



Review

Cytokine-Based Immunotherapy of Allergic Disease

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Abstract. Human immediate hypersensitivity diseases are strongly associated with an excessive type 2 response to normally innocuous environmental antigens, and are a growing health care concern in developed nations. Commonly prescribed treatments provide effective symptomatic relief, but are unable to consistently ameliorate the underlying cause of allergic disease: the excessive generation of allergen-specific Th2 cells. IL-12 and IL-18 are potent inducers of type 1 immunity, and, as such, have been proposed as candidates for treatment of allergic diseases. This review critically assesses the potential of recombinant IL-12 and IL-18 immunotherapy to redirect both *de novo* and established allergic responses in animal models of human allergic disease to clinically protective immune responses.

Key words: IL-12; IL-18; immunotherapy; immediate hypersensitivity.

Introduction

CD4⁺ T cells play a pivotal role in regulating both the type and intensity of immune responses. The principal model of T cell function views them as subsets which are defined by the cytokine repertoire they produce. In the murine system, Th1 cells promote type 1 immunity (producing IFN- γ which drives cell-mediated immunity and IgG_{2a} synthesis), while Th2 cells enhance type 2 immunity (producing IL-4, IL-5 and IL-13, which promote antibody-mediated responses and class switching to IgG₁ and IgE)^{8, 40}. This model has proven useful for predicting and understanding a wide range of *in vivo* immune responses. Type 1 responses are protective in some situations (viral and protozoal infections) while type 2 responses are protective in others (helminthic infections). These immune responses are detrimental when not appropriate (i.e. autoimmune diseases) or when excessive (e.g. allergic diseases). At the same time, while the Th1/Th2 paradigm is useful,

there is growing awareness that it is an oversimplification of a complex process^{30, 41, 66}.

Much work has been done to determine what causes differentiation of naive T cells into Th1- vs Th2-like patterns. Many factors have been implicated, but one of the most important is the cytokine mixture present at the onset of the immune response. IL-12 and IL-18 both drive differentiation of Th1 cells (reviewed in^{16, 35}), while IL-4 favors differentiation towards Th2 patterns (reviewed in⁴⁶).

Allergic disease is one of the most prevalent chronic health problems in developed nations²⁵. Many factors influence generation of the allergic response, including the genetic background of the host, the intrauterine environment, pollution and infection (or more specifically, lack thereof – reviewed in¹⁷). At present, the most widely prescribed strategy for immunologic manipulation of allergic diseases is allergen immunotherapy, which involves repeated injections of increasing doses of allergen (reviewed in⁶⁹). This is an attempt to

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clinically tolerize the allergic individual by inducing immunologic anergy or redirecting the immune response from type 2 domination to a more balanced type 1/type 2 profile. However, the technique is associated with frequent failure and has the potential to induce fatal anaphylactic reactions⁶⁹. Other current therapies (β 2-agonists, anti-leukotrienes, anti-histamines and corticosteroids) effectively relieve the symptoms of allergic disease, but do little to address the underlying cause of the disorder: the continuous generation of allergen-specific Th2 cells. Experimentation in murine models of allergic disease reveals that IL-12 (and IL-18, indirectly) have the capacity to promote differentiation of naive T cells into Th1-like cells. This review focuses on experimental data obtained in animal models of human immediate hypersensitivity with particular emphasis on the potential utility of exogenous IL-12 and IL-18 administration for immune redirection.

Regulation of IL-12 Expression

IL-12 is a unique member of the cytokine family in that it exists as a heterodimer. Subunits of 35 and 40 kDa, (p35, p40) are disulfide linked to form bioactive IL-12 (p70)^{35, 59}. p40 expression is restricted to cells capable of secreting p70⁵⁹. p35 mRNA is ubiquitously expressed, but protein cannot be secreted in the absence of p40. Much more p40 is synthesized than p35, and excess can be secreted in the form of free p40 or p40 homodimer (p40)₂¹³. While there was initial speculation that (p40)₂ production *in vivo* may play a role as a constitutive inhibitor of IL-12 function by antagonizing binding of p70 to the IL-12 receptor³⁸, recent data have failed to extend this work. Expression of p35 and p40 are independently regulated and, because p40 can be secreted as free p40, (p40)₂, or within p70, a change in mRNA levels for a single constituent cannot be readily related to p70 secretion. As such, IL-12 p70 production should be measured directly whenever possible.

Sources of IL-12

IL-12 is produced by certain types of B cells⁵⁸, polymorphonuclear cells, and subsets of mast cells (reviewed in ³⁵). However, IL-12 is produced at the highest levels by dendritic cells (DCs) and macrophages via both T cell-dependent and -independent pathways. IL-12 production is rapidly induced in response to bacterial products or intracellular pathogens^{3, 19, 36}. However, IL-12 production in response to T cell-dependent

antigens requires the ligation of CD40 on the surface of the DC with T cell-expressed CD40L¹⁵. Antigen-specific T cells expressing CD40L, which interact with DCs, are exposed to high local concentrations of IL-12 and will preferentially differentiate into Th1 cells. Exposure of antigen-presenting cells (APCs) to IFN- γ prior to the IL-12-inducing signal has a potent effect on enhancing IL-12-synthesis²³. Thus, since IL-12 can itself induce the production of IFN- γ from NK cells and T cells (see below), IFN- γ -mediated enhancement of IL-12 production provides a positive feedback loop, whereby the Th1 nature of an immune response can be strengthened.

IL-12 Receptor

The IL-12 receptor (IL-12R) consists of two subunits (β 1 and β 2), one of which mediates high-affinity IL-12 binding (β 1)⁵³ and the other signaling (β 2)^{53, 74}. A naive T cell rapidly increases production of IL-12R β 2 when it recognizes peptide in the context of MHC class II, enhancing commitment to type 1 cytokine production profiles. In contrast, exposure to IL-4 potently inhibits expression of the β 2 subunit, rendering these T cells less responsive to IL-12 and taking a committed step towards becoming Th2 cells⁶¹. However, if exposed to IL-4 plus IFN- γ , expression of the β 2 subunit is maintained, and the cell remains responsive to IL-12. Cells exposed to IL-4 and IFN- γ are able to secrete IL-4, but also to respond to IL-12 by secretion of IFN- γ ⁶¹. *In vivo*, this may represent a mechanism to prevent the generation of unalterable (or excessive) type 2 responses. Numerous studies have been published which have examined the kinetics and regulation of the IL-12R on Th2 clones and the reversibility of Th2 clones to Th1^{28, 42, 51}. Regulation of this pivotal receptor on fresh, normal T cells is currently under investigation in a number of laboratories.

Biological Activities of IL-12

IL-12 has many biologic activities (see Table 1), but, importantly, deficiencies in the IL-12/IL-12R system are thought to contribute to the pathogenesis of human allergic disease. IL-12 mRNA and protein synthesis in culture of cells from allergic asthma patients following stimulation with bacterial products is lower than in non-atopic controls^{43, 68}. Other studies indicate that the production of IL-12 is identical in allergic individuals, but it is rather the responsiveness to IL-12

Table 1. Biologic activities of IL-12 and IL-18

Cytokine	Function
IL-12	Induces production of IL-2 ⁵² , IL-8 ⁴⁴ and IL-10 ¹² Important role in host defence ^{11, 39} Up-regulates IL-18R ¹ Induces differentiation of Th1 cells
IL-12, IL-18	Induces IFN- γ from NK and T cells ^{2, 48, 73} Activates and increases cytotoxicity of NK cells ^{4, 14, 29} Mediates endotoxin induced shock ^{4, 45, 62, 65}
IL-18	Induces IL-5 and IL-13 production ^{27, 74} Induces release of IL-4 from basophils ⁷⁴ Induces development of effector CD8 ⁺ T cells ⁴⁷

which is diminished²⁴. In animal studies using IL-12 deficient mice, exogenous antigen induced IFN- γ synthesis is depressed while IL-4 levels are elevated following restimulation of spleen cell cultures³⁷. Consistent with observed increases in IL-4, IgE production has been reported to be enhanced in IL-12-deficient mice^{18, 57}. These data argue for an essential inhibitory role for IL-12 in Th2 regulation.

However, we recently published evidence that in IL-12-deficient mice, IL-4, IL-5, IL-13 and antigen-specific IgE responses to exogenous protein antigen are identical to those of wild-type mice, despite 90% reductions in the intensity of type 1 responses⁵⁴. In models of viral infection, Th2 cytokine production was not enhanced in the absence of endogenous IL-12 and, strikingly, the Th1 response (as measured by cytokine responses to viral Ags and survival) was unaltered^{50, 57}. Collectively, these studies suggest that, while IL-12 is important in promoting optimal type 1 responses, it is **not** critical for the inhibition of type 2 responses.

Regulation of IL-18 Production

IL-18 is a single subunit cytokine of 18 kDa⁴⁹, with significant structural homology to IL-1 β ⁵. IL-18 mRNA is constitutively expressed in many cell types, including pancreas, kidney, skeletal muscles, liver, lungs, peripheral blood mononuclear cells (PBMC) and the adrenal cortex^{10, 67}. Although widely expressed, it encodes a propeptide with no biologic activity. Processing with caspase-1 (IL-1 β -converting enzyme – ICE) is required to remove 35 amino acids and generate functional IL-18^{21, 22}. Studies examining the production of IL-18 should thus ideally focus on protein production rather than mRNA expression.

IL-18 Receptor

Two subunits of the IL-18R have been identified to date. The first, IL-18R α , is a member of the IL-1R family (previously IL-1Rrp) that facilitates low-affinity binding of IL-18⁶⁴. The second subunit (IL-18R β), also a member of the IL-1R family (previously IL-1RAcPL), is necessary for high-affinity binding and IL-18-mediated signaling⁷. Differential expression of IL-18R is seen on T cell subsets. Activated CD4⁺ T cells express low levels of IL-18R, while activated CD8⁺ T cells and CD4⁺/CD8⁺ T cells express high levels of IL-18R protein⁶³. In studies of T cell clones, expression of IL-18R α mRNA is found to be restricted to Th1 clones and is augmented by IL-12⁷¹. The ability of IL-12 to augment IL-18R expression may explain the synergy seen between IL-12 and IL-18 in inducing the expression of IFN- γ ¹. The expression of higher levels of IL-18R on CD8⁺ and CD4⁺/CD8⁺ T cells (perhaps representing NK T cells) suggests a greater role for IL-18 following infection with intracellular pathogens than in exogenous antigen-driven responses. This hypothesis remains to be tested.

Whether naive T cells express IL-18R is a contentious issue. TOMURA et al.⁶³ report that resting lymph node T cells from naive mice do not constitutively display IL-18R. IL-12 treatment of lymph node cells from naive animals was necessary to induce IL-18 responsiveness. Looking at human PBMC, however, KUNIKATA et al.³³ found that a high percentage (70–80%) of peripheral, resting CD8⁺ cells and B cells had constitutive expression of IL-18R protein (in the absence of IL-12). Expression on CD4⁺ cells was low (14%).

This paradox may be a result of different methods used to detect IL-18R. In the study by TOMURA et al.⁶³, IL-18R expression was evaluated by incubating the cells with IL-18 followed by an incubation with labeled α IL-18 antibodies. KUNIKATA et al.³³, however, made use in their study of monoclonal antibodies to IL-18R α . Constitutive expression of IL-18R α is possible (and not without precedent, e.g. the IL-2R β / γ complex). This subunit, mediating only low affinity interactions with IL-18 and not representing functional responsiveness to IL-18, would be detected in the Kunikata study. The Tomura study, using a more complex procedure which is likely blind to low-affinity interactions allowed by expression of IL-18R α alone, would detect high-affinity interactions between the entire IL-18R complex and IL-18, which would be more indicative of functional IL-18R expression. A further examination of the kinetics and regulation of both (known) receptor subunits needs to be completed before this controversy can be resolved.

Biologic Activities of IL-18

IL-18 shares many activities with IL-12 (Table 1). IL-18-deficient mice, despite having normal IL-12 levels, synthesize substantially less IFN- γ following lipopolysaccharide (LPS) challenge⁶², suggesting an important role for IL-18 in endotoxin-mediated responses. Additional studies suggest IL-18 involvement in the maturation of CD8⁺ T cells⁴⁷. Despite initial controversy, IL-18, unlike IL-12, is not able to directly cause naive CD4⁺ T cell differentiation into Th1 cells. However, due to its substantial capacity to induce IFN- γ synthesis, IL-18 has the potential to enhance IL-12-driven differentiation of naive T cells⁵⁶.

IL-18 is also a regulator of the allergic response. In the absence of endogenous IL-18 (a result of neutralizing antibodies or genetic knockout), bronchoalveolar lavage (BAL) fluid eosinophilia was more rapidly induced, being enhanced at 8 h (5-fold)⁷ and 24 h (1.5-fold)³², but indistinguishable from control mice at 72 h⁷. Airway responsiveness in IL-18-deficient mice, measured in anesthetized, artificially respired animals, was either twice that of⁷ or indistinguishable from³² control mice. Collectively, the data argue that endogenous IL-18 limits the allergic response *in vivo*.

As well as promoting type 1 responses, there is growing evidence that IL-18 can induce production of type 2 cytokines. IL-18 induces IL-13 synthesis from NK and T cells^{27, 74} and IL-4 release from basophils⁷⁴. This property of IL-18 is markedly enhanced in the absence of IFN- γ ²⁷. Thus, *in vivo*, IL-18, originally discovered by virtue of its potent IFN- γ -promoting properties, may serve as more than a promoter of type 1 immunity. The additional factors which dictate the type of response IL-18 elicits remain a key area of investigation in the potential therapeutic applicability of rIL-18.

IL-12 Therapy of Established Allergic Responses

Initial studies assessing the potential of IL-12 as a therapeutic agent in animal models of human immediate hypersensitivity focused on administration of IL-12 as an adjuvant at the time of allergen sensitization. While large doses of IL-12 are successful at redirecting a *de novo* allergic response to a clinically protective phenotype^{20, 31, 34, 60}, the protective effects of IL-12 are highly transient in nature, and the response rapidly returns to type 2-dominated patterns of antibody and cytokine synthesis⁵⁵. The true utility of a therapeutic agent for allergy lies in its ability to alter **ongoing** allergic responses. Despite the transient nature of the successes of IL-12

in redirecting *de novo* allergic responses in animal models, it is seen as an attractive candidate for a cytokine based therapy for established allergic disease.

In animal models of immediate hypersensitivity, allergen challenge is administered systemically or directly to the airways via intratracheal immunization⁶⁰ or inhalation³¹. In most studies, evaluation of total and antigen-specific IgE indicates that administration of IL-12 during an **ongoing** immune response has no inhibitory effects on IgE synthesis^{31, 60}. Examination of BAL fluids revealed significant (75–95%) decreases in the number of eosinophils in the airways of IL-12-treated mice^{31, 60}. Analysis of airway responsiveness yields conflicting results. In one study, airway responsiveness, measured by tracheal ring reactivity to acetylcholine, was unchanged following IL-12 administration⁶⁰. Another found a striking decrease in lung resistance in response to carbachol in anesthetized, artificially respired mice (to levels lower than unchallenged controls) following IL-12 treatment. Taken together, it is clear that the efficacy of IL-12 administration during **ongoing** type 2 responses is not as striking as that seen when IL-12 treatment is given at time of sensitization. Secondary IgE responses are unaltered (or somewhat increased) following IL-12 treatment and airway responsiveness is variably affected. While eosinophilia is lessened, it should be noted that this measurement was made at a single time point in all published experimental models, so it is difficult to critically assess the benefits of IL-12 administration. Long-term effects of IL-12 on airway eosinophilia need to be assessed. In particular, the question of whether its administration provides protective effects during subsequent challenges in the absence of exogenous IL-12, or if the phenotype of the response reverts to the type 2-dominated patterns normally seen in an allergic response, needs to be addressed.

Combined IL-12/IL-18 Therapy of Allergic Diseases

IL-12 and IL-18 synergistically induce production of IFN- γ . As such, simultaneous administration of both IL-12 and IL-18 would be expected to induce a more strongly polarizing signal for type 1 immunity than either cytokine alone. Proceeding on the hypothesis that such treatment may be strong enough to override existing type 2 responses, a number of studies have been carried out in experimental animal models to determine the potential for IL-18 in combination with IL-12 to redirect allergic responses.

Administration of either *Nippostrongylus brasiliensis* or anti-IgD to mice is a potent inducer of polyclonal IgE synthesis. While high-dose IL-12 or IL-18 therapy alone to parasite-infected mice has the capacity to reduce serum IgE by 3- and 2-fold respectively, co-administration of IL-12 and IL-18 results in a 95% reduction of IgE production⁷². Interestingly, administration of IL-12 and IL-18 to *N. brasiliensis* or anti-IgD-treated IFN- γ ^{-/-} mice serves to **enhance** IgE production 3-fold⁷². Thus, while the concentrations of IL-12 and IL-18 used in this study were high, and the experimental stimuli were not directly applicable to human immediate hypersensitivity, this work demonstrates the potential of IL-12 and IL-18 co-administration to inhibit ongoing type 2 responses.

In an antigen-specific system, IL-12 or IL-18 administration alone, timed to coincide with aerosol challenges, had no detectable effect on total IgE synthesis one day after final allergen challenge²⁶. However, when administered together, IL-12 and IL-18 inhibited total IgE synthesis by 2.5-fold²⁶. Similarly, airway responsiveness (measured by whole-body plethysmography) was unaffected by IL-12 or IL-18 alone, but if administered together, airway responsiveness was decreased to levels akin to unchallenged mice²⁶. Finally, an examination of BAL fluid following the final allergenic challenge revealed that IL-12 and IL-18 administered together virtually abolished cellular infiltration of both eosinophils and neutrophils (88 and 80% decrease, respectively)²⁶. Administration of either cytokine alone had no significant effect on cell numbers in the BAL fluid.

While this study also suggests the potential of IL-12 and IL-18 co-administration, showing clear and marked decreases in all measures of allergic disease examined, there remain important caveats. First, the stability of the IL-12- and IL-18-induced changes were not examined. If treatment does not induce a durable change in the underlying immune response, subsequent allergen exposures may elicit type 2-dominated responses and clinical sensitivity. Secondly, the total dose of cytokine administered was 800 ng IL-12 and 4 μ g IL-18 per mouse. This is an extremely large dose of cytokine, so the potential for toxicity would need to be carefully assessed.

IL-18 Treatment of Allergic Diseases

While co-administration of IL-12 and IL-18 facilitates redirection of existing allergic responses, administration of IL-18 alone has dramatically different re-

sults. In pretreatment studies, BAL fluid from mice given multiple doses of IL-18 intraperitoneally prior to immunization contained 1.5 times more eosinophils than untreated mice⁷⁰. Similarly, *in vitro* allergen restimulation of cultured spleen cells from IL-18-treated mice induced significantly higher levels of the type 2 cytokines IL-4 and IL-5 (2- and 3-fold, respectively) as compared with untreated mice⁷⁰. IgE production was unaltered by IL-18 pre-treatment. The type 2-promoting effects were even greater if IL-18 was administered locally via intranasal administration. IL-18 increased the BAL eosinophil count 450%, the IgE titre by 66-fold, and the allergen-stimulated spleen cell IL-4 and IL-5 synthesis by 63- and 44-fold, respectively. These alterations strongly suggest a potential exacerbation of the allergic phenotype following localized IL-18 administration at the time of initial allergen sensitization.

Again, the capacity of IL-18 to redirect existing immune responses is the more clinically relevant question. Administration of a high local dose of IL-18 (200 ng directly to the trachea) to mice undergoing an allergic response increases the number of both peribronchial and BAL eosinophils by 2- and 3-fold, respectively, 24 h after allergen challenge⁷. IL-18 also has the capacity to induce eosinophilia in unsensitized animals 24 h after administration⁷. This increase in eosinophil number is the result of significant IL-18-induced eotaxin production from macrophages and epithelial cells⁷. Eotaxin^{-/-} mice demonstrate no increase in eosinophil number following treatment with IL-18⁷. A high systemic dose of IL-18 (3 μ g), however, induces an 89% decrease in BAL eosinophils⁷⁰. Interestingly, administration of multiple large doses of IL-18 induces significant (5-fold) increases in total serum IgE levels⁷⁰ of unsensitized animals. While conflicting, these studies indicate again that exacerbation of the allergic phenotype is a distinct possibility following administration of IL-18 alone to allergic individuals.

While the reports of the effects of IL-18 are contradictory, it is worthwhile to point out that in studies where the role of endogenous IL-18 has been assessed (either through the use of genetic knockout mice or neutralizing antibodies) the results have come up consistently in favor of a constitutive protective role of IL-18 in inhibiting development of allergic disease (see section on biologic activities of IL-18). In stark contrast, most of the indicators of allergic disease examined in studies to evaluate the potential of pharmacological administration of IL-18 were elevated. The dichotomy seen between the actions of endogenous and exogenous IL-18 might indicate that the balance of IL-18 and other factors present (perhaps IL-12 or IFN- γ)

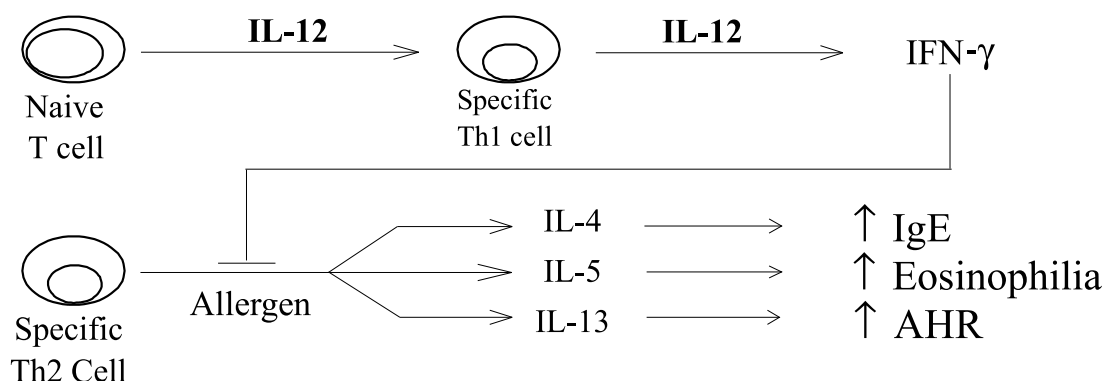
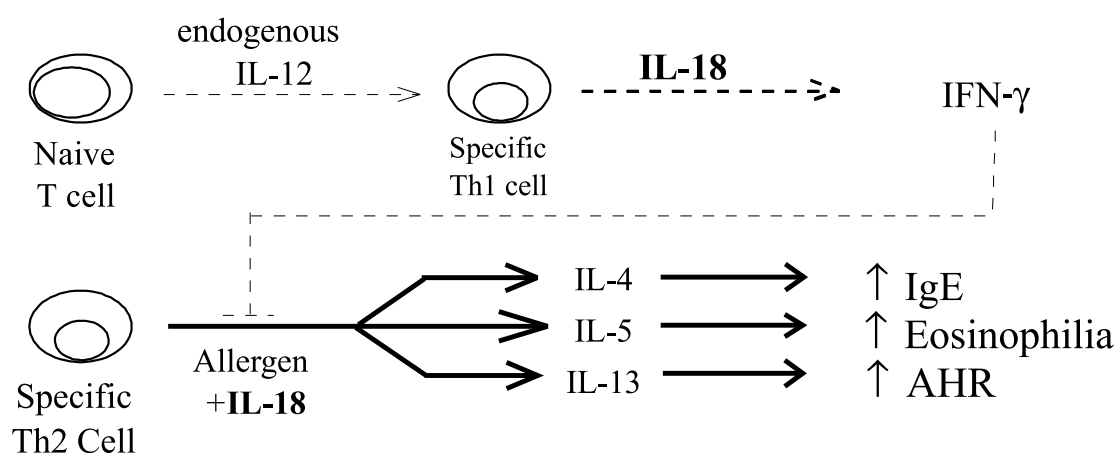
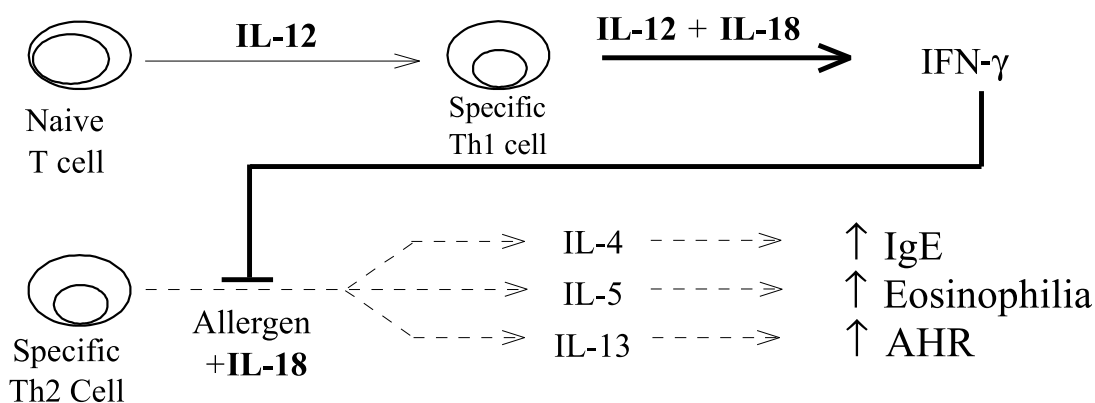
A) Administration of IL-12 alone**B) Administration of IL-18 alone****C) Administration of IL-12 and IL-18**

Fig. 1. Possible mechanisms of action of exogenously administered cytokine. IL-12 administration alone (**A**) induces the production of IFN- γ which transiently inhibits allergen induced type 2 cytokine synthesis. Administration of IL-18 alone (**B**) results in weak IFN- γ synthesis which is unable to prevent IL-18 induced enhancement of type 2 cytokine synthesis and exacerbation of the allergic condition. Administration of IL-12 and IL-18 (**C**) induces higher levels of IFN- γ synthesis which potentially decrease the type 2 nature of the allergic response and overcome type 2 promoting effects of IL-18

is most important in dictating which effect of IL-18 dominates. Administration of large amounts of IL-18 would be expected to upset this balance and allow for enhancement of existing type 2 responses. Administration of much lower doses of IL-18, closer to those seen in physiological systems, or co-administration of IL-12 and IL-18 (thus synergistically increasing the levels of IFN- γ in the system) might maintain the balance and promote the formation of type 1-dominated responses, even in the face of strong, ongoing type 2 responses. This hypothesis needs to be further explored.

Summary of IL-12- and IL-18-Induced Changes

Co-administration of IL-12 and IL-18 strongly induces synthesis of IFN- γ . IL-12 and IL-18 induced IFN- γ inhibits type 2 cytokine production, subsequently inhibiting eosinophilia, airway responsiveness and production of IgE (see Fig. 1). In the absence of high levels of IFN- γ , a known inhibitor of type 2 responses, IL-18 promotes production of IL-4, IL-5, and IgE, and thus has the potential to markedly exacerbate the allergic response^{7, 32, 70}. Indeed, in the absence of endogenous IFN- γ (as in IFN- $\gamma^{-/-}$ mice) co-administration of IL-12 and IL-18 enhances IgE synthesis⁷². Furthermore, the administration of IL-18 increases the levels of total IgE seen in the serum in naive mice⁷⁰. In light of this, administration of IL-18 to allergic individuals (who, by the very nature of allergic disease, have low allergen-induced IFN- γ production) may do more harm than good. The effects of exogenous IL-18 on the allergic response and the role of endogenous IL-18 in an immune response need to be further elucidated before this cytokine can be safely used in any cytokine-based therapy for allergic diseases.

Conclusion

The increasing prevalence of allergic disease in the developed world makes the need for an effective treatment (or prophylaxis) a high priority. Classical allergen immunotherapy, still widely in practice today, has variable efficacy. Administration of IL-12 as an adjuvant to redirect allergic responses has also been plagued by questions of efficacy, stability and safety⁹. The therapeutic potential of IL-18 is, at present, poorly understood. It is clear that IL-18 is a powerful regulator of the immune response, and that it has the potential to enhance both type 1 and type 2 responses. Unfortunately, it is not yet clear how IL-18 specifically promotes one or the other response. A great deal of work must

be done to understand the biology of IL-18 before it can be considered as a potential treatment for allergic disease.

While safe and effective therapies for allergic disease based on the administration of exogenous cytokine are at best several years away, recent studies have indicated that induction of endogenous IL-12 and IL-18 is of importance in redirecting allergic responses in other animal models (e.g. CpG and pDNA immunization), confirming the potential of this cytokine pair. The pressing need for consistently effective treatments of allergic diseases, taken with the effects seen upon co-administration of IL-12 and IL-18, dictates that work must continue in earnest to decipher the intricacies and vagaries of exogenous administration of these cytokines.

Acknowledgment. This work was supported by the Canadian Institutes of Health Research. Ian P. Lewkowich holds a Tom and Mindel Olenick Research Award in Immunology, Kent T. HayGlass a Canada Research Chair in Immune Regulation.

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Received in May 2000

Accepted in June 2000