (which are difficult to cultivate *in vitro*) (19) and conventional Th2 cells are similar. Furthermore, it is possible that anergized/unresponsive T cells, which are capable of secreting IL-4 but not IL-2, are also similar to Th2 cells (20, 21). Whether heterogeneity exists among T cells that secrete IL-4 is an important issue to clarify, particularly with regard to allergic disease, since exposure to antigen at mucosal surfaces (e.g., in the gastrointestinal or respiratory tracts) is associated with tolerance (with high antigen doses), or with the development of regulatory cells producing cytokines such as IL-4 and TGFβ (with moderate antigen doses) (22). Allergic rhinitis and asthma, then, may represent a pathological aberration of oral/mucosal tolerance, where T cells that normally develop into "Th2" regulatory/suppressor cells instead develop into "Th2" cells that initiate and intensify allergic inflammation.

Does the Predisposition to Produce Polarized Cytokine Profiles in a Given Individual Apply to All Antigens?

Although it is generally agreed that allergy is caused by the development of allergen-specific Th2 cells in al-

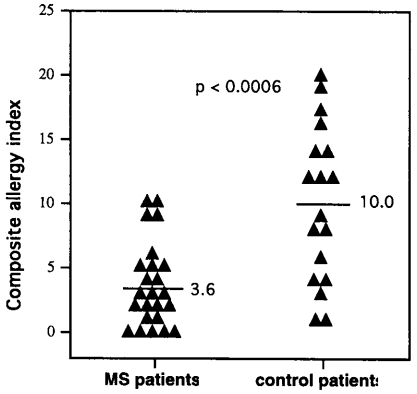

Figure 3. Composite allergy index is lower in patients with multiple sclerosis compared with control neurology patients. Composite allergy index incorporated symptom scores and serologic studies for allergen-specific IgE. Each triangle represents the index for a single patient. The mean index in the multiple sclerosis (MS) patient group was 3.6 compared with 10.0 for control subjects ($P < 0.0006$, Mann-Whitney test). (Reproduced by permission from Ref. 24.)

lergic individuals, it is not clear if allergic individuals are predisposed to producing Th2 responses to all encountered antigens (e.g., infectious agents or self antigens). If this is true, then allergic individuals should be protected from the development of autoimmune inflammatory diseases, since autoantigen-specific cells producing Th2 instead of Th1 cytokines would protect against autoimmune disease. Conversely, patients with autoimmune inflammatory diseases should be protected from the development of allergy, since allergen-specific T cells producing Th1 cytokines in these individuals would protect against allergy. In mice, this seems to be the case, in that mice predisposed towards the production of IL-4 are less capable (but not incapable) of developing delayed type hypersensitivity responses and cell-mediated immunity, and seem to be protected to some extent from developing autoimmune inflammatory disease (23). Is this true in humans? Recently, we demonstrated support for this theory by finding that patients with multiple sclerosis, a disease caused by excess production of Th1 cytokines in myelin-reactive T cells, were much less likely to develop allergic disease, as defined by allergy symptom scores and positive allergen-specific IgE tests, when compared with control subjects with noninflammatory neuro-convulsive disorders (Fig. 3) (24). These results support the hypothesis that, in humans, genetic factors that promote susceptibility to the Th1-mediated inflammatory disease, multiple sclerosis, protect against the development of Th2-mediated diseases. Whether this is true for other inflammatory diseases, such as rheumatoid arthritis or diabetes mellitus, is not yet clear. Nevertheless, these results support the concept that in humans, regulation of cytokine synthesis is a significant factor in the development of particular diseases, and indicate that genetic predisposition towards the production of a particular polarized cytokine profile in a given individual may be a global pattern, which predisposes that individual towards the development of the same polarized cytokine profile in response to a broad range of unrelated antigens, including autoantigens and allergens.

What Is the Underlying Immunological Defect in Allergic Individuals?

If T cells in allergic individuals produce greater quantities of Th2 cytokines than T cells from nonallergic individuals, what is the underlying immunological defect in allergic individuals that results in increased Th2 cytokine synthesis? One approach to answering this question is to find genes or genetic loci that are linked to atopy, or asthma, and determine if these genes affect cytokine synthesis. For many years, genes in the MHC were examined for linkage to allergy, but for the most part, no MHC-related genes causing allergic disease in a broad atopic/asthmatic population could be identified. In mice, the MHC does not appear to regulate the development of Th1 versus Th2 responses, although antigen peptide affinity for specific MHC antigens can affect T-cell activation (25). Stronger binding of peptide to MHC results in greater T-cell receptor ligation, which may increase the likelihood of Th1 cytokine synthesis (26).

Several non-MHC genes have been found associated with atopy. The first of these was the gene for the β subunit of the high-affinity immunoglobulin E receptor (FcεR1β) on chromosome 11 (27). This finding has been controversial and has not been reproduced by other groups, possibly because of differences in study populations. However, mast cells do produce a large number of cytokines, including IL-4 and IL-5, and, therefore, variation in mast cell FcεR1β may affect activation and production of IL-4.

A second non-MHC gene locus has been found associated with atopy. Marsh *et al.* have found that total serum IgE concentration is linked to markers on chromosome 5q31.1, a region that includes genes for IL-4, IL-5, and IL-13 (28, 29). No linkage was found between these markers and specific IgE antibody concentrations. However, another group has found that bronchial hyperresponsiveness was also linked to this region, confirming previous studies associating elevated IgE and risk for developing asthma (30) and suggesting that asthma/atopy is related to polymorphisms in Th2 cytokine genes and alteration in the cytokine gene regulation. Exactly how polymorphisms in this region might increase production of Th2 cytokines in atopic individuals is not known (e.g., do they directly increase IL-4 gene transcription, or are transcription inhibitory mechanisms inactivated?).

T-Cell and Non–T-Cell Compartments Can Independently Determine Resistance to Leishmania Major

Another approach to identifying the underlying immunological defect in allergic individuals that results in

increased Th2 cytokine synthesis is to identify specific mechanisms that differentially regulate cytokine synthesis in allergic and nonallergic individuals or in distinct strains of mice. Shankar *et al.* (31) examined the regulation of IL-4 synthesis in strains of mice that differ in their response to infection with *Leishmania major*, using C57BL/6 mice (which preferentially produce Th1 cytokines and are resistant to infection with Leishmania) and BALB/c mice (which preferentially produce Th2 cytokines and are susceptible to infection with Leishmania). They constructed T-cell chimeric mice using adult thymectomized lethally irradiated, bone marrow–reconstituted (ATXBM) animals of C57BL/6 or BALB/c backgrounds. BALB/c ATXBM hosts given C57BL/6 T cells responded on antigen challenge (infection with Leishmania major) with typical Th1 cytokine profiles, indicating that factors intrinsic to T cells control the development of Th1/Th2 cytokine synthesis. However, C57BL/6 ATXBM hosts given BALB/c T cells were also resistant to infection with Leishmania major, and produced Th0 T cells, indicating that non-curing (BALB/c) T cells can mediate cure in a curing environment. These authors concluded, therefore, that both T-cell and non–T-cell compartments can affect cytokine synthesis in T cells.

Default T Helper Phenotype Development *in Vitro*

The idea that factors intrinsic to T cells are responsible for Th subset development was confirmed by studies by Hsieh *et al.* (32), who generated α/β-TCR transgenic mice of several different genetic backgrounds. They found that when T cells from TCR transgenic mice were activated *in vitro* under "neutral" conditions in which exogenous cytokines were not added, TCR transgenic T cells from B10.D2 (C57BL/6 background) mice defaulted to the Th1 phenotype, while TCR transgenic T cells from the BALB/c background preferentially developed the Th2 phenotype, which correlated with *in vivo* Th responses to Leishmania in these strains. These studies are therefore consistent with the idea that Th phenotype development is mainly affected by factors intrinsic to the T cell, at least in mice.

Downregulation of Receptors for IL-12 as a Mechanism Intrinsic to T Cells

A molecular mechanism intrinsic to T cells that governs Th phenotype development may involve receptors for IL-12. IL-12 is a heterodimeric cytokine produced by antigen-presenting cells and plays a critical role in regulating cytokine production by enhancing IFN-γ synthesis by T cells and natural killer (NK) cells (33). Szabo *et al.* have found that, as $CD4^+$ T cells differentiate towards the Th2 (but not the Th1) phenotype, they quickly lose expression of the second chain of the IL-12 receptor, IL-12Rβ2, which normally phosphorylates the transcription factors Stat3 and Stat4. The loss of IL-12Rβ2 by Th2 but not Th1 cells provides a biochemical difference between Th1 and Th2 cells. Furthermore, IL-12Rβ2 is lost more readily in BALB/c (Th2 predisposed) than in B10.D2 (Th1 predisposed) mice (34) and enhances early commitment to Th2 differentiation in BALB/c mice. This strain difference in IL-12 receptor function extinction provides a molecular mechanism for the predisposition of BALB/c mice to produce Th2-dominated cytokine profiles and for the susceptibility of BALB/c mice to pathogens such as *L. major* (34). Similar observations with the IFN-γ receptor indicate that the second chain of the IFN-γ receptor (IFN-γRβ) is lost by developing Th1 but not Th2 cells. This phenomenon explains the inhibitory effects of IFN-γ on Th2 but not Th1 cells (35), which allow IFN-γ to enhance the emergence of Th1-dominated responses. However, strain differences in this process have not been described.

Non–T-Cell Compartments in Atopic Diseases

It is not yet clear if rapid loss of IL-12Rβ2 expression in developing Th2 cells is the immunologic cause of atopic disease in humans. Another mechanism that might account for atopic disease in humans is increased production of IL-10 by antigen-presenting cells (APC) from atopic individuals (36), or a reduced production of IL-12. Since 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 towards Th2 rather than Th1 responses. Ohmen *et al.* (36) found evidence for this possibility by examining skin biopsy specimens from patients with atopic dermatitis, allergic contact dermatitis, and positive tuberculin reactions. They observed that IL-10 mRNA was greatest in the skin of patients with atopic dermatitis, whereas mRNA for IFN-γ was greatest in tuberculin skin reactions. These authors concluded that the relative overexpression of IL-10 in atopic dermatitis may contribute to the upregulation of humoral responses and the downregulation of Th1 responses. Limitation in the production of IL-12 has not yet been demonstrated in humans, although, in a mouse model, we have demonstrated that antigen-presenting cells from BALB/c mice (Th2 predisposed) have been shown to produced substantially less IL-12 than those from C57BL/6 and DBA/2 mice (Th1 predisposed) (37).

Another mechanism that might cause atopic disease is the overexpression by APC of an accessory molecule, B7-2. B7-1 (CD80) and B7-2 (CD86) are both ligands for CD28, which is the most important co-stimulatory signaling molecule on T cells (38). Kuchroo *et al.*, in a murine EAE system, and Freeman *et al.*, using human

Table II. Therapies to Alter Cytokine Profiles of $CD4^+$ T-Cell Subsets

Inhibit Th2 cytokine production (e.g., in allergic disease)
Administration of larger dose of antigen
Immunize while neutralizing IL-4 and IL-10
Immunize with IL-12 as adjuvant
Target antigen to macrophage
Tolerize Th2 cells with high dose of peptide
Inhibit Th1 cytokine production (e.g., in autoimmune inflammatory disease)
Administer IL-4 or IL-10 (with or without antigen)
Neutralize IFN-γ or IL-12
Target antigen to B cells
Use agents that stimulate cAMP production, such as PGE_2, or pentoxifylline
Immunize with antigen orally or by inhalation to induce regulatory cells
Tolerize Th1 cells with high dose of antigen peptide

cells, found that co-stimulation of T cells with B7-2 rather than B7-1 resulted in generation of Th2-dominated responses (39, 40). However, studies using other disease models have produced conflicting results (41), and other investigators suggest that the B7-1 and B7-2 antigens bind equally well to CD28. Nevertheless, blocking both B7-1 and B7-2 interaction with CD28 and its related receptor, CTLA-4 (42–44), blocks Th2 cytokine synthesis (45), indicating that these molecules play important roles in cytokine regulation. T-cell activation is complex, and further study is required to determine if differential use of B7 antigens are involved in the generation of the atopic state.

New Therapies for Allergic Diseases

Past therapies for allergic diseases have focused on the mast cell and mast cell products, but newer therapies have been proposed to deal with the dysregulation of cytokine synthesis and the inappropriate production by allergen-specific $CD4^+$ T cells of IL-4 and IL-10 but not IFN-γ (46). These new therapies (Table II), discussed below, focus on reducing IL-4 and IL-10, and upregulating IFN-γ synthesis in allergen-specific $CD4^+$ T cells. Such therapies however, are difficult to develop given that the cytokine profiles of memory effector cells are relatively resistant to change (14, 15). In addition, although the Th1/Th2 paradigm suggests that treatment should focus on the enhanced development of Th1 cells which are protective against atopy, Th1 cells are very capable of causing significant tissue damage (e.g., in inflammatory bowel disease). This suggests that conversion to vigorous allergen-specific Th1 responses that inhibit IgE synthesis might potentially cause damage to epithelial surfaces in the respiratory tract. Therefore, other therapies (Table II) attempting to inactivate allergen-specific T cells are also being developed, as discussed below.

Neutralization IL-4 and IL-10 on Immunization or at Time of Antigen Challenge. The most direct method to deal with excessive Th2 cytokine synthesis is to neutralize these cytokines. In one model of respiratory disease with respiratory syncytial virus (RSV), mice immunized with formalin-inactivated RSV developed on subsequent infection with live RSV, severe lung pathology, characterized by the presence of T cells producing IL-4. Neutralization of IL-4 and IL-10 at the time of challenge with live RSV abrogated the lung pathology. These data indicate that immunization with killed RSV primes for a Th2 inflammatory response to subsequent RSV infection, and that the Th2 inflammatory response can be modified by neutralization of some Th2 cytokines at the time of challenge (47). Interestingly, administration of anti–IL-4 monoclonal antibody during immunization with inactivated virus primed the mice for a Th1-like protective responses (48). Similar studies with other infectious agents have been also described (49). Neutralization of IL-4 and IL-10, however, is difficult in humans, since neutralization must occur at the time of immunization or at the time of challenge.

Manipulation of Antigen Dose. It has long been known that antigen dose can influence whether cell-mediated or humoral immunity is elicited, and the Th1/Th2 paradigm suggests that this is largely due to the development of $CD4^+$ T helper cells producing distinct sets of cytokines. The ability of antigen dose to direct the development of a Th1 or Th2 phenotype from naïve $CD4^+$ T cells was recently demonstrated by Hosken *et al.* (50), who showed that antigen dose used in primary cultures could directly affect Th phenotype development using TCR αβ transgenic $CD4^+$ T cells. Using antigen peptide, they found very high (>10 μ*M*) or very low (<0.05 μ*M*) concentrations directed the development of Th2-like cells that produced increasing amounts of IL-4 and diminishing levels of IFN-γ. In contrast, midrange peptide concentrations (0.3–0.6 μ*M*) induced the development of Th0/Th1-like cells, which produced moderate amounts of IFN-γ. These findings, along with those of Constant *et al.* (26), suggest that antigen dose can alter cytokine synthesis in naïve $CD4^+$ T cells. Therefore *prevention* of allergic disease might occur by administration of intermediate doses of allergen (51).

Individuals already allergic to antigen may be similarly treated with elevated doses of allergen (52). Secrist *et al.* found that $CD4^+$ T cells from allergic donors produced high levels of IL-4 when stimulated *in vitro* with low concentrations of allergen (0.003–0.01 μg/ml), particularly when B-cell–enriched populations presented antigen. In contrast, the same responding $CD4^+$ T-cell population produced little IL-4 when stimulated with high concentrations of allergen (10–30 μg/ml), especially when monocytes were used as antigen-presenting cells. These studies indicated that the circumstances under which memory T cells are activated, as well as the

strength of the proliferative signal to T cells, greatly affect the quantity of IL-4 produced, and that the cytokine profile of allergen-specific memory $CD4^+$ T cells could be modulated by the antigen dose and APC type. These studies suggest that methods that preferentially enhance allergen uptake by monocytes with high antigen doses and that enhance T-cell proliferation will improve the clinical efficacy of immunotherapy in the treatment of allergic disease. Allergen immunotherapy with high doses of allergen does appear to switch production of Th2 to Th1 cytokines in allergen-specific T cells (53–55), indicating that the cytokine profiles of memory $CD4^+$ T cells are mutable and not fixed. However, it is not clear, at the single cell level, whether such therapy selectively expands an existing population of antigen specific Th1 cells or whether memory cells destined to become Th2 cells become diverted into Th1 cells.

Therapy with IL-12. IL-12, because it enhances IFN-γ synthesis by T cells and NK cells (33), has been proposed as a treatment of Th2-mediated diseases. For example, IL-12 can function as an effective treatment for cutaneous leishmaniasis by initiating a protective Th1 immune response if given early during infection (56), or when used as an adjuvant in a vaccine against *L. major* by directing the development of Leishmania-specific $CD4^+$ T cells (57). In addition, treatment with IL-12, in combination with the antimony-based leishmanicidal drug Pentostam, induces healing in *L. major*–infected mice, and apparently induces a switch from a Th2 to a Th1 response (58), indicating that in a chronic infectious disease, an inappropriate Th2 response can be switched to an effective Th1 response. IL-12 administration induces Th1 cells and accelerates autoimmune diabetes in NOD mice (59), whereas antibodies against IL-12 prevent experimental autoimmune encephalomyelitis (60).

With regard to therapy for allergic disease, administration of IL-12 in a mouse model for asthma has been shown to inhibit antigen-induced airway hyperresponsiveness and Th2 cytokine expression. Sensitized A/J mice developed airway hyperresponsiveness and increased numbers of eosinophils and other inflammatory cells after single or repeated intratracheal challenges with antigen. Administration of IL-12 intratracheally once and intraperitoneally on four additional occasions around the time of a single antigen challenge abolished the airway hyperresponsiveness and pulmonary eosinophilia normally associated with antigen challenge. It also promoted an increase in IFN-γ and a decrease in IL-4 and IL-5 expression. Treatment of mice with IL-12 at the time of a second antigen challenge also prevented airway hyperresponsiveness and significantly reduced numbers of BAL inflammatory cells, suggesting that ongoing inflammation can be inhibited with IL-12, and that the responses of memory T cells can be altered with IL-12. Thus, local administration of IL-12 may provide effective immunotherapy for the treatment of pulmonary allergic disorders such as atopic asthma (61).

In studies with human antigen–primed T cells, which are thought to have relatively fixed cytokine profiles, Marshall *et al.* showed that IL-12 dramatically inhibited the development of IL-4 and IL-10 synthesis, while it enhanced T-cell secretion of IFN-γ and IL-2, and enhanced antigen-specific proliferation (62). The inhibitory effect of IL-12 on IL-4 synthesis was greatest when IL-12 was added at the initiation of culture, and was minimal when added late, indicating that resting memory $CD4^+$ T cells were more sensitive than activated $CD4^+$ T cells to the effects of IL-12. These results were consistent with the idea that resting memory $CD4^+$ T cells are not fully committed to a specific cytokine profile and can be reprogrammed (63). In addition, these studies were consistent with the fact that activated Th2 effector cells lose IL-12 receptor function and cannot respond to IL-12 (34). At the single cell level, it was not clear whether IL-12 selectively expanded antigen-specific T cells with Th1 profiles or whether memory cells destined to become Th2 cells were diverted into Th1 cells. Nevertheless, these studies indicated that IL-12 might be therapeutically beneficial in the treatment of allergic diseases by altering cytokine production in resting allergen-specific T cells by reversing the enhanced Th2 cytokine production. Although we have also demonstrated that IL-12 inhibits IL-4 synthesis in murine memory $CD4^+$ T cells (64), further *in vivo* studies with IL-12 must be performed, since IL-12, particularly when given in large doses, appears paradoxically to enhance IL-4 synthesis *in vivo* possibly by generating a rebound increase in IL-10 production (65, 66).

Immunotherapy with Antigen–IL-12 Fusion Proteins. More recently, we demonstrated that IL-12, when covalently linked by genetic means to antigen, could function effectively in reversing a Th2-dominated immune response *in vivo* in an antigen-specific fashion.[1] Like conventional immunotherapy with allergen, we demonstrated that an antigen–IL-12 fusion protein inhibited IL-4 and enhanced IFN-γ synthesis in memory $CD4^+$ T cells in an antigen-specific fashion. Moreover, this effect was achieved after two to four "vaccinations" with the fusion protein rather than after the several years of therapy that is required for conventional allergen immunotherapy to be effective. A most surprising characteristic of IL-12 when covalently linked to OVA was that the effects of IL-12 appeared to be confined to OVA-specific cells. Whereas the presence of free

[1] Kim TS, DeKruyff RH, Maecker HT, Levy S, Umetsu DT. An OVA-IL-12 fusion protein is more effective than OVA plus IL-12 in inducing a Th1-dominated immune response and inhibiting antigen-specific IgE production. J Immunol, in press, 1997.

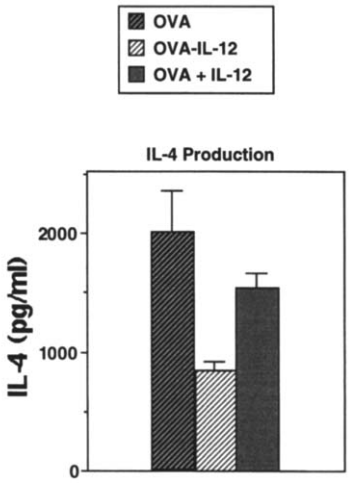

Figure 4. Cytokine production in OVA (alum)-primed BALB/c mice immunized with the OVA–IL-12 fusion protein. BALB/c mice were immunized in the foot pads with OVA (10 μg) in alum to induce a Th2-dominated immune response. Three weeks later, the mice were immunized with OVA–IL-12 (50 p*M*) or recombinant OVA (50 p*M*), or with a mixture of recombinant OVA (50 p*M*) and free rIL-12 (1 p*M*) (four times every other day). Ten days after the last injection, the lymph node cells were prepared and incubated *in vitro* with OVA (4×10^5 cells/well, 100 μg/ml) for 4 days. IL-4 was determined by ELISA, and values represent the mean ± SD of triplicate determinations.

recombinant IL-12 induced a great increase in antigen-nonspecific IFN-γ production, as seen by the *in vitro* production by lymph node cells of IFN-γ in the absence of antigenic stimulation, immunization with the OVA–IL-12 fusion protein, in both naïve and antigen-primed mice, resulted in enhanced *in vitro* IFN-γ production, which was elicited only on stimulation with antigen (Fig. 4). In addition, the OVA–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. Thus antigen-nonspecific effects of IL-12 were greatly reduced by covalent linkage of IL-12 to OVA, while such linkage enhanced the antigen-specific effects of IL-12.

Immunotherapy with antigen has also been attempted with two additional methodologies. First, it has been reported that immunization with plasmid DNA containing cDNA for antigen could induce a Th1 response that dominates over an ongoing protein-induced Th2 response in an antigen-specific manner (67). However, the long-term implications of vaccination with naked DNA is unclear. In addition, since short immunostimulatory DNA sequences that contain CpG dinucleotides may enhance IFN-α and IFN-β synthesis (68), the specific mechanisms by which plasmid DNA containing cDNA for antigen enhances Th1 cell type bias is not clear. The second methodology for treating atopic disease and excess production of Th2 cytokines involves tolerization or induction of unresponsiveness in allergen specific T cells. Such tolerization might be achieved with the administration of high doses of peptide fragments of allergens. Exposure of the immune system to immunodominant T-cell epitopes/peptides of antigens under nonstimulatory conditions has been shown to induce unresponsiveness to later antigen challenge (69), or if given under stimulatory conditions to induce apoptosis (70). Immunodominant peptides for dust mite antigen (*Der. p.* 1), cat allergen (*Fed. d* I) 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* (71). Human studies with allergen peptides are currently in progress as therapy for atopic disease, and preliminary data have demonstrated its effectiveness in reducing allergy symptoms in patients (72).

1. Lukacs NW, Strieter RM, Kunkel SL. Leukocyte infiltration in allergic airway inflammation. Am J Respir Cell Mol Biol **13:**1–6, 1995.
2. Marfaing-Koka A, Devergne O, Gorgone G, Portier A, Schall TJ, Galanaud P, Emilie D. Regulation of the production of the RANTES chemokine by endothelial cells. Synergistic induction by IFN-gamma plus TNF-alpha and inhibition by IL-4 and IL-13. J Immunol **154:**1870–1878, 1995.
3. Pattison JM, Nelson PJ, Krensky AM. The RANTES chemokine. A new target for immunomodulatory therapy? Clin Immunother **4:**1–8, 1995.
4. Bochner BS, Schleimer RP. The role of adhesion molecules in human eosinophil and basophil recruitment. J Aller Clin Immunol **94:**427–438, 1994.
5. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med **326:**298–304, 1992.
6. Mosmann TR, Coffman RL. Two types of mouse helper T-cell clone: Implications for immune regulation. Immunol Today **8:**223–226, 1987.
7. Umetsu DT, Jabara HH, DeKruyff RH, Abbas AK, Abrams JS, Geha RS. Functional heterogeneity among human inducer T cell clones. J Immunol **140:**4211–4216, 1988.
8. Paul WE, Seder RA. Lymphocyte responses and cytokines. Cell **76:**241–251, 1994.
9. Parish CR, Liew FY. Immune response to chemically modified flagellin. III. Enhanced cell-mediated immunity during high and low zone antibody tolerance to flagellin. J Exp Med **135:**298, 1972.
10. Kamogawa Y, Minasi LE, Carding SR, Bottomly K, Flavell RA. The relationship of IL-4– and IFN-γ–producing T cells studied by lineage ablation of IL-4–producing cells. Cell **75:**985–995, 1993.
11. Romagnani S. Lymphokine production by human T cells in disease states. Annu Rev Immunol **12:**227–257, 1994.
12. Bradley, LM, Duncan DD, Tonkonogy S, Swain SL. Characterization of antigen-specific $CD4^+$ effector T cells in vivo: Immunization results in a transient population of $MEL\text{-}14^-$, $CD45RB^-$ helper cells that secretes interleukin 2 (IL-2), IL-3, IL-4, and interferon. J Exp Med **174:**547–559, 1991.
13. Marshall J, Wen Y, Abrams JS, Umetsu DT. In vitro synthesis of IL-4 by human $CD4^+$ T cells requires repeated antigenic stimulation. Cell Immunol **152:**18–34, 1993.
14. Swain SL. Generation and in vivo persistence of polarized Th1 and Th2 memory cells. Immunity **1:**543–552, 1994.
15. DeKruyff RH, Fang Y, Secrist H, Umetsu DT. IL-4 synthesis by in vivo–primed memory $CD4^+$ T cells: II. The presence of IL-4 is not required for IL-4 synthesis in primed $CD4^+$ T cells. J Clin Immunol **15:**105–115, 1995.

16. Del Prete GF, De Carli M, Almerigogna F, Daniel CK, D'Elios MM, Zancuoghi G, Vinante F, Pizzolo G, Romagnani S. Preferential expression of CD30 by human CD4+ T cells producing Th2-type cytokines. FASEB J **9:**81–86, 1995.
17. Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. Science **265:**1237–1240, 1994.
18. Akhtar I, Gold JP, Pan LY, Ferrara JL, Yang XD, Kim JI, Tan KN. CD4+ beta islet cell-reactive T cell clones that suppress autoimmune diabetes in nonobese diabetic mice. J Exp Med **182:**87–97, 1995.
19. Salgame P, Abrams JS, Clayberger C, Goldstein H, Convit J, Modlin RL, Bloom BR. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. Science **254:**279–282, 1991.
20. Gajewski TF, Qian D, Fields P, Fitch FW. Anergic T-lymhocyte clones have altered inositol phosphate, calcium, and tyrosine kinase signaling pathways. Proc Natl Acad Sci **91:**38–42, 1994.
21. Gajewski TF, Lancki DW, Stack R, Fitch FW. "Anergy" of Th0 helper T lymphocytes induces downregulation of Th1 characteristics and a transition to a Th2-like phenotype. J Exp Med **179:**481–491, 1994.
22. Weiner HL, Friedman A, Miller A, Khoury SJ, Nussenblatt RB, Trentham DE, Hafler DA. Oral tolerance: Immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. Annu Rev Immunol **12:**809–837, 1994.
23. Scott B, Liblau R, Degermann S, Marconi L, Ogata L, Caton A, McDevitt H, Lo D. A role for non-MHC genetic polymorphism in susceptibility to spontaneous autoimmunity. Immunity **1:**73, 1994.
24. Oro AS, Guarino TJ, Driver R, Steinman L, Umetsu DT. Regulation of disease susceptibility: Decreased prevalence of IgE-mediated allergic disease in patients with multiple sclerosis. J Aller Clin Immunol **97:**1402–1408, 1996.
25. Pfeiffer C, Stein J, Southwood S, Ketelaar H, Sette A, Bottomly K. Altered peptide ligands can control CD4 T lymphocyte differentiation in vivo. J Exp Med **181:**1569–1574, 1995.
26. Constant S, Pfeiffer C, Woodward A, Pasqualini T, Bottomly K. Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. J Exp Med **182:**1591–1596, 1995.
27. Shirakawa T, Li A, Dubowitz M, Dekker JW, Shaw AE, Faux JA, Ra C, Cookson WO, Hopkin JM. Association between atopy and variants of the β subunit of the high-affinity immunoglobulin E receptor. Nat Genet **7:**125–130, 1994.
28. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. Science 264:1152–1156, 1994.
29. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Schou C, Beaty TH. Genetic basis of IgE responsiveness: Relevance to the atopic diseases. Intern Arch Aller Immunol **107:**25–28, 1995.
30. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med **320:**271, 1989.
31. Shanker AH, Titus RG. T cell and non-T cell compartments can independently determine resistance to Leishmania major. J Exp Med **181:**845–855, 1995.
32. Hsieh C-S, Macatonia SE, O'Garra A, Murphy KM. T cell genetic background determines default T helper phenotype development in vitro. J Exp Med **181:**713–721, 1995.
33. Trinchieri G. Interleukin-12: A proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol **13:**251–276, 1995.
34. Szabo SJ, Jacobson NG, Dighe AS, Gubler U, Murphy KM. Developmental commitment to the Th2 lineage by extinction of IL-12 signaling. Immunity **2:**665–675, 1995.
35. Pernis A, Gupta S, Gollob KJ, Garfein E, Coffman RL, Schindler C, Rothman P. Lack of interferon γ receptor β chain and the prevention of interferon γ signaling in Th1 cells. Science **269:**245–247, 1995.
36. Ohmen JD, Hanifin JM, Nickoloff BJ, Rea TH, Wyzykowski R, Kim J, Jullien D, McHugh T, Nassif AS, Chan SC, Modlin RL. Overexpression of IL-10 in atopic dermatis. Contrasting cytokine patterns with delayed type hypersensitivity reactions. J Immunol **154:**1956–1963, 1995.
37. Gieni RS, Fang Y, Trinchieri G, Umetsu DT, DeKruyff RH. Differential production of IL-12 in distinct mouse strains controls the development of Th1 versus Th2 cytokine profiles in primed CD4 lymphocytes. Int Immunol **8:**1511–1520, 1996.
38. Allison JP. CD28-B7 interactions in T-cell activation. Curr Opin Immunol **6:**414, 1994.
39. Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N, Glimcher LH. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: Application to autoimmune disease therapy. Cell **80:**707–718, 1995.
40. Freeman GJ, Boussiotis VA, Anumanthan A, Bernstein GM, Ke X, Rennert P, Gray GS, Gridden JG, Nadler LM. B7-1 and B7-2 do not deliver identical costimulatory signals since B7-2 but not B7-1 preferentially costimulates the initial production of IL-4. Immunity **2:**523–532, 1995.
41. Lenshow DJ, Ho SC, Sattar H, Rhee L, Gray G, Nabavi N, Herold KC, Bluestone JA. Differential effects of anti–B7-1 and anti–B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. J Exp Med **181:**1145–1155, 1995.
42. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med **182:**459–465, 1995.
43. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multi-organ tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 3:541–547, 1995.
44. Gribben JG, Freeman GJ, Boussiotis VA, Rennert P, Jellis CL, Greenfield E, Barber M, Restivo VA Jr., Ke X, Gray GS, Nadler LM. CTLA4 mediates antigen-specific apoptosis of human T cells. Proc Natl Acad Sci **92:**811–815, 1995.
45. Lu P, Zhou XD, Chen SJ, Moorman M, Schoneveld A, Morris S, Finkelman FD, Linsley P, Claassen E, Gause WC. Requirement of CTLA-4 counter receptors for IL-4 but not IL-10 elevations during a primary systemic in vivo immune response. J Immunol **154:**1078–1087, 1995.
46. Kapsenberg ML, Wierenga EA, Bos JD, Jansen HM. Functional subsets of allergen-reactive human CD4+ T cells. Immunol Today **12:**392–395, 1991.
47. Connors M, Giese NA, Kulkarni AB, Firestone C-Y, Morse HCI, Murphy BR. Enhanced pulmonary histopathology induced by respiratory syncytial virus (RSV) challenge of formalin-inactivated RSV-immunized BALB/c mice is abrogated by depletion of interleukin-4 and IL-10. J Virol **68:**5321–5325, 1994.
48. Tang Y-W, Graham BS. Anti–IL-4 treatment at immunization modulates cytokine expression, reduces illness, and increases cytotoxic T lymphocyte activity in mice challenged with respiratory syncytial virus. J Clin Invest **94:**1953–1958, 1994.
49. Coffman RL, Varkila K, Scott P, Chatelain R. Role of cytokines in the differentiation of CD4+ T-cell subsets in vivo. Immunol Rev **123:**189–207, 1991.

50. Hosken NA, Shibuya K, Heath AW, Murphy KM, O'Garra A. The effect of antigen dose of CD4+ T helper cell phenotype development in a T cell receptor-αβ-transgenic model. J Exp Med **182:**1579–1584, 1995.
51. Holt PG. Immunoprophylaxis of atopy: Light at the end of the tunnel? Immunol Today **15:**484–489, 1994.
52. Secrist H, DeKruyff RH, Umetsu DT. Interleukin 4 production by CD4+ cells from allergic individuals is modulated by allergen concentration and antigen-presenting cell type. J Exp Med **181:**1081–1090, 1995.
53. Varney VA, Hamid QA, Gaga M, Ying S, Jacobson M, Frew AJ, Kay AB, Durham SR. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. J Clin Invest **92:**644–651, 1993.
54. Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. J Exp Med **178:**2123–2130, 1993.
55. Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. J Immunol **154:**4187–4194, 1995.
56. Sypek JP, Chung CL, Mayor SE, Subramanyam JM, Goldman SJ, Sieburth DS, Wolf SF, Schaub RG. Resolution of cutaneous leishmaniasis: Interleukin 12 initiates a protective T helper type 1 immune response. J Exp Med **177:**1797–1802, 1993.
57. Afonso LCC, Scharton TM, Vieira LQ, Wysocka G, Trinchieri G, Scott P. IL-12 functions as an effective adjuvant in a vaccine against Leishmania major by directing the development of leishmanial specific CD4+ T cells. Science **263:**235, 1994.
58. Nabors GS, Afonso LC, Farrell JP, Scott P. Switch from a type 2 to a type 1 T helper cell response and cure of established Leishmania major infection in mice is induced by combined therapy with interleukin 12 and Pentostam. Proc Natl Acad Sci **92:**3142–3146, 1995.
59. Trembleau S, Penna G, Bosi E, Mortara A, Gately MK, Adorini L. Interleukin 12 administration induces T helper Type 1 cells and accelerates autoimmune diabetes in NOD mice. J Exp Med **181:**817–821, 1995.
60. Leonard JP. Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. J Exp Med **181:**381–386, 1995.
61. Gavett SH, O'Hearn DJ, Li X, Huang S-K, Finkelman FD, Wills-Karp M. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. J Exp Med **182:**1527–1536, 1995.
62. Marshall JD, Secrist H, DeKruyff RH, Wolf SF, Umetsu DT. IL-12 inhibits the production of IL-4 and IL-10 in allergen-specific human CD4+ T lymphocytes. J Immunol **155:**111–117, 1995.
63. Murphy E, Shibuya K, Hosken N, Openshaw P, Maino V, Davis K, Murphy K, O'Garra A. Reversibility of T helper 1 and 2 populations is lost after long-term stimulation. J Exp Med **183:**901–913, 1996.
64. DeKruyff RH, Fang Y, Wolf SF, Umetsu DT. IL-12 inhibits IL-4 synthesis in keyhole limpet hemocyanin-primed CD4+ T cells through an effect on antigen-presenting cells. J Immunol **154:**2578–2587, 1995.
65. Finkelman FD, Madden KB, Cheever AW, Katona IM, Morris SC, Gately MK, Hubbard BR, Gause WC, Urban JF Jr. Effects of interleukin 12 on immune responses and host protection in mice infected with intestinal nematode parasites. J Exp Med **179:**1563–1572, 1994.
66. Bliss J, Van CV, Murray K, Wiencis A, Ketchum M, Maylor R, Haire T, Resmini C, Abbas AK, Wolf SF. IL-12, as an adjuvant, promotes a T helper 1 cell, but does not suppress a T helper 2 cell recall response. J Immunol **156:**887–894, 1996.
67. Hsu CH, Chua KY, Tao MH, Lai YL, Wu HD, Huang SK, Hsieh KH. Immunoprophylaxis of allergen-induced immunoglobulin E synthesis and airway hyperresponsiveness in vivo by genetic immunization. Nature Medicine **2:**540–544, 1996.
68. Sato Y, Roman M, Tighe H, Lee D, Corr M, Nguyen MD, Silverman GJ, Lotz M, Carson DA, Raz E. Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. Science **273:**352–354, 1996.
69. Wallner BP, Gefter ML. Immunotherapy with T cell reactive peptides derived from allergens. Allergy **49:**302–308, 1994.
70. Critchfield JM, Racke M, Zuniga-Pflucker JC, Cannella B, Raine CS, Goverman J, Lenardo MJ. T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis. Science **263:**1139–1143, 1994.
71. Briner TJ, Kuo M-C, Keating KM, Rogers BL, Greenstein JL. Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I. Proc Natl Acad Sci **90:**7608–7612, 1993.
72. Norman PS, Ohman MD, Long AA, Creticos PS, Gefter ML, Shaked Z, Wood RA, Eggleston PA, Lichtenstein LM, Jones NH, Nicodemus CF. Follow on study of the first clinical trial with T cell defined peptides from cat allergen Fel d 1. J Aller Clin Immunol **95:**A259, 1995.