

Antagonizing the differentiation and functions of human T helper type 2 cells

Jan E de Vries, José M Carballido, Thierry Sornasse and Hans Yssel

DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, USA

Allergen-specific T cells from atopic patients generally belong to the T-helper type 2 subset. IL-4 and IL-13 produced by these cells induce IgE synthesis by B cells and play a major role in allergic disease mediated by IgE. Recent advances in our understanding of the differentiation and IgE-inducing activities of T-helper type 2 cells suggest that targeting allergen-specific T cells may provide a novel way to intervene in allergy.

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Introduction

Allergen-specific T cells generally belong to the T-helper type 2 subset, producing relatively high levels of IL-4, IL-5 and IL-13, but undetectable, or low levels of IFN- γ following activation [1–3]. Both IL-4 and IL-13 induce the synthesis of human IgE [4–6]. In addition to these cytokines, contact-mediated signals between CD40 ligand (L), which is transiently induced on T cells following their activation, and CD40, which is constitutively expressed on B cells, are required for productive interactions between T cells and B cells resulting in the synthesis of IgE [7]. From these observations, it is clear that antagonizing the biological activities of IL-4 and IL-13, or inhibition of Th2-cell development, will provide an efficient way to intervene in the production of IgE and IgE-mediated allergic disease.

In this review, we will discuss the effects of a human IL-4 and IL-13 receptor (R) antagonist on human IgE synthesis induced by IL-4 and IL-13 *in vitro*. Furthermore, its effects on ongoing human IgE synthesis by severe combined immune deficient (SCID)-hu mice *in vivo* will be discussed. In addition, it we will describe that the IL-4R and IL-13R antagonist blocks IL-4-driven differentiation of Th2 cells producing IL-4, IL-5 and IL-13 *in vitro*. Finally, we will describe that triggering of a novel 70 kDa receptor expressed on T cells enhances antigen-specific T-cell expansion and strongly upregulates IFN- γ production, even in allergen-specific Th2 clones.

IL-4R and IL-13R are complex structures sharing the IL-4R α chain

IL-4 and IL-13 share most biological activities on B cells, monocytes, natural killer (NK) cells and endothelial

cells [8–10]. In contrast to IL-4, however, IL-13 does not act on human T cells [8,11*], B cells transformed by Epstein-Barr virus (EBV), or Burkitt's lymphoma cells [12]. Binding studies carried out with radiolabeled IL-13 have indicated that human T cells do not express IL-13R [11*]. Thus, the high affinity receptors for IL-4 and IL-13 are distinct receptors. This notion was confirmed by the observation that IL-13 does not bind to the 130 kDa IL-4R α chain transfected into heterologous cells [13]. However, IL-13 can partially compete with IL-4 for binding to the TF-1-cell line, which responds both to IL-4 and IL-13. In addition, a human IL-4 mutant protein, hIL-4.Y124D, in which the Tyr residue at position 124 in the carboxy-terminal α helix is replaced by Asp, inhibits the biological activities of both IL-4 and IL-13 ([13–15]; see below). These observations indicate that IL-4R and IL-13R are complex receptors, which share a component required for signal transduction. More recently, it has been shown that this common component is the IL-4R α chain, because blocking studies carried out with anti-hIL-4R α monoclonal antibodies (mAbs) not only inhibited both the binding and biological activities of IL-4 on B cells or monocytes, but also blocked the biological activities of IL-13 [16*,17*]. In addition, both IL-4 and IL-13 stimulate tyrosine phosphorylation of the IL-4R α chain [18*]. Thus, the IL-13R consists of the IL-4R α chain (which cannot bind IL-13), and a primary IL-13R-binding protein designated IL-13R α . To date, no information is available about the IL-13R α chain, but cross-linking studies identified a ~65 kDa protein, which specifically associates with IL-13R, and which likely represents the IL-13R-binding protein [7].

It has been established that the 75 kDa IL-2R γ subunit is a component of the IL-4R, which functions in signal transduction and slightly enhances the ability of IL-4R α to bind IL-4 [19,20]. The IL-2R γ chain is also a shared

Abbreviations

APC—antigen-presenting cell; h—human; IFN—interferon; IL—interleukin; mAb—monoclonal antibody; R—receptor; SCID—severe combined immune deficient; TCR—T-cell receptor; Th2—T-helper type 2.

component of the receptors for IL-7, IL-9 and IL-15 and is therefore, called the common γ (γ_c) chain [21–24]. Recent studies have indicated that B cells from X-linked SCID patients, who have mutations in their γ_c chain, fail to respond to IL-2, but respond normally to IL-13 and IL-4, indicating that IL-4 can signal via binding to the IL-13R complex [25]. In contrast, T cells from these patients do not respond to IL-4, indicating that the γ_c chain is required for IL-4 signal transduction in these cells. This implies that two types of IL-4R exist (see Fig. 1): one that consists of the IL-4R α and γ_c chain, and another that is comprised of IL-4R α chain and the primary IL-13-binding protein, designated the IL-13R α chain. This latter receptor functions also as the IL-13R. Both types of IL-4R are expressed on B cells, monocytes, NK cells and endothelial cells, whereas T cells express only the IL-4R α - γ_c chain complex. The γ_c chain is not a component of the mouse IL-13R, since neutralizing anti- γ_c chain mAbs, which blocked IL-4-induced proliferation of the mouse mast-cell line MC/9, failed to affect IL-13-induced proliferation of these cells, indicating that the IL-13 effects occur independently of the presence of a γ_c chain [26]. Recent studies have shown that both IL-4 and IL-13 were able to upregulate the expression of CD54 on human renal cell carcinoma cells, which lack γ_c chain expression, indicating that the γ_c chain is also not a subunit of the human IL-13R [27]. The finding that IL-4.Y124D acts as an antagonist of IL-4R and IL-13R can now be explained by the fact that the IL-4 mutant protein, because of its high-affinity binding to the IL-4R α chain (without receptor activation), competes with IL-4 for binding to both receptors. In addition, binding of IL-4.Y124D to the IL-4R α inhibits activation of IL-13R, probably by preventing the binding of IL-13 to the IL-13R α chain, or by preventing association between IL-4R α and IL-13R α .

Production of IL-4 and IL-13

Both peripheral blood CD4⁺ and CD8⁺ T cells produce IL-13 and IL-4 following their activation [11]. IL-13 is also produced by naive CD4⁺ CD45RA⁺ T cells (T Sornasse *et al.*, unpublished data). In contrast, CD45RA⁺ T cells fail to produce detectable levels of IL-4, but they can be induced to produce IL-4 following repetitive activation via the TCR in the presence of IL-4 [28]. CD4⁺ CD45RO⁺ 'memory' T cells produce both IL-4 and IL-13 [28]. In addition, analysis of IL-13 production by cloned human CD4⁺ T-helper subsets showed that IL-13 is produced by Th0, Th1 and Th2 T-cell clones [11]. The average levels of IL-13 production by these various T-cell subsets were within the same range, suggesting that IL-13, in contrast to IL-4, is not a typical Th2 cytokine, because human Th1-like cells are either very poor producers of IL-4, or fail to do so [29–31]. In addition, the majority of the CD8⁺ T-cell clones produced low levels of IL-13. Finally, concentrated culture supernatants of EBV-transformed B-cell lines secreted very low levels of

IL-13 constitutively, indicating that IL-13 production is not restricted to T cells [11].

Interestingly, IL-4 and IL-13 production by human T cells follow different kinetics. The level of expression of both IL-4 and IL-13 mRNA was highest at two to four hours after activation, but considerable levels of only IL-13 mRNA expression and IL-13 protein production could be detected at >48 hours after activation. In contrast, the expression of IL-4 mRNA was undetectable at 24 hours after activation, which correlated with the production of IL-4 protein, which was also transient [11]. Thus, activated human T cells still produce IL-13 after they cease to produce IL-4, which may have important biological consequences.

Inhibition of IgE synthesis by an antagonist of IL-4R and IL-13R

Both IL-4 and IL-13 induce IgE synthesis by human B cells *in vitro* [4–6]. In addition, enhanced production of IL-4 has been shown to be associated with increased levels of IgE observed in atopic patients [32,33]. Little is known, however, about the role of IL-13 in allergic disease. Although the relative contribution of IL-4 and IL-13 to IgE synthesis *in vivo* remains to be determined, it is clear that inhibition of the effects of both IL-4 and IL-13 is required for optimal intervention in the production of IgE and IgE-mediated disease.

The human IL-4 mutant protein IL-4.Y124D binds with high affinity to the IL-4R α chain without receptor activation, and therefore acts as an antagonist for both the IL-4R and IL-13R [13–15]. IL-4.Y124D inhibits proliferation of the TF-1-cell line induced by IL-4 or IL-13 [14]. Furthermore, IL-4.Y124D blocks both IL-4-induced and IL-13-induced proliferation of B cells activated by anti-CD40 mAbs. In addition, the mutant IL-4 protein efficiently inhibited IL-4- and IL-13-induced IgG₄ and IgE production *in vitro* [15]. In general, >90% inhibition of the synthesis of IgG₄ and IgE was observed at concentrations of the mutant protein that were 20–50-fold greater than the concentrations of IL-13 or IL-4. Despite the fact that IL-4.Y124D and human IL-4 proteins differ in only one amino acid, IL-4.Y124D had minimal agonistic activity, which was detectable only at concentrations that were >100-fold higher than the optimal concentration of IL-4 [15].

In order to determine whether IL-4.Y124D was also effective *in vivo*, we established a SCID-hu mouse model. For this purpose, SCID mice were transplanted with pieces of human fetal bone (as a source of bone marrow) and fetal thymus. Two to three months following transplantation, considerable numbers of human CD4⁺ and CD8⁺ T cells, B cells, monocytes and other myeloid cells could be detected in these mice. All SCID-hu mice spontaneously produced human IgM and all human IgG subclasses in the same ratios as observed in normal individuals. In addition, 80% of the mice produced human IgA, whereas approximately

one third of the mice also produced human IgE spontaneously [34•]. These observations indicate that productive interactions between human T-helper cells and B cells, probably in response to environmental antigens, result in human Ig isotype switching *in vivo*. Administration of IL-4.Y124D to SCID-hu mice, with ongoing human IgE synthesis, by subcutaneously implanted osmotic minipumps at concentrations of 20 µg per day for three weeks, reduced the circulating levels of human IgE by >90%. No agonistic activity of IL-4.Y124D, as judged by the increase in the level of the expression of CD23 on human B cells, could be detected following this treatment period. The effects of IL-4.Y124D were specific for IgE, as total human IgG levels were not affected. In addition, administration of phosphate buffered saline (PBS) as a control failed to reduce the levels of human IgE in these mice [34•]. These results indicate that relatively large polypeptides, like hIL-4.Y124D, are effective for reducing ongoing human IgE synthesis in SCID-hu mice *in vivo*, and therefore may have potential clinical use in reducing allergen-specific IgE levels in allergic individuals, thereby ameliorating allergic disease.

The effects of IL-4.Y124D on T-helper cell type 2 differentiation

Allergy is a disease mediated by Th2 cells. Allergen-specific T cells generally belong to the Th2 subset, which produces relatively high levels of IL-4, IL-5, IL-13 and low, or undetectable, levels of IFN-γ following activation [1–3,29]. Th2 cells are found at sites of allergic reactions following allergen challenges in atopic or asthmatic patients [35–38]. Activation of allergen-specific Th2 cells by the relevant allergens results in expansion of these cells and production of IL-4 and IL-13, amongst other cytokines. The mechanisms by which Th2 cells are selected in atopic patients are still ill-defined; however, mouse studies have shown that the presence of IL-4 during the initial priming of naive T cells by antigen results in the generation of Th2 cells that produce IL-4 and IL-5, both *in vitro* and *in vivo*, whereas the differentiation of IFN-γ-producing Th1 cells is inhibited [39–41]. In contrast, IL-12, which is produced by macrophages, dendritic cells and B cells, is the dominant factor in inducing the differentiation of naive CD4⁺ cells to IFN-γ-producing antigen-specific Th1 cells [42–44,45•]. In addition, IL-12 transiently induces, or enhances, IFN-γ production by established allergen-specific human Th2-like clones, indicating that the cytokine profile of polarized human Th2 cells still can be modulated [46•,47•].

IL-12, IFN-γ and IFN-α can also enhance, to various extents, the development of cells producing IFN-γ in cultures initiated with anti-CD3 activated CD4⁺ T cells derived from cord blood, whereas the generation of T cells producing IL-4 is downregulated by the same cytokines. In an opposite fashion, IL-4 enhanced the generation of T cells that produce IL-4 and

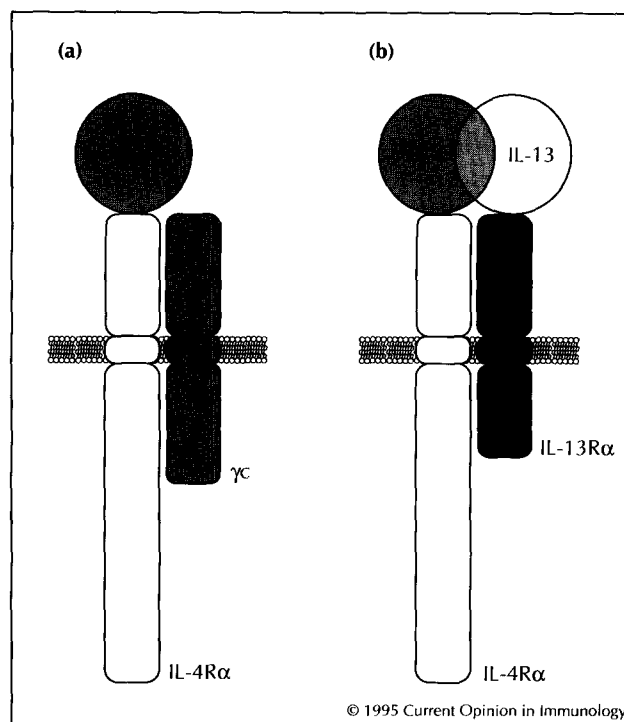


Fig. 1. Schematic representation of the two types of IL-4R. (a) One type of IL-4R consists of the IL-4Rα chain and the γc chain. (b) The other type of IL-4R consists of the IL-4Rα chain and the primary IL-13-binding unit, designated the IL-13Rα chain. This latter receptor functions also as the IL-13R.

IL-5, whereas the development of IFN-γ-producing T cells was suppressed [48,49,50•]. Furthermore, *in vitro* activation of peripheral blood mononuclear cells from allergic patients by allergens in the presence of IFN-γ, IFN-α or IL-12, resulted in a preferential outgrowth of cells with a Th1-like phenotype, instead of the normal Th2-like cytokine profile [51,52]. These results suggest that human T cells that have been exposed to allergens *in vivo* for prolonged periods of time can still be redirected *in vitro*. Alternatively, it cannot be ruled out that the apparent reversal of the cytokine production profile of this human T-cell subset is a result of the differentiation of uncommitted T cells. Fully polarized murine Th1 or Th2 cells seem to be more resistant to a reversal in their cytokine production patterns [53•,54•]. In particular, mouse Th2 cells that produce IL-4 appear to be resistant to the effects of IL-12 and anti-IL-4 mAbs, which may reflect a downregulation of receptors for Th1 induction [54•]. A recent report [55•], however, has shown that IL-12, when given together with a standard drug to reduce the parasite load, reverses established Th2 responses to chronic infection with *Leishmania major* to healing Th1 responses, indicating that polarized mouse Th2 responses still can be modulated under certain conditions *in vivo* [55•].

Thus far, most studies of human Th-cell subset development have been carried out with cells that have already been primed *in vivo* [51,52], or with cells that have been stimulated in an antigen-specific fashion and analyzed at the population level [48,49,50•,51].

Recently, we demonstrated that IL-4 could also drive the differentiation of human Th2 cells at the single-cell level. The repetitive activation of highly purified naive CD4⁺ CD45RA⁺ T cells isolated from cord blood with anti-CD3 mAbs cross-linked by human CD32 (FcγRII) transfected into mouse fibroblasts (L cells) which were co-transfected with CD58 and CD80 in the presence of IL-4, resulted in the generation of relatively high numbers of individual cells producing IL-4, IL-5, IL-10 and IL-13, as judged by the intracellular presence of these cytokines (T Sornasse *et al.*, unpublished data). In contrast, the frequencies of cells producing IFN-γ were low, but not completely absent, under these culture conditions, indicating that the differentiation of Th1 cells producing IFN-γ was not totally prevented by IL-4. As IL-4 is species specific, and does not act on mouse L cells, it has to be concluded that its capacity to induce Th2-cell differentiation reflects a direct effect on T cells. In contrast, IL-13 was ineffective in this culture system, which is compatible with the observation that IL-13 does not act on T cells. More importantly, it illustrates that the absence of IL-13R on T cells may have major biological consequences in that IL-13, in contrast to IL-4, cannot direct Th2-cell development. It is clear that these results need to be confirmed in *in vivo* experiments. This is of importance, as IL-13, through its capacity to downregulate the production of IFN-α and IL-12 (both of which direct Th1 development) by monocytes or dendritic cells, may still indirectly favor the generation of Th2 cells [56].

Repetitive activation of the CD45RA⁺ cord blood T cells in the presence of IL-12 induced, as expected, high frequencies of cells producing IFN-γ. In addition, IL-12 induced consistently low levels of IL-4 protein, which was predominantly secreted by Th0 cells which simultaneously produced IFN-γ. IL-12 also induced moderate levels of IL-13 and high levels of IL-10 production in these cultures.

Interestingly, IL-4.Y124D added at a concentration of 100 ng per ml, which is a tenfold excess compared to the concentration of IL-4, completely prevented the differentiation of Th2 cells producing IL-4 and IL-5 in these cultures, indicating that this IL-4 mutant protein effectively antagonizes Th2-cell development driven by IL-4 *in vitro*. The production of IL-10, however, was only partially inhibited by IL-4.Y124D, indicating that the differentiation of naive T cells into T cells producing IL-10 is not solely dependent on the presence of IL-4. Furthermore, this observation confirms the notion that IL-10 is not a typical Th2 cytokine [57,58]. In contrast, IL-13 production was abolished by the IL-4 mutant protein, indicating that, under these conditions, the differentiation of IL-13-producing cells is completely dependent on IL-4 (T Sornasse *et al.*, unpublished data). Thus, in addition to its capacity to antagonize IL-4-induced and IL-13-induced B-cell proliferation and IgE synthesis, the IL-4 mutant protein prevents the IL-4-driven differentiation of human Th2 cells *in vitro*.

T-cell stimulation via SLAM upregulates IFN-γ production

Stable mouse Th1 and Th2 clones are generally established from highly polarized T-cell subsets, which develop in response to hyperimmunization or chronic infection. In humans, highly polarized Th1 and Th2 clones have been established from patients with chronic infectious diseases or allergy [1,2,30]. A number of studies, however, have shown that most allergen-specific CD4⁺ T-cell clones obtained from atopic individuals, who often have been chronically exposed to allergens for years, still produce low levels of IFN-γ in addition to high levels of IL-4 and IL-5 following stimulation with allergen and antigen-presenting cells (APCs). Because of their high IL-4 : IFN-γ ratios, these clones are considered to be Th2-like [59–62]. Similarly, Th clones specific for the 65 kDa heat shock protein of *Mycobacterium lepra* and derived from tuberculoid leprosy patients produced high levels of IFN-γ following activation, but generally also low levels of IL-4 [30]. These clones were considered to be Th1-like because of their high IFN-γ : IL-4 ratios. The low levels of IFN-γ production by allergen-specific Th2-like clones were strongly upregulated following activation by Ca²⁺ ionophore and the phorbol ester TPA, indicating that the IFN-γ gene is not silent in these cells and that their cytokine production phenotype can be modulated depending on the mode of activation [57].

Recently, we found that costimulation of human T cells through a novel 70 kDa T-cell surface receptor, SLAM, also strongly enhanced the production of IFN-γ [63*]. SLAM is a novel member of the Ig gene superfamily and is constitutively expressed on CD4⁺ CD45RO⁺ memory T cells and CD4⁺ CD8⁺ thymocytes, a proportion of single positive CD4⁺ or CD8⁺ thymocytes, peripheral blood and splenic B cells. The expression of SLAM, however, is rapidly induced on all B cells and CD4⁺ and CD8⁺ T cells following activation. Engagement of SLAM by an agonistic mAb results in the enhanced proliferation of antigen-specific T cells, indicating that SLAM acts as a costimulatory molecule for human peripheral blood T cells. In addition, once the T cells are activated, ligation of SLAM results in direct T-cell proliferation. Moreover, antigen-induced cytokine production by CD4⁺ T-cell clones belonging to the various T-cell subsets was upregulated after engagement of SLAM by the agonistic mAb. Particularly strong increases (up to 20-fold) in IFN-γ production were observed in allergen-specific Th2-like clones. Following activation by allergen and APCs in the presence of the anti-SLAM mAb, the levels of IFN-γ production by these Th2 clones were comparable to those induced by antigen and APCs in Th1 and Th0 clones.

In contrast to its effect of inducing IFN-γ production by Th2 clones, the anti-SLAM mAb failed to induce the production of IL-4 and IL-5 by Th1 clones. Furthermore, ligation of SLAM had little (less than twofold), or no enhancing effects on the production IL-4

and IL-5 by antigen-activated Th2 and Th0 clones [63]. Collectively, these results indicate that the triggering of SLAM during antigen-specific T-cell activation results not only in further expansion of these cells, but also in a preferential induction of IFN- γ production, even in allergen-specific Th2 clones. Furthermore, they suggest that engagement of SLAM by its putative ligand can reverse the cytokine production profile of these cells to a Th0 cytokine phenotype, whereas the cytokine production pattern defining Th1 clones is not altered by concomitant stimulation via SLAM. This confirms the notion that human Th2-like clones, which produce high levels of IL-4 and IL-5, still have the capacity to produce IFN- γ [46*,47*,61]. On the basis of these observations, it is tempting to speculate that interactions between SLAM and its putative ligand may also favor the development of cells producing IFN- γ *in vivo*, thereby preventing the generation of Th2 responses, including allergen-specific Th2 responses. In addition, ligation of SLAM may, through enhanced IFN- γ production, counteract the IgE-inducing effects of IL-4 and IL-13 [5].

Conclusions

Allergen-specific CD4⁺ Th2-like cells, which produce IL-4, IL-5 and IL-13, play a pivotal role in allergic diseases. Expansion of these cells in response to allergen challenge, together with a downregulation of Th1 cells producing IFN- γ , most likely accounts for the enhanced IgE levels observed in atopic patients. Therefore, one efficient way to intervene in the allergic disease process is to target allergen-specific Th2 cells. We have described here that an IL-4 mutant protein efficiently inhibits human IgE synthesis induced by IL-4 and IL-13 *in vitro*, which is consistent with the notion that the IL-4R and IL-13R share the IL-4R α chain, which is involved in signal transduction. More importantly, the mutant protein also efficiently inhibited ongoing human IgE synthesis in a SCID-hu mouse model, proving the point that this mutant protein is also effective *in vivo* and may therefore have potential clinical use in downregulating allergen-specific IgE levels in atopic patients and reducing IgE-mediated allergic disease. As the IL-4 mutant protein also blocked IL-4-induced Th2 development *in vitro*, it is presently not clear whether the downregulation of human IgE synthesis in SCID-hu mice can be attributed to the inhibition of Th2-cell differentiation, antagonizing the IgE inducing effects of IL-4 and IL-13, or both. Taking into account that IL-13, in contrast to IL-4, does not induce Th2 development *in vitro*, which is the hallmark of allergic disease, one could speculate that IL-4 may be the more dominant cytokine in driving and maintaining allergic responses. First, however, it needs to be confirmed that IL-13 indeed fails to induce Th2 development in *in vivo* models in which IL-4 is effective. On the other hand, the observation that IL-13, in contrast to IL-4, is produced by naive CD45RA⁺ T cells, and that T cells continue to produce IL-13 after the production of IL-4 by these

cells has ceased, raises the possibility that IL-13 may play a unique role in both the initiation and maintenance of IgE production.

Polarized mouse Th1 cells, and particularly Th2 cells, established following chronic antigen stimulation seem to be more resistant to phenotype reversal than human Th1 and Th2 populations. Nevertheless, deadly Th2 responses to *L. major* in BALB/c mice could, under certain conditions, still be reversed to a healing Th1 response by the administration of IL-12 [55*]. Although highly polarized human Th1 and Th2 clones have been described, evidence is emerging that human T-cell clones (even T-cell clones obtained from patients who had been chronically exposed to infectious agents, or allergens) are less polarized than their mouse counterparts. This may be the reason why these clones, which are categorized as Th1-like or Th2-like on the basis of their high IFN- γ :IL-4 or IL-4:IFN- γ production ratios, respectively, are more amenable to modulation. This has been illustrated by the capacity of IL-12, or anti-SLAM mAbs to induce or enhance IFN- γ production by allergen-specific Th2-like clones established from allergic patients who were chronically exposed to the relevant allergens. Whether IL-12 or anti-SLAM mAb treatment will indeed be effective in redirecting established allergen-specific Th2 responses *in vivo* remains to be determined. However, the present information indicates that IL-4 mutant protein, because of its ability to inhibit IL-4-driven Th2 differentiation and ongoing human IgE synthesis in SCID-hu mice, may have clinical potential in the treatment of allergic diseases.

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- JE de Vries, JM Carballido, T Sornasse and H Yssel, DNAX Research Institute of Molecular and Cellular Biology, Human Immunology Department, 901 California Avenue, Palo Alto, CA 94304-1104, USA.