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Mechanisms Controlling the Human Immunoglobulin E Response: New Directions in the Therapy of Allergic Diseases

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ABSTRACT. The IgE response plays a critical role in the pathogenesis of human allergic diseases. A detailed understanding of the mechanisms underlying the regulation of IgE synthesis is thus important in the development of new treatments for allergic diseases. It is now well established that the induction of IgE synthesis in human B cells requires two signals. The first signal is delivered by IL-4, which induces Ig gene switching to the ϵ locus. The second signal can be delivered by a number of B-cell activators which, in combination with IL-4, cause the expression of productive ϵ mRNA transcripts and the synthesis of IgE protein. These second signals include contact-mediated signals delivered by T cells via cognate or noncognate interactions, Epstein-Barr virus infection, hydrocortisone, and MAb to the B-cell antigen CD40. Cytokines such as IL-5 and IL-6 significantly amplify IgE synthesis, whereas interferon gamma (IFN-γ) inhibits IL-4–induced IgE synthesis. The production of cytokines is frequently compartmentalized to specific T-cell subsets: TH1, but not TH2, cells produce IL-2, IFN-γ and lymphotoxin, whereas TH2 but not TH1 cells produce IL-4, IL-5, IL-6, and IL-10. These two T-cell subsets therefore produce cytokines that functionally antagonize each other, *e.g.* IFN-γ inhibits proliferation of TH2 cells. Recent studies indicate that severe allergic diseases such as atopic dermatitis are associated with expansion of TH2 cells. These observations provide a rationale for the use of agents that interfere with IL-4 production or action, or alternatively, the use of IFN-γ in the treatment of severe allergic diseases. (*Pediatr Res* 33 (Suppl): S56–S62, 1993)

Abbreviations

AD, atopic dermatitis
EBV, Epstein-Barr virus
IFN, interferon
LPR, late phase response
LPS, lipopolysaccharide
PBMC, peripheral blood mononuclear cells
r, recombinant
sIgE, surface-bound IgE

The principal feature that distinguishes atopic individuals from nonatopic individuals is their capacity to develop a sustained IgE response to environmental allergens. Clinically significant allergen-induced reactions are generally characterized by an IgE-dependent biphasic response (1). Within minutes of exposure to allergen, mast cells bearing IgE directed to the relevant allergen become activated and release a variety of mediators, chemotactic factors, and cytokines into the local tissue. This immediate reaction is generally clinically evident within 15 min of allergen challenge and subsides within 30 to 90 min later. Three to four h after the immediate reaction begins to subside, there is frequently the onset of an intense inflammatory reaction, termed the "late phase response" (LPR). This is associated with the expression of leukocyte-adhesion molecules on the local vascular endothelium and the concomitant infiltration of eosinophils, neutrophils, and mononuclear cells (2, 3). This IgE-mediated LPR is thought to play a critical role in the pathogenesis of chronic allergic diseases. The clinical manifestations of a particular hypersensitivity reaction depend on the extent of the reaction and anatomical location of mast cell degranulation.

It has become increasingly appreciated that the IgE-mediated LPR plays an important role in the pathogenesis of chronic allergic diseases such as asthma, AD, and allergic rhinitis. For example, it is thought that the local deposition of eosinophil-derived major basic protein during the LPR contributes to the respiratory epithelial damage and lung inflammation found in asthma (4). The LPR may be sustained by infiltrating cells that bear surface-bound IgE and can therefore release cytokines and mediators on exposure to specific allergens. The importance of the LPR is further supported by the observation that the intensity of nonspecific bronchial hyperreactivity in asthmatic reactions after antigen bronchoprovocation is proportional to the intensity of the LPR (5). Clinical improvement in asthmatic symptoms after allergen immunotherapy correlates with a marked attenuation of LPR after bronchoprovocation challenge (6).

Recent studies have found a correlation between serum IgE levels and airway responsiveness in asthmatics and normals (7). A correlation between serum IgE level and severity of skin disease in AD has also been reported (8). These observations suggest that a detailed understanding of the mechanisms that control IgE synthesis will provide considerable insight into the pathogenesis of allergic diseases. This review focuses on recent advances in our knowledge regarding the regulation of IgE synthesis and the implications these findings may have for the development of new therapy for allergic diseases.

HISTORICAL BACKGROUND

Studies on rodents dating back to the early 1970s have demonstrated that T cells play an important role in the development of IgE responses (9–11). In these studies, neonatally thymectomized rats were unable to produce IgE antibody. Whole-body irradiation or administration of immunosuppressive agents con-

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verted rats from a low-IgE response to a high-IgE responder state. This pattern of high-IgE response to allergens could be terminated by passive transfer of syngeneic T cells. These studies suggested that T cells were also critical in suppressing IgE responses in low-IgE (*i.e.* nonatopic) responder animals.

Until recently, studies of IgE synthesis in humans have been limited because polyclonal B-cell activators that induce IgG, IgA, and IgM synthesis were not able to induce IgE synthesis by normal lymphocytes (12). Based on these observations, two possibilities were considered: *1*) precursor IgE B cells may be absent from the circulation of nonatopic donors, and *2*) the activation of IgE-producing B cells may require T-cell–derived signals not generated under IgG-inducing conditions. It was subsequently shown that B cells from nonatopic donors bearing the appropriate alloantigen can be induced to synthesize Ig of all isotypes, including IgE, upon cognate interaction with selected human alloreactive (13) or autoreactive (14) helper T-cell clones. These data indicated that normal individuals possess circulating B cells with the potential to differentiate into IgE-secreting cells.

The first clue that T-cell–derived soluble factors were involved in the regulation of human IgE synthesis came from the observation that supernatants from T cells of patients with markedly elevated serum IgE levels secrete soluble helper factor(s) that induce IgE but not IgG synthesis by B-cell–enriched populations from normal donors (15). The major breakthrough came in 1986, when Coffman and Carty (16) reported that the T-cell–derived lymphokine IL-4 induced IgE production *in vitro* by LPS-stimulated murine B-cell blasts. The *in vivo* significance of IL-4 has subsequently been demonstrated in studies showing that anti–IL-4 antibody inhibits IgE responses in experimental animals (17, 18).

REQUIREMENTS FOR INDUCTION OF IgE SYNTHESIS IN HUMANS

In humans, IL-4 induces IgE synthesis by normal unfractionated PBMC (19, 20). The actual mechanism by which IL-4 contributes to the induction of IgE synthesis has been actively investigated by many laboratories but remains incompletely understood. Studies to date indicate that IL-4 acts as a crucial signal for isotype-switching to IgE. This has been shown by examination of single B cells stimulated by murine and human T-cell clones as well as limiting dilution experiments with LPS-stimulated B cells in mice (21–23). Incubation of B cells with rIL-4 induces a 1.8-kb germline Cϵ RNA transcript but not the 2.2-kb productive ϵ mRNA (24). In murine models, it has been demonstrated that Ig heavy chain switching is preceded by expression of the corresponding germline transcript (23). Thus, these observations are consistent with the hypothesis that IL-4 controls IgE isotype-switching by modulating the accessibility of the ϵ switch region to a putative common switch recombinase.

IL-4 is necessary but not sufficient for the induction of IgE synthesis on highly purified B cells. Addition of rIL-4 alone or in combination with a variety of other cytokines, including IL-5 and IL-6, is ineffective in inducing IgE synthesis in highly purified B-cell suspensions (25). Additional signals are required for IL-4–induced IgE synthesis to occur. These *second* signals can be provided by T cells (26, 27) or a variety of B-cell activators including EBV infection (28, 29), MAb to the B-cell antigen CD40 (30–32), and hydrocortisone (33–34).

T-CELL–DEPENDENT INDUCTION OF IgE SYNTHESIS

T cells can play two important roles in the induction of IgE synthesis. First, they provide a source of IL-4. This may account for the observation that selected, but not all, alloreactive T-cell clones can induce IgE synthesis by B cells bearing the relevant alloantigen. Second, in addition to IL-4, physical contact between T and B cells is required for IgE synthesis (26). Mixtures of T and B cells synthesize IgE upon incubation with IL-4 only when the T and B cells are cultured in the same well, but not when they are separated by a semipermeable membrane. Furthermore, IL-4–induced IgE synthesis is strongly inhibited by MAb specific for cell adhesion molecules (26).

Although initial studies suggested that cognate interaction between T and B cells (recognition by the T-cell receptor/CD3 complex on CD4+ T cells of MHC class II antigen plus peptide on B cells) was required for induction of IgE synthesis, more recent studies indicate that noncognate T cell/B cell interaction, in which the T-cell receptor does not recognize the B-cell MHC class II antigen plus peptide complex, can also support IL-4–dependent IgE synthesis (35). The antigens involved in the latter interaction are still poorly characterized. Using T-cell clones to further study this interaction, Gascan *et al.* (36) found that the T-cell clones needed to be activated to induce IgE synthesis. Furthermore, intact activated CD4+ T-cell clones could be replaced by membranes of these cells, suggesting that an inducible membrane-associated molecule is involved in the B-cell differentiation pathway that results in IgE production. One speculation is that this molecule may represent the ligand for a B-cell activation antigen.

T-cell clones that are defective in IL-4 production can induce germline IgE transcripts via a contact-mediated signal. However, both IL-4 and the contact-mediated signal provided by T cells are required to induce 2.2-kb productive ϵ-mRNA transcripts and IgE synthesis (32). The molecular mechanisms that underlie the production of productive ϵ mRNA in humans are presently a matter of speculation (reviewed in ref. 37).

T-CELL–INDEPENDENT SYSTEMS OF IgE INDUCTION

In addition to T cells, there is a growing list of direct (T-cell–independent) B-cell activators that can synergize with IL-4 to induce IgE synthesis. In this regard, it has been shown that stimulation with IL-4 and EBV induces T-cell–independent IgE synthesis in human B cells (28, 29). IgE production in this system was shown to be due to *de novo* induction of isotype switching rather than expansion of a precommitted sIgE+ B-cell population, which has undergone Cϵ switching *in vivo*, because sIgE-negative B-cell precursors could be stimulated by EBV and IL-4 to produce IgE. IgE-secreting B cells obtained by activation with EBV and IL-4 contained both the 1.8-kb germline Cϵ RNA transcript and the 2.2-kb productive ϵ mRNA (29).

More recently, several investigators have reported that highly purified B cells costimulated with rIL-4 and various MAb to CD40 synthesize high levels of IgE antibody (30, 31). Stimulation of B cells from nonatopic donors with anti-CD40 MAb, in the absence of IL-4, results in a small increase in IgG synthesis but no IgE or IgM synthesis. When both anti-CD40 and rIL-4 are added, IgG production increases slightly; however, large amounts of IgE are synthesized.

CD40 stimulation alone, however, can enhance IgE production from *in vivo* driven IgE-producing cells from atopic patients (31). In this regard, rIL-4 has been demonstrated to up-regulate CD40 expression on B cells. B cells from atopic donors have been found to have increased expression of CD40 on their cell surface (38). Thus, the capacity of anti-CD40 alone to enhance IgE production by B cells from atopic donors may reflect *in vivo* exposure to IL-4. These data suggest that the signals delivered for IgE production by IL-4 and CD40 stimulation could serve as a model for the activation of IgE synthesis seen *in vivo* in human allergic disease.

The natural ligand of CD40 is unknown. Molecular cloning has demonstrated that the CD40 gene is closely related to receptors for nerve growth factor (39) and tumor necrosis factor-α (40). The various anti-CD40 MAb have not been able to induce calcium mobilization (41). In the presence of primary B-cell activators such as phorbol esters or anti-IgM, however, anti-CD40 MAb can deliver a strong progression signal to resting B cells (42, 43). Thus, it has been suggested that CD40 may be

involved in the regulation of B-cell activation and growth. One possibility that has been suggested is that T cells express a ligand for CD40 on activation. This would implicate CD40 in noncognate, contact-dependent T-cell interactions.

Hydrocortisone has been found to up-regulate IL-4–dependent IgE synthesis by normal unfractionated mononuclear cells (33). These observations have been extended to demonstrate that sIgE− B cells from nonatopic donors can be induced to synthesize IgE when incubated with a combination of hydrocortisone and rIL-4 (34). The mechanisms by which hydrocortisone synergizes with IL-4 are unknown. However, these *in vitro* observations provide an immunologic basis for the *in vivo* studies, which demonstrate that atopic individuals frequently have an increase in serum IgE during the first 2 wk after systemic steroid therapy.

Taken together, these data indicate that the second signal(s) required for IgE production can be delivered to B cells through different activation pathways. In particular, it has been shown that anti-CD40 MAb and CD4+ T-cell clones act with rIL-4 to induce IgE switching in purified B cells via different signaling pathways (32). The exact mechanisms by which diverse signals such as T cells, EBV, hydrocortisone, and anti-CD40 stimulate IgE synthesis in the presence of rIL-4 remain to be elucidated. It is possible, however, that these different pathways will share the ability to activate switch recombination in B cells that have been incubated with IL-4 to render the Cε locus accessible.

MODULATION OF IgE SYNTHESIS BY CYTOKINES

Although IL-4 is critical for the induction of IgE synthesis, other cytokines have been found to modulate IL-4–induced IgE synthesis. In particular, IL-5, a nonisotype-specific B-cell growth factor (44), and IL-6, a B-cell differentiation factor, up-regulate IgE synthesis induced by IL-4 in PBMC (25). Indeed, endogenous IL-6 seems to be critical for IL-4–induced IgE synthesis in PBMC, inasmuch as anti–IL-6 antibody strongly inhibits the production of IgE in such cultures. In contrast, IL-1 and tumor necrosis factor-α have no effect on this response. Although some laboratories have reported that IL-2 enhances IL-4–induced IgE synthesis, others have found no significant effect.

IFN-γ, IFN-α, and transforming growth factor-β have been reported to suppress IL-4–induced IgE synthesis in experimental animals and humans (45–48). Of these three cytokines, IFN-γ has been studied most extensively for its capacity to inhibit IgE synthesis *in vitro* and *in vivo*. The capacity of human and mouse T-cell clones to induce IgE synthesis has been found to be directly correlated with the ratio of secreted IL-4 to IFN-γ (47). IFN-γ also antagonizes the effects of IL-4 in other systems, *e.g.* IFN-γ has been reported to inhibit the IL-4–dependent induction of CD23 on B cells (49). Of particular interest is the finding that the production of IFN-γ is down-regulated by IL-4 (27, 50). The capacity of IL-4 to induce IgE synthesis thus may reflect not only its ability to induce germline ε transcripts but also its ability to suppress the production of IFN-γ, a potent antagonist of IL-4 action.

The mechanism by which IFN-γ inhibits IgE synthesis remains unknown. It has been reported that IFN-γ inhibits the expression of ε germline transcripts in murine B cells stimulated with IL-4 and LPS (51). However, no inhibition of ε germline transcripts has been observed when IFN-γ is added to purified human B cells stimulated with IL-4 (24). These observations suggest that activation of ε germline transcription and switch recombination are separate events. IFN-γ may thus prevent recombination events without affecting the expression of ε-mRNA transcripts. It has also been observed that stimulation of IgE synthesis induced by IL-4 and anti-CD40 is not inhibited by IFN-γ (31, 32). IFN-γ can, however, inhibit IgE synthesis induced by IL-4 and EBV (28) and can inhibit ε germline transcription when B cells are stimulated with IL-4 in the presence of T cells (24). These data suggest that the capacity of IFN-γ to inhibit IgE synthesis may depend on the pathway of B-cell activation. Furthermore, the possibility remains that IFN-γ may act indirectly on T cells to either down-regulate IL-4 production or induce the production of another cytokine that can inhibit IgE synthesis.

COMPARTMENTALIZATION OF T CELLS BY CYTOKINE PROFILES

In 1986, Mosmann *et al.* (52) described two distinct types of cloned mouse helper T-cell lines that were defined primarily by differences in the pattern of lymphokines synthesized. TH1 but not TH2 cells produce IL-2, IFN-γ, and lymphotoxin, whereas TH2 but not TH1 cells produce IL-4, IL-5, IL-6, and IL-10 (53). IL-3 and granulocyte-macrophage colony stimulating factor are secreted by both types. TH1 and TH2 cells have been reported to remain well defined and functionally stable, at least in tissue culture.

Although both TH1 and TH2 cells can enhance B-cell proliferation, TH2 but not TH1 cells support B-cell antibody secretion (53). This may be due to the ability of TH1 cells to kill B cells, probably via IFN-γ and lymphotoxin production, as well as the capacity of TH2 but not TH1 cells to produce IL-6, a B-cell differentiation factor. The primary function of TH1 cells is thought to be involvement in mediating delayed-type hypersensitivity responses (54).

The selective expansion of TH2 cells is thought to play a critical role in inducing IgE synthesis because of the selective ability of IL-4 secreted by TH2 cells to induce Ig gene switching to the ε locus (22–24). The cytokines secreted by these two helper cells appear to be more important than the cell type providing the help because TH1 cells can provide help for IgE production in the presence of exogenous rIL-4 and anti–IFN-γ (53). In addition to stimulating IgE synthesis via IL-4, TH2 cells also enhance two other features of allergic responses. First, at least in mice, IL-3 and IL-4 are mast cell growth factors (55). Second, in humans and in mice, IL-5 induces the proliferation and differentiation of eosinophils both *in vitro* and *in vivo* (20, 56).

The mechanisms that regulate the differentiation of resting T cells into TH1 *versus* TH2 cytokine secretion phenotypes are not well understood. In mice, the cytokine environment has been identified as one important influence on the type of helper T cell generated. Gajewski *et al.* (57) have shown that TH1 cells are preferentially generated when CD4+ cells are cloned in the presence of IFN-γ. Conversely, Swain *et al.* (58) have reported that the presence of IL-4 during helper T-cell effector generation *in vitro* enhances the development of IL-4– and IL-5–secreting effectors while suppressing the development of effectors that can secrete IL-2 and IFN-γ.

Cytokines also influence the activation and growth of fully differentiated TH1 and TH2 cells. IFN-γ inhibits the proliferation of TH2-cell lines responding to either IL-2 or IL-4 but does not inhibit the proliferation of TH1-cell lines (59). IL-10 inhibits the synthesis of cytokines by TH1 cells but not TH2 cells (60). It is also likely that the nature of the antigen, as well as the antigen-presenting cell itself, can regulate the differentiation of helper T cells. In this regard, Finkelman *et al.* (48) recently presented evidence supporting the notion that antigen-presenting cells, by secreting IFN-α early in the course of an immune response, *e.g.* to an infectious agent, can down-regulate the IgE response but enhance IgG2a synthesis in mice.

RELEVANCE OF TH1/TH2 CELLS TO HUMAN ALLERGIC DISEASE

Although many mouse T-cell clones can be classified into either the TH1- or the TH2-cell pattern, a number of laboratories have shown that other cytokine secretion patterns can be observed in mouse T-cell clones (61, 62). Such patterns include the TH0-cell pattern, in which IL-2, IFN-γ, IL-4, and IL-5 are present. Other intermediate patterns have been seen, particularly when unimmunized mice are used as donors for T cell cloning.

Normal unimmunized mouse spleen T cells, termed "THp cells," when first stimulated produce high levels of IL-2 but not other T-cell cytokines (63, 64). Repeated antigen stimulation, however, results in the ability to produce other cytokines, such as IL-4 and IFN-γ. These experiments provide evidence for the hypothesis that there are precursor T cells that differentiate into TH1, TH2, and TH0 cells.

These results also fit well with data obtained with many human T-cell clones. In this regard, most alloreactive or phytohemagglutinin-induced human T-cell clones derived from the peripheral blood of normal donors have intermediate patterns of cytokine production that do not fit clearly into TH1 or TH2 cells. However, T cells isolated from diseased tissues or peripheral blood of patients with active disease have been found to exhibit TH1- or TH2-like cytokine profiles. Thus, CD4+ T cells isolated from thyroid glands of patients with autoimmune thyroiditis, when cloned by limiting dilution with phytohemagglutinin, develop into T-cell clones that produce IFN-γ but not IL-4. In contrast, most T cells infiltrating the conjunctiva of patients with vernal conjunctivitis develop into T-cell clones that produce high levels of IL-4 but not IFN-γ (65). Using *in situ* hybridization, Kay *et al.* (66) have also reported increased mRNA expression of IL-3, IL-4, IL-5, and granulocyte-macrophage colony stimulating factor but no IFN-γ mRNA expression in skin biopsies of allergen-induced late-phase reactions in atopic subjects.

The nature of the antigenic stimulus may play an important role in influencing T-cell differentiation. In this regard, the majority of allergen-specific T-cell clones from atopic individuals produce high levels of IL-4 and IL-5 but low levels of IFN-γ after antigen stimulation (67, 68). In contrast, virtually all T-cell clones specific for bacterial components, *e.g.* tetanus toxoid established from the same donors, produce high levels of IL-2 and IFN-γ (68). Del Prete *et al.* (69) also derived 60 T-cell clones specific for purified protein derivative of *Mycobacterium tuberculosis* and 69 T-cell clones specific for *Toxocara canis* from two healthy individuals. Their data indicate that the great majority of purified protein derivative-specific T-cell clones secreted IL-2 and IFN-γ but no or limited amounts of IL-4 and IL-5. In contrast, most *T. canis*-specific T-cell clones secreted IL-4 and IL-5 but no or limited amounts of IL-2 and IFN-γ.

The potential importance of a dysregulation of IL-4 and IFN-γ production in allergic diseases is further supported by immunologic characterization of PBMC from patients with AD and markedly elevated serum IgE levels. B cells and monocytes from AD patients express increased levels of the CD23 (low-affinity IgE receptor) surface antigen (70). Furthermore, peripheral blood B cells from AD patients spontaneously produce high levels of IgE (13). Because IL-4 plays an important role in the induction of IgE synthesis as well as CD23 expression on B cells (49) and monocytes (71), these observations suggest that AD is associated with increased secretion of IL-4 *in vivo*. In this regard, several investigators have reported that the increased spontaneous production of IgE *in vitro* by lymphocytes from AD patients can be inhibited by the addition of anti–IL-4 (72, 73). Furthermore, T cells from selected AD patients have been found to spontaneously secrete increased amounts of IL-4 (73).

PBMC from AD patients have also been found to have a decreased capacity to produce IFN-γ in response to a number of stimuli (73, 74). A significant correlation has been reported between IFN-γ generation *in vitro* and IgE serum concentrations *in vivo* in AD (74). Spontaneous IgE production by PBMC from AD patients can also be suppressed by the addition of IFN-γ (73). Taken together, these data suggest that an imbalance of IL-4 and IFN-γ production may account for many of the immunologic features found in patients with severe allergic disease and elevated IgE levels.

IMMUNOMODULATORY STRATEGIES IN CONTROL OF ALLERGIC DISEASES

The current management of allergic diseases is generally directed toward relief of the clinical symptoms present at any given time. Because patients with allergic diseases manifest abnormalities in immune regulation, there has been much interest in the development of therapy directed toward correcting their immune dysfunction or restoring the differential balance of cytokines involved in the allergic inflammatory response. As already discussed, IL-4 plays a critical role in the induction of IgE synthesis. In this regard, antibodies to IL-4 or its receptor completely block IgE responses in experimental animals (17, 18). As humanized antibodies directed to such specificities and cytokine or cytokine receptor antagonists become available, the safety and efficacy of such therapy may be tested. The recent cloning and expression of a recombinant extracellular domain of the human IL-4 receptor that inhibits the *in vitro* biologic effects of IL-4 on T and B lymphocytes also offers a novel therapeutic approach to counteracting the effects of IL-4 (75).

Several experimental immunomodulators have recently been studied in clinical trials for the treatment of AD. In a study by Leung *et al.* (76), thymopentin, a synthetic pentapeptide that promotes differentiation of thymocytes and T-cell production of IL-2 and IFN-γ, was found in a double-blind, placebo-controlled study of 100 patients to cause significant relief of pruritus and erythema due to AD. As already discussed, IFN-γ suppresses the *in vitro* IgE response. Several investigators have therefore assessed the *in vivo* effect of IFN-γ in patients with hyper IgE syndrome and AD. King *et al.* (77) reported that two patients with hyper IgE syndrome had a decrease in serum IgE levels while receiving IFN-γ by s.c. injection. In an open-label study, Boguniewicz *et al.* (78) presented preliminary data in 23 patients with chronic AD suggesting a marked reduction in clinical severity after therapy with rIFN-γ. In this study, there was significant inhibition of spontaneous IgE synthesis by circulating lymphocytes while patients were on therapy. However, no effects on serum IgE levels were noted, even in patients on long-term maintenance therapy. These results suggest that the clinical efficacy of IFN-γ in AD is not due to an effect on serum IgE levels.

More recently, in a double-blind, placebo-controlled trial, rIFN-γ therapy was found to be significantly more effective than placebo in reducing the clinical severity of AD (79). During this trial, AD patients treated with IFN-γ had a significant reduction in numbers of circulating eosinophils. These observations are intriguing because IL-4 and IL-5 production compartmentalizes to the TH2-cell subpopulation. Recent studies have demonstrated that allergen-specific T cells isolated from the AD skin lesion produce high levels of IL-4 but no or low levels of IFN-γ, *i.e.* a TH2 cytokine phenotype (80). Furthermore, in mice, it has been shown that IFN-γ inhibits the proliferation of TH2 cells (59). Taken together, these observations suggest that the administration of rIFN-γ *in vivo* may act by inhibiting TH2-cell expansion and therefore result in a reduction of IgE synthesis and eosinophil differentiation by indirectly inhibiting IL-4 and IL-5 production. Further studies are needed to test this hypothesis by examining the effects of IFN-γ on human TH2 cells.

Other immunomodulatory approaches to the treatment of allergic diseases have also been used. These include the use of cyclosporine, a drug that reduces IgE production and inflammation possibly by inhibiting cytokine production by T cells (81). Intravenous immune globulin, given at high doses, has been reported to have immunomodulatory effects that include the inhibition of IgE synthesis and reduction of allergen-induced immediate skin test reactivity in patients with severe asthma (82). In the future, these approaches to therapy as well as other immunologic approaches to the treatment of allergic diseases are likely to arise as we better understand the mechanisms that underlie allergic diseases. If further controlled studies confirm the safety and efficacy of these immunologic treatment approaches, these may represent exciting alternatives to be used in patients who are resistant to currently available therapy.

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FLOOR DISCUSSION

Dr. Levinson: A recounting of your work and Dr. Geha's work that deals with IgE-binding factors was conspicuously absent from your excellent review. For those of us who grew up during the era of IgE-binding factors with your work and Ishizaka's work, can you tell us where that stands at the present time?

Dr. Leung: The reason for not including it is primarily because of the inability to clone and better characterize that factor. There is still reason to believe that there may be such factors; however, the closest that has been really gotten to cloning an IgE-binding factor has been the soluble CD23 molecule that both Jan de Vries' group and Andy Saxon's group have shown induces IgE synthesis. Certainly, that molecule is very similar to the binding factors that we originally described, in the sense that it seems these IgE-binding factors are B-cell differentiation factors. They are certainly not like IL-4, in the sense that they are not isotype-switch factors.

Dr. Hill: In our chronic granulomatous disease study, we used the drug three times a week. As I understand it, you used it every day in those patients. Was there a reason for doing that? And also, did you see any toxicity with the lower dose that you indicated that you used?

Dr. Leung: With the very lowest dose, 10 $\mu g/M^2$, there is absolutely no toxicity. It was only at the 50, and of course at the 100-μg level, that 25% of the doses were associated with flu-like symptoms. Coming back to the issue of three times a week *versus* daily, I agree that one would have liked to have had even three arms in that study to look at that, and certainly that might be a basis for a phase IV trial. The reason for picking daily doses was really for statistical reasons. Based on the number of people entered, we had to pick some sort of a schedule. In this regard, the original open label study showed that even within 2 d of discontinuing IFN-γ there was a tendency for a rebound in terms of worsening skin symptoms, as well as a rebound in IgE synthesis. That was our reason for deciding to go with the daily doses. But I agree there are patients even on prophylaxis now or maintenance who are doing pretty well at three times a week, and the issue is titrating the IFN-γ dose.

Dr. Hill: Did you see neutropenia or lymphopenia developing at any time?

Dr. Leung: There was some, particularly in patients early on, but this was a reversible phenomenon. As they stayed on the IFN-γ longer, in almost all cases their neutrophil counts went back to normal. Indeed, with the eosinophil data that I showed, we were concerned that maybe the reduction in eosinophils was due to this granulocytopenia effect, but in fact an analysis demonstrated no correlation between reduced white cell counts and reduction in eosinophil counts.

Dr. Ochs: Since you and Dr. Gelfand are now teamed up, what about teaming up IFN-γ and IVIG (intravenous immune globulin)? We have for years heard Dr. Gelfand telling us that we should use IVIG in hyper-IgE syndromes and in asthmatic patients, and now you have an IFN-γ that is reasonably well tolerated, and it works in some patients. Is it too much to ask you to do this kind of a study?

Dr. Leung: We have submitted a grant application to study this further. Let me just say that I think IFN-γ is probably going in that direction, not to be used as an exclusive drug, but as an adjuvant or in combination with other drugs. I don't know if it will be used with IVIG because that works well alone.

Dr. Gulati: Do you foresee any role for IVIG and IFN-α?

Dr. Leung: There might be. The problem is that the companies who have IFN-α do not appear to be interested, at this time, in using it clinically in this setting, partly because there are more side effects from IFN-α than IFN-γ. One of the things I think that has not been well studied is whether patients who fail on doses of IFN-γ at 50 $\mu g/M^2$ might not do better on higher doses.

What we are looking at now is whether one can direct the cytokine therapy into particular organs to minimize side effects. As an example, Ron Crystal's group published a paper in the *Journal of Clinical Immunology*, in May 1991, showing the feasibility of nebulizing IFN-γ into the lung. We are beginning to look at some trials along those lines, because when you nebulize IFN-γ into the lung, you do not get the systemic side effects, even giving 10 times higher doses than we give subcutaneously.

Dr. Levinson: You have shown a lot of nice associations in terms of things working in the way we might like them to work, vis-à-vis with IFN-γ. The eosinophils are going down, but the total IgE isn't going down. But it is not clear to me that the effects that you are seeing are in fact mediated through TH2 cells or even through T cells themselves. Let me throw out another possibility for consideration. Several years ago, a dermatologist showed that if you treat patients with AD with erythromycin, they improve markedly because they are always colonized with staphylococci. Is it possible that all you are doing, in fact, is activating neutrophils to make them behave better, whereas they may not behave too well in the first place in AD? Maybe you are clearing staph or doing something that might not be too interesting to you as an immunologist but would certainly be interesting to you as a clinician.

Dr. Leung: Those are very interesting questions. We did superoxide generation studies and looked at neutrophil chemotaxis in AD patients treated with IFN-γ *versus* placebo. There was no difference between the placebo and the IFN-treated group, although Harry Hill has certainly described the effects of IFN-γ on neutrophil chemotaxis in the hypo-IgE syndrome. I think, like everything else, IFN-γ is a pleiotropic drug. It is going to work on many factors. This, of course, was just a hypothesis.

Dr. Gelfand: With erythromycin, the interest is also as a macrolite antibiotic; it does have some effects on T cells, like troleandomycin, which is also like the cyclosporin FK5 or 6 effects. You may be modulating different cytokines.

Dr. Schiff: With your observations that the eosinophil count is going down, have you treated any patients with hyper-eosinophil syndrome or eosinophilofasciitis? Do you think it would have any role in these patients? I am particularly interested in production of IL-5. We are looking at that now in a couple of patients, trying to treat them by modulating T cells rather than trying to use cytotoxic drugs.

Dr. Leung: We have not seen any hyper-eosinophil syndrome patients. The problem is that the disease itself is so rare that it would be hard to do a controlled trial to convince yourself you were seeing an effect. It would almost be anecdotal, but potentially it is worth trying on a case-by-case basis.

Dr. Schreiber: Following up on the comment about IFN-γ effect on phagocytes, I want to suggest an experiment in terms of looking at chemotaxis or other factors. I think it is probably a more sensitive indicator. We have shown that very low doses of IFN-γ will up-regulate at least two different Fc receptor gene products, one of which is a very potent mediator of a phagocytic signal in our hands. So I think that would be the direction to look at if you wanted to address that specifically.

Dr. Leung: There is a problem in AD, and this is really a bias on my part, but when I was in Boston, we decided that since staph was so important in eczema, we would try a bacterial desensitization schedule, and actually try to introduce staph 502A into the patients' flora. We found that when we gave them a regimen of Bactrim or rifampin, these patients would improve dramatically, but when we did staph counts, there was no change in the number of *Staphylococcus aureus*. So I suspect that part of it may even be modulation of the toxin production rather than enhanced phagocytosis or eradication of *S. aureus*. We have never personally been able to demonstrate any neutrophil defect, at least in AD.

Dr. Schreiber: On the other hand, you can get up-regulation of a couple of receptors. Even though they might not be defective, they may perform much more effectively because you are dealing with an important regulatory molecule in that pathway.