

EDITORIAL

Interleukin-12 and the development of atopy

Introduction

Atopic individuals are characterized by the propensity to synthesize and secrete immunoglobulin (Ig)E in response to various, otherwise essentially harmless, environmental antigens. This feature differentiates atopic from normal individuals whose immune response to the same antigens is mainly constituted by IgM or IgG.

Allergic inflammation and IgE synthesis are known to be promoted by a distinctive allergen-specific T helper (Th)2 cell subset defined by a cytokine profile represented by interleukin (IL)-4, IL-5 and IL-13 in contrast to Th1 cells which synthesize mainly IL-2 and IFN- γ [1–3]. The single most powerful factor inducing the synthesis of interferon (IFN)- γ by CD4⁺ T cells is IL-12. IFN- γ and IL-12 are reciprocally regulated; in fact IL-12 stimulates IFN- γ release and, in turn, IFN- γ is able to both augment IL-12 production and enhance cell responsiveness to IL-12 by increasing the expression of the high-affinity IL-12 receptor (IL-12R β_2). As a result of this IL-12/IFN- γ loop, T helper cells will differentiate into a predominant Th1 phenotype being able to synthesize IFN- γ following antigen recognition. Besides these effects of Th1 differentiation, IL-12 is able to inhibit the differentiation of T cells towards IL-4-secreting Th2 cells and to suppress IgE production [4,5] and allergic inflammation that result after allergen exposure.

In this issue of Clinical and Experimental Allergy, Janefjord *et al.* present a study aimed at understanding whether the decreased IFN- γ production seen in atopic children is due to a reduced lymphocyte responsiveness to IL-12. The authors found that peripheral blood mononuclear cells (PBMC) from atopic children have reduced levels of IFN- γ , lower expression of CD2 and, when stimulated through CD2, express less IL-12R β_2 compared to normal children. These results led the authors to the conclusion that reduced levels or function of CD2 could be responsible for the altered responsiveness to IL-12 and consequently to the impaired IFN- γ production and T cell differentiation [6].

The induction of a type 1 or 2 response involves many factors: the genetic background, type of antigen-presenting cells (APC), the presence, type and/or strength of the interaction between pairs of costimulatory molecules (e.g. CD28/CD80-86, CD2/CD58), and the nature of the antigenic stimulus. It is, however, clear that the presence

of cytokines like IL-4 or IL-12, IL-18 and IFN- γ in the microenvironment where T cell priming and activation take place, represent the single most important factor influencing the differentiation of CD4 T cells into Th2 or Th1, respectively [4,7,8].

Interleukin 12 is a cytokine produced by dendritic cells, monocyte-macrophages and other APC [9]. Structurally, IL-12 is a heterodimer composed of a 35 and a 40 kDa peptide. The transcription of both the p35 and p40 genes, located on human chromosomes 3p12-q13.2 and 5q31-q33, respectively, is required for production of active IL-12 [10].

Once released by APC, IL-12 promotes Th1 responses, stimulating NK cells and differentiated and memory Th1 cells maximally to produce IFN- γ [11,12]. IL-12 acts by binding to its own receptor (IL-12R), a heterodimer composed of two subunits, β_1 and β_2 , that is expressed on activated T and NK cells but not on resting T cells or B cells [13]. Of these two subunits, the β_2 chain is essential for IL-12 signalling and its expression is highly controlled and selectively restricted to some cell subtypes [14]. Both subunits are required for binding IL-12 with high affinity.

In human T cells, the IL-12R β_2 expression on the cell surface is tightly regulated by cytokines (positively by IFN- γ and IFN- α [15] and negatively by IL-4, IL-13 and IL-10) and by the interaction between costimulatory molecules expressed on the surface of APC and T cells (Fig. 1). Because the expression of IL-12R β_2 is influenced by the cytokine milieu, and since IFN- γ powerfully up-regulates the levels of this receptor, it is now appreciated that IL-12R β_2 expression is restricted to Th1 cells and that its levels correlate with IL-12 responsiveness. It can therefore be speculated that the fine modulation of IL-12R β_2 expression may constitute a pivotal mechanism for regulating IL-12 responsiveness and hence the maturation of Th phenotype. Studies have demonstrated that fully differentiated human Th2 cells lose IL-12R β_2 , becoming resistant to the effects of IL-12, as shown by their failure to produce IFN- γ or to exhibit STAT-4 phosphorylation following IL-12 stimulation [15,16].

CD2 and IL-12 responsiveness

In addition to cytokines, the synthesis of IL-12 and its biological actions are also dependent on the presence of accessory molecules expressed by APC and T cells. One of these accessory molecules is the CD2 receptor, a

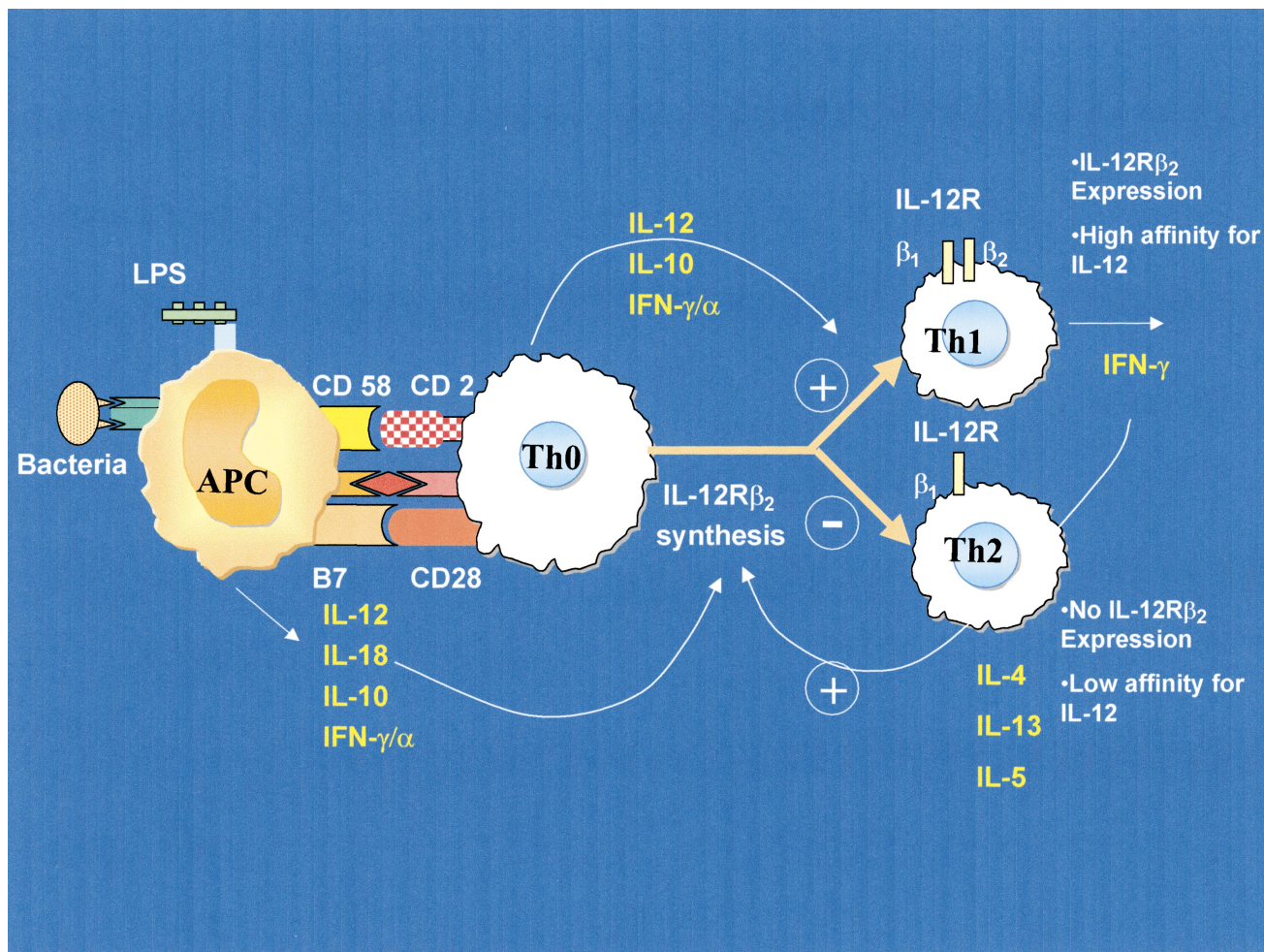


Fig. 1. Factors involved in the regulation of IL-12R β_2 expression on human T helper cells and their phenotypic differentiation.

glycoprotein belonging to the immunoglobulin superfamily, highly expressed on all mature human T cells. The expression of this surface antigen characteristically increases from the fetal state to adult life, providing evidence that this molecule can be important in the process of maturation of the immune system [17]. In fact, it has been shown that the rate of postnatal maturation of T cells may be slower in children at high genetic risk of atopy compared to their normal counterparts. By interacting with its ligand CD58 (LFA-3), CD2 serves different functions: it enhances T cell activation by delivering a second (costimulatory) signal, and it is involved in the regulation of cytokine production and T cell differentiation mainly by reinforcing the IL-12/IFN- γ positive feedback loop. This would provide a key functional link that controls T cell responsiveness to IL-12. Blockade of CD2–CD58 interactions inhibits IL-12-induced proliferation and IFN- γ production. Furthermore, by using activating pairs of CD2 antibodies Gollob *et al.* showed that CD2 stimulation

strongly synergized with IL-12 in inducing T cell proliferation and IFN- γ production [18] possibly by increasing IL-12R β_2 expression.

Dysregulation of IL-12 production in human allergy

The 'Th2 hypothesis' of allergy implies that atopic patients have a predominant Th2 response with a reduced Th1 response [19]. The low levels of IL-12 observed in atopic patients lead to aberrant Th2 development impeding the normal maturation of the immune system.

A number of previous studies have suggested that a deficiency in IL-12 may skew the phenotype of the inflammatory cells infiltrating the airways towards a Th2-type immune reaction. Naseer *et al.* showed that bronchial biopsies from atopic asthmatic subjects exhibit a lower expression of IL-12 mRNA compared to those from normal controls [20]. Similarly, van der Pouw Kraan *et al.* using whole blood cultures, with different stimuli, found a

2.6-fold reduction in IL-12 production in patients with allergic asthma compared to controls [21].

The mechanisms for the impaired IL-12 production in allergic patients are not fully elucidated but they may result from either a defect of the genes encoding for IL-12 or from mutations of IL-12R β_2 gene [22], from an imbalance between factors able to stimulate or inhibit IL-12 production (e.g. IFN- γ vs. IL-4 [23,24], or as a result of a loss in IL-12 responsiveness and therefore a deficit in the IFN- γ /IL-12 loop [25]. IL-12 responsiveness in Th1 cells has been shown to correlate with the mRNA encoding for IL-12R β_2 and various allergic conditions have been associated with altered IL-12R β_2 gene expression. The study by Janefjord *et al.* shows that another mechanism can account for the observed deficit in IL-12 and that is mediated by CD2–CD58 interaction [6].

Further evidence of the importance of IL-12 in regulating the allergic immune response has been provided by *in vivo* studies where exogenous IL-12 has shown an anti-allergic activity when administered systemically in a murine model of allergic airway inflammation. In particular, IL-12 was able to prevent antigen-induced increases in airway hyper-responsiveness, lung eosinophilia and Th2 cytokine expression [26], and to inhibit IgE synthesis [27]. Moreover, experiments employing mucosal gene transfer resulting in IL-12 expression in the lung have shown inhibition of the local Th2 cytokine production, thus preventing the development of allergic disease and airway hyper-responsiveness [28]. Unfortunately these data have not been confirmed in a phase-I trial of IL-12 in human asthma. In this trial subcutaneous injections of IL-12 caused a significant reduction of peripheral blood and sputum eosinophils but no effects were observed on allergen-induced early- and late-phase response [29].

A different approach to studying the relevance of IL-12 in modulating the immune response *in vivo*, comes from models where the production of IL-12 is induced as a result of cell activation by stimuli like bacteria or bacterial products. These stimuli are known to boost the innate immune response by stimulating IL-12 in addition to up-regulating costimulatory molecules on the surface of APC and T cells. In this context lipopolysaccharide (LPS), a component of the cell membrane of gram-negative bacteria, widely distributed in the environment, is able to increase IL-12 production by PBMC and modify the T cell phenotype to an extent greater than IL-12 alone (personal unpublished data). Furthermore, a non-pathogenic species of Mycobacteria like *M. vaccae* can evoke an enhanced production of IL-12 and IFN- γ and can also increase the levels of costimulatory molecules necessary to increase the sensitivity to IL-12 and provide the necessary link to complete the IL-12/IFN- γ loop. In addition to these effects on cytokine profile, *M. vaccae* when given subcutaneously

to allergic asthmatic individuals, has been shown to cause a significant reduction in the late-phase response following allergen challenge [30].

In conclusion, there are now several studies that demonstrate that there is a deficit of IL-12 production in atopic individuals and that this deficit can result in the amplification and maintenance of chronic allergic inflammation. Regulation of IL-12 is one of the mechanisms whereby current treatments available for allergy, such as steroids or immunotherapy, may exert their effects [31]. The available evidence provides a strong rationale for developing more effective therapeutic approaches in order to regulate IL-12 production in human allergic disease.

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