

INTERLEUKIN-10 (IL-10) is a cytokine derived from CD₄⁺ T-helper type 2 (T_{H2}) cells identified as a suppressor of cytokines from T-helper type 1 (T_{H1}) cells. Interleukin-12 (IL-12) is produced by B cells, macrophages and dendritic cells, and primarily regulates T_{H1} cell differentiation, while suppressing the expansion of T_{H2} cell clones. Interferon- γ (IFN- γ) is a product of T_{H1} cells and exerts inhibitory effects on T_{H2} cell differentiation. These cytokines have been implicated in the pathogenesis of asthma and allergies. In this context, IL-12 and IFN- γ production in asthma have been found to be decreased, and this may reduce their capacity to inhibit IgE synthesis and allergic inflammation. IL-10 is a potent inhibitor of monocyte/macrophage function, suppressing the production of many pro-inflammatory cytokines. A relative under-production of IL-10 from alveolar macrophages of atopic asthmatics has been reported. Therapeutic modulation of T_{H1}/T_{H2} imbalance in asthma and allergy by mycobacterial vaccine, specific immunotherapy and cytosine–guanosine dinucleotide motif may lead to increases in IL-12 and IFN- γ production. Stimulation of IL-10 production by antigen-specific T-cells during immunotherapy may lead to anergy through inhibition of CD28-costimulatory molecule signalling by IL-10s anti-inflammatory effect on basophils, mast cells and eosinophils.

Key words: Interleukin-10, Interleukin-12, interferon- γ , T-helper 1 and 2 cells, Asthma, Allergy

Anti-inflammatory cytokines in asthma and allergy: interleukin-10, interleukin-12, interferon- γ

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Introduction

The chronic airway inflammation of asthma is characterised by infiltration of the airway wall by diverse effector cells, including T lymphocytes, eosinophils, monocytes/macrophages, mast cells, and occasionally, neutrophils.^{1–3} The airway wall undergoes chronic structural changes labelled as remodelling, which include thickening of the airway smooth muscle due to hypertrophy and hyperplasia, myofibroblast activation with increase in subepithelial basement membrane collagen deposition, angiogenesis and increase in submucosal blood vessels and an increase in goblet cell numbers in the airway epithelium.^{4,5} The mobilisation, activation and trafficking of effector cells to the airway are controlled by a complex cytokine milieu derived from activated CD4⁺ T_{Helper} (T_H) cells and also from other resident airway cells including airway smooth muscle and epithelial cells. T_{Helper} cells of type 2 variety (T_{H2}) secrete a T_{H2} profile of cytokines, after cognate stimulation of the naïve T-cell by antigen presenting cells, such as the dendritic cell and the alveolar macrophage. T_{H2} cytokines include IL-4, IL-5, IL-9, IL-10 and IL-13. These cytokines promote various elements of allergic inflammation including propaga-

tion of the T_{H2} phenotype, isotype-switching from IgG₁ to IgE synthesis, eosinophil mobilisation, maturation and activation and mast cell activation.⁶ Chronic airway structural changes too are variably influenced by T_{H2} cytokines, and certain growth factors.

T_{H1} cells form a natural counterbalance to T_{H2} cells driving protective cell-mediated immunity, and are induced on exposure to foreign agents including protozoa, bacteria and viral particles. T_{H1} responses are characterised by the induction of cell mediated immune responses and the synthesis of IgG_{2a}, while T_{H2} responses are of humoral-type, inciting the production of IgE and IgG₄. T_{H1} responses inhibit T_{H2} responses through the production of cytokines such as IL-12 and IFN- γ . There is evidence for a preferential skewing to the expansion of the CD4⁺ T_{H2} lymphocyte subset in allergic processes, while CD4⁺ T_{H1} responses may be subdued and this is a likely crucial forerunner to development of allergic asthma. In this context, the T_{H1} cytokines, IL-12 and IFN- γ , may be considered to be 'anti-inflammatory' in that they inhibit certain responses attributable to T_{H2} cell activation, while the T_{H2} cytokine, IL-10, under certain circumstances, may inhibit pro-inflammatory activation of certain cells. The manipulation of the immune allergic system to promote T_{H1} responses

involves the upregulation of IL-12 and $\text{INF}\gamma$, and under certain circumstances, of IL-10, which can prevent the establishment of allergy and of various manifestations of allergic inflammation.

In this review, the important roles of $\text{INF}\gamma$, IL-10 and IL-12 in inducing the therapeutic benefits of various immune therapies for allergy and asthma will be examined first, followed by a systematic review of these anti-inflammatory cytokines.

Immunological imbalance of T_{H1} & T_{H2} cells

The vast majority of asthmatics have an atopic background, in whom the inflammatory process of asthma may be driven following sensitisation and exposure to common aeroallergens to which they become sensitised to. Allergen-derived peptides are usually taken up by specialised cells on mucosal surfaces such as dendritic cells, and subsequently processed and presented to naïve T-cells. The activation of naïve T-cells requires direct signalling by 2 distinct pathways: firstly via the $\text{CD4}+$ T-cell receptor through the antigen-presenting cell (APC)-bound antigen to MHC-II complex and, secondly, via the co-stimulatory pathway linked by the B7 family and T-cell bound CD28.⁷ T-cells stimulated via the T-cell receptor (TCR) in the absence of co-stimulatory signalling are incapable of IL-2 secretion and subsequent activation and therefore enter an anergic state.⁸ CD28 itself has 2 major ligands, B7.1 which inhibits T_{H2} cell activation and development, and B7.2, which induces T-cell activation, and T_{H2} development. An important third ligand, CTLA4, is expressed on activated T cells, binds CD28 with enhanced avidity and acts as a negative regulator of T-cell function by inhibiting T_{H2} differentiation.^{9,10} In those who later develop an allergic response, naïve T cells differentiate into the T_{H2} subtype. This differentiation is induced by cytokines themselves, and may be secreted by antigen-stimulated T cells themselves, or by other cells that become activated by T cell cytokines. $\text{INF}\gamma$ secreted by T_{H1} cells activate macrophages and dendritic cells, and in turn induces them to produce IL-12, the main T_{H1} -inducing cytokine. T_{H1} and T_{H2} cells produce cytokines that cross-regulate and antagonise each other's activity and development. IL-4 can inhibit IL-12 production, and T_{H2} cytokines (IL-4, -10 and -13) antagonise the macrophage-activating properties of T_{H1} cytokines. $\text{INF}\gamma$ is involved in the T_{H1} inhibition of the effects of T_{H2} cells, such as bronchial hyper-responsiveness, eosinophilia and mucus goblet cell hyperplasia.^{11,12}

T-cell profiles in the newborn demonstrate a T_{H2} bias suggesting that prenatal influences are involved in T-cell priming.¹³ During the course of maturation of the normal infant, however, increased T_{H1} expression

occurs and the T_{H2} imbalance is overcome.¹⁴ Delay or failure of this T_{H1} response may result in T_{H2} persistence and atopy or atopic disease, and in infants destined to become atopic, circulating lymphocytes have impaired production of $\text{INF}\gamma$.¹⁵ Recently, a 'hygiene hypothesis' has been put forward stating that a diminished induction of T_{H1} responses is a potential explanation for the rising prevalence of atopy and asthma.^{13,16} Cross-sectional surveys have identified inverse relationships between prior microbial exposure and development of atopy.^{15,17} Further, respiratory allergy appears less frequently in those heavily exposed to orofaecal and foodborne microbes.¹⁶ Improved hygiene, early infection and antibiotic use, and a westernised or semi-sterile diet may facilitate atopy by influencing exposure to commensals and pathogens that stimulate immune cell populations such as gut-associated lymphoid tissue.^{18,19} Thus, early environmental exposure may be a determinant of the development of atopy in the adult.²⁰ The identification of ways to prevent, control or even reverse the process of T_{H2} immunodeviation has become a focus for the development of new strategies to control asthma and allergies.

Therapeutic T_{H1}/T_{H2} modulation: roles of IL-10, IL-12 and $\text{INF}\gamma$

The potential therapeutic roles of IL-10, IL-12 and $\text{INF}\gamma$ in allergies and asthma are well illustrated by various potential treatments. Specific immunotherapy (SIT) by administration of allergen extracts is a treatment aimed at the induction of specific unresponsiveness, or anergy, in peripheral T-cells to peptide epitopes. This treatment has been successfully used in asthma and allergic rhinitis and for venom allergy,^{21,22} but is usually most useful in subjects allergic to single allergens such as grass pollens, and in subjects with mild forms of allergic diseases.^{23,24} Peripheral T-cells following SIT are characterised by reduced IL-4 and IL-5, and increased $\text{INF}\gamma$ production by $\text{CD4}+$ T cells²⁵⁻²⁷ and by attenuated proliferative responses to specific allergens.²⁸ This process is initiated by an autocrine effect of IL-10 produced by antigen-specific T-cells.²⁹⁻³¹ IL-10 induces anergy by inhibition of CD28-costimulatory molecule signaling,^{32,33} and also by anti-inflammatory effects on basophils, mast cells and eosinophils. There are reduced levels of IL-4 and IL-5 and increased $\text{INF}\gamma$ production, indicating a shift of the T cells towards an increased T_{H1} response at the expense of T_{H2} responses.^{25,26} After successful grass pollen immunotherapy, there is also an increase in IL-12 and $\text{INF}\gamma$ mRNA expression in tissues.^{34,35}

The potential benefit of the mycobacterial vaccine, Bacille-Calmette-Guerin (BCG) (*Mycobacterium bovis*) vaccination, in atopic diseases was suggested

by the association between BCG vaccination and diminished incidence of atopy and allergic disease in Japanese schoolchildren, indicating a role for early mycobacterial exposure in the subsequent development of atopic responsiveness.¹⁵ Mice vaccinated with BCG prior to allergen sensitisation had increased IFN- γ and decreased IL-4 and IL-5 expression along with reduced levels of airway T-cells and eosinophilia and bronchial reactivity.^{36,37} Local production of IFN- γ induced by the BCG vaccine was the major cytokine involved in blocking atopic disease in the lung.³⁶ *Mycobacterium vaccae* is ubiquitously present in the soil as a saprophyte and can evoke a strong production of IFN- γ . Suppression of T_{H2} activation has been demonstrated using heat-killed *M. vaccae* in mice.³⁸⁻⁴⁰

The cytosine-guanosine dinucleotide repeat, when present in a particular base context, is known as a CpG motif and is an important prokaryotic immunomodulatory effector whose role is probably that of a warning or priming agent against bacterial infection.⁴¹ This motif is expressed at very low frequency in vertebrates, in a non-functional methylated form, and hence is without function. CpG vaccination directly induces antigen-presenting cells and B-lymphocytes to release IL-12, IL-18 and TNF γ effectively, suppressing T_{H2} responses by inducing the T_{H1} phenotype. These CpG oligonucleotides (ODN) prevent the development of eosinophilic airway inflammation, allergen-induced elevation of serum IgE and bronchial hyper-responsiveness in murine asthma models,⁴² and can also reduce established inflammation.⁴²⁻⁴⁴ The protective effects of CpG-ODN appear to be due to an induction of IL-12 and IFN- γ .^{42,45} Other mechanisms may also be involved.⁴⁶

Specific cytokine profiles

Interleukin-10

Synthesis and release.

IL-10, previously known as cytokine synthesis inhibitor factor (CSIF), was originally identified as a product of murine T helper (T_{H2}) clones that suppressed the production of cytokines by T_{H1} clones responding to stimulation of antigen.⁴⁷ In humans, T_{H0}, T_{H1}, and T_{H2}-like CD4⁺ T cell clones, cytotoxic T-cells, activated monocytes and peripheral blood T cells including CD4⁺ and CD8⁺ T-cells have the capacity to produce IL-10.^{48,49} An autocrine action of IL-10 with increased production of IL-10 by antigen-specific cells may be responsible for the induction of anergy.³¹ Mast cells also have the capacity to produce IL-10. Constitutive IL-10 secretion occurs in the healthy lung with the major source being the alveolar macrophage; however, the circulating monocyte elaborates more IL-10 than the alveolar macrophage.⁵⁰

Receptors and signalling pathways.

IL-10R has been characterised and cloned from a human lymphoma cell line,⁵¹ and is expressed in several lymphoid and myeloid cells,⁵² and also on NK cells.⁵³ The IL-10R is made up of 2 subunits, which belong to the same class of receptor family that also contains receptors for IFN- γ .⁵⁴ The IL-10 receptor α -chain (110 kDa) mediates high-affinity ligand binding and signal transduction,^{51,55} while the β subunit (40 kDa) is required for signalling.⁵⁶ The functional IL-10R complex is a tetramer consisting of 2 IL-10 α -chains and 2 IL-10 β -chains. Activation of IL-10R leads to the activation of JAK-STAT signalling pathway, with activation of JAK-1 by the α chain and TYK2 by the β chain. These kinases then phosphorylate specific tyrosine residues on the intracellular domain of the IL-10R α chain. Once phosphorylated, these tyrosine residues serve as temporary docking sites for the latent transcription factors STAT1, STAT3 and STAT5.^{57,58} STAT3 is directly recruited to the IL-10R α and becomes phosphorylated by receptor-associated JAK kinases. STAT1 is also activated in macrophages. Upon phosphorylation, STAT1 and STAT3 homo/heterodimerise and translocate to the nucleus where they bind with high affinity to STAT-binding elements in the promoters of various IL-10-responsive genes. One of these genes, SOCS-3 (suppressor of cytokine signalling-3) is a member of a newly identified family of genes that inhibit JAK/STAT-dependent signalling. The ability of IL-10 to inhibit gene expression in monocytes is associated with its ability to rapidly induce the synthesis of SOCS-3.

Effects.

IL-10 is a pleiotropic cytokine that can exert either immunosuppressive or immunostimulatory effects on a variety of cell types. IL-10 is a potent inhibitor of monocyte/macrophage function, suppressing the production of a number of pro-inflammatory cytokines, including TNF- α , IL-1 β , IL-6, MIP-1 α and IL-8,⁵⁹⁻⁶¹ although the release of MCP-1 is increased.⁶¹ IL-10 inhibits monocyte MHC Class II, B7.1/B7.2 and CD23 expression and accessory cell function. Accessory signals mediated by B7 molecules through CD28 on the surface of T-cells are essential for T-cell activation. Expression of IL-10 by antigen-presenting cells may be an established pathway for the induction of antigen-specific tolerance, such as that to allergens.⁶² By contrast, IL-10 up-regulates the monocyte expression of IL-1ra, another anti-inflammatory cytokine.⁶³ IL-10 suppresses the synthesis of superoxide anions and NO by activated monocytes/macrophages.⁶⁴ An IL-10 antibody enhances the release of cytokines from activated monocytes, suggesting that this cytokine may play an inhibitory role when the cell is stimulated.⁶⁰ IL-10 inhibits the stimulated release of RANTES and IL-8 from human airway smooth muscle cells in culture.^{65,66}

IL-10 inhibits the production of IFN- γ and IL-2 by T_{H1} lymphocytes,⁴⁷ and IL-4 and IL-5 production by T_{H2} cells by interfering with B7-CD28-dependent signals.^{67,68} IL-10 inhibits the proliferative T cell response in peripheral blood mononuclear cells to various antigens, and the superantigen staphylococcal enterotoxin B. IL-10 selectively inhibits the anti-CD28 stimulated proliferation of purified CD45RO+ T cells. There is a constitutive association of CD28 with the IL-10 receptor, and IL-10 acts directly on the CD28 signalling pathway to inhibit CD28 tyrosine phosphorylation and binding to phosphatidylinositol 3-kinase.⁶⁹ IL-10 also inhibits eosinophil survival and IgE synthesis induced by IFN- γ . On the other hand, IL-10 acts on B-cells to enhance their viability, cell proliferation, immunoglobulin secretion with the isotype switch and class II MHC expression. IL-10 decreases IL-4-induced IgE switch in peripheral blood mononuclear cells, but potentiates IgE production in B-cells that are already switched to produce IgE.⁷⁰ IL-10 is also a growth co-stimulator for thymocytes and mast cells,⁷¹ as well as an enhancer of cytotoxic T cell development.⁷² IL-10 increases Bcl-2 expression and survival of primary human CD34+ progenitor cells committed to the myeloid lineage.⁷³ IL-10 also activates the transcription of genes for mast-cell derived proteases. IL-10 enhances the production of the tissue inhibitor of metalloproteinases of monocytes and tissue macrophages while decreasing metalloproteinase biosynthesis.⁷⁴

Role in allergy and asthma.

There is significantly less IL-10 mRNA and protein expressed in alveolar macrophages of asthmatic subjects compared to those from non-asthmatic individuals.^{75,76} Triggering of CD23 molecule by anti-CD23 monoclonal antibodies induces IL-10 production by human monocytes.⁷⁷ An IL-10 polymorphism on the transcription initiation site could be responsible for reduced IL-10 release.⁷⁵ Another polymorphism upstream from this site was associated with elevated total serum IgE.⁷⁸ Inhaled corticosteroid therapy restores the reduced IL-10 release from macrophages of asthmatics⁷⁶ and theophylline increases IL-10 secretion.⁷⁹ On the other hand, other studies indicate that there are increased number of macrophages and T-cells expressing IL-10 mRNA in bronchoalveolar lavage fluid of patients with asthma.⁸⁰

Studies regarding the role of IL-10 in allergic mouse models have provided some conflicting results. Co-instillation of IL-10 by the intranasal route significantly inhibits the peritoneal and lung eosinophilia induced by ovalbumin in immunised mice,⁸¹⁻⁸³ however, IL-10 augmented airway responsiveness. Concurrent expression of IL-10 and GM-CSF in the airway epithelium led to a suppression of GM-CSF-driven ovalbumin-specific eosinophilic inflammation with decreased IL-4, IL-5 and TNF γ expression. However, in

IL-10 gene knock-out mice, allergen-induced eosinophilic inflammation, IL-5 production and airway hyper-responsiveness were inhibited.⁸⁴⁻⁸⁶ These studies in mice indicate that IL-10 may augment or decrease allergic inflammatory responses, but could worsen airway responsiveness.

IL-10 is involved in the induction of specific anergy in peripheral T cells during specific immunotherapy, characterised by suppressed proliferative and cytokine responses. IL-10 administration before allergen exposure induces antigen-specific T cell tolerance.³¹ IL-10-derived regulatory CD4⁺ T cells producing IL-10 but not IL-2 and IL-4 suppresses antigen-specific T cell response *in vitro* and prevented antigen-induced murine colitis.⁸⁷ Administration of IL-10 to normal volunteers induced a fall in circulating CD2, CD3, CD4 and CD8 lymphocytes with suppression of mitogen-induced T-cell proliferation and reductions in TNF- α and IL-1 β production from whole blood stimulated with endotoxin *ex-vivo*.⁸⁸

Interferon- γ

Synthesis and release.

IFN- γ was originally identified as a product of mitogen-stimulated T lymphocytes that inhibited viral replication in fibroblasts. The known sources of IFN- γ are CD4⁺ and CD8⁺ T-cells and NK cells.

Receptors and signalling.

The receptor is expressed on T-cells, B-cells, monocytes/macrophages, dendritic cells, granulocytes and platelets. Epithelial and endothelial cells also express these receptors. IFN- γ receptor consists of an α -chain (90 kDa) which is the major ligand binding subunit, and of a β chain (65 kDa) which increases the affinity of the α chain for its ligand, but is obligatory for transducing the IFN- γ signal.^{89,90} Signalling through the IFN- γ receptor is mediated through JAK1 and JAK2, which are constitutively associated with specific membrane proximal residues on the cytoplasmic domain of the receptor.^{91,92} Activated JAKs phosphorylate a specific tyrosine residue, to which STAT-1 binds through its SH2 domain.⁹³ STAT-1 is in turn phosphorylated by receptor-associated JAKs and homodimerisation of 2 STAT-1 proteins form a protein complex, GAF (gamma-activated factor).⁹⁴ STAT-1 homodimer translocates to the nucleus where it binds to a consensus sequence leading to modulation of the expression of many genes. The recently identified SOCS (suppressors of cytokine signalling) family of proteins inhibits IFN- γ signalling through the prevention of JAK kinase activation.⁹⁵

Effects.

IFN- γ has extensive and diverse immunoregulatory effects on various cells. It is produced by T_{H1}-cells and exerts an inhibitory effect on T_{H2}-cells.⁹⁶ IFN- γ

inhibits antigen-induced eosinophil recruitment in the mouse.⁹⁷ However, IFN- γ may also have proinflammatory effects and may activate airway epithelial cells to release cytokines and express adhesion molecules.⁹⁸ IFN- γ has an amplifying effect on the release of TNF- α from alveolar macrophages induced by IgE triggering or by endotoxin^{99,100} and increases the expression of class I and class II MHC molecules on macrophages and epithelial cells. IFN- γ is a powerful and relatively specific inhibitor of IL-4-induced IgE and IgG4 synthesis by B-cells.

IFN- γ increases the production of IL-1, PAF and H₂O₂ from monocytes, in addition to down-regulating IL-2-induced IL-8 mRNA expression.¹⁰¹⁻¹⁰³ IFN- γ also synergises the effects of TNF- α in the production of RANTES from airway smooth muscle cells.⁶⁵ On the other hand, IFN- γ inhibits IL-10 production from monocytes,¹⁰⁴ which in turn leads to an up-regulation of TNF- α transcription.¹⁰⁵ Thus, IFN- γ promotes cell-mediated cytotoxic responses, while inhibiting allergic inflammation and IgE synthesis. IFN- γ up-regulates class II molecules on monocytes/macrophages and dendritic cells, and induces *de novo* expression on epithelial, endothelial and other cells, thus making them capable of antigen presentation.

Role in allergy and asthma.

There is reduced production of IFN- γ by T-cells of asthmatic patients and this correlates with disease severity.^{106,107} No polymorphism of the IFN- γ gene has been associated with asthma.¹⁰⁸ Administration of exogenous IFN- γ prevents the airway eosinophilia and hyper-responsiveness following allergen exposure in mice.^{109,110} Liposome-mediated gene transfer of IFN- γ to the pulmonary epithelium in sensitised mice before secondary antigen exposure also inhibited the pulmonary allergic response.¹¹¹ IFN- γ receptor knock-out mice develop a prolonged airway eosinophilia in response to allergen.¹¹² IFN- γ inhibits allergic eosinophilia^{109,113} and airway hyper-responsiveness, probably by inducing the formation of IL-10. These studies indicate that IFN- γ has a potential modulating effect on allergen responses. The inhibition of the effects of allergen-specific T_{H2} cells, such as eosinophilia, mucus goblet cell hyperplasia and bronchial hyper-responsiveness, by allergen-specific T_{H1} cells is mediated by IFN- γ .^{11,12} Allergen immunotherapy of asthmatic patients results in increased production of IFN- γ by circulating T-cells¹¹⁴ and in an increase in IFN- γ producing T-cells in nasal biopsies.¹¹⁵ Corticosteroid treatment also increases IFN- γ expression in asthmatic airways,¹¹⁶ but in corticosteroid-resistant patients IFN- γ is unexpectedly reduced.¹¹⁷ In asthmatic patients, nebulised IFN- γ reduces the number of eosinophils in bronchoalveolar lavage, indicating its therapeutic potential in asthma.¹¹⁸

Interleukin-12

Synthesis and release.

IL-12 was initially recognised as a cytokine capable of synergising with IL-2 to increase cytotoxic T-lymphocyte responses, and also as an inducer of IFN- γ synthesis by resting human peripheral blood mononuclear cells *in vitro*. IL-12 is secreted by antigen-presenting cells, including B lymphocytes, monocytes/macrophages and dendritic cells.^{119,120} IL-12 mRNA has been localised to eosinophils in bronchoalveolar lavage fluid and in peripheral blood.¹²¹

Receptors and signalling.

The IL-12 receptor is composed of two subunits (β 1 and β 2), which are members of the haemopoietin receptor superfamily with strong homology to the gp130 receptor.¹²² The β 1 receptor is not able to transduce an IL-12-mediated signal, while the β 2 subunit when co-expressed with the β 1 subunit forms a high-affinity receptor for IL-12 and results in signalling.¹²³ IL-12R β 2 expression is differentially regulated in T_{H1} as opposed to T_{H2} cells,¹²⁴ while T_{H1} cells but not T_{H2} cells express the β 2 subunit.¹²⁵ On binding of IL-12 to IL-12R, activation of JAK kinases occurs, leading to phosphorylation of the receptor, which become binding sites for STATs that are rapidly recruited to the receptor and tyrosine-phosphorylated by JAK kinases. Tyrosine phosphorylation of STAT proteins induces their dimerisation and translocation to the nucleus where they bind to specific sequences and regulate transcription. IL-12 stimulation causes TYK2 and JAK2 phosphorylation, and interaction of TYK2 with IL-12R β 1 subunit and interaction of JAK2 with the IL-12R β 1 subunit.^{126,127} In T_{H1} cells and NK cells, IL-12 induces STAT4 to be tyrosine phosphorylated and activated.^{126,128} STAT1, STAT3 and STAT5 can also be activated by IL-12.^{128,129}

Effects.

IL-12 enhances the growth of activated T-cells and NK cells¹³⁰⁻¹³³ and enhances cytotoxic T-cell and NK activity.^{130,134,135} IL-12 stimulates NK cells and T-cells to produce IFN- γ ,^{134,136-138} promotes *in vitro* differentiation of mouse and human T-cells that secrete IFN- γ and TNF- α ,^{132,136,139,140} and inhibits the differentiation of T cells into IL-4 secreting cells.^{139,140} IL-12 indirectly inhibits IL-4-induced human IgE responses by IFN- γ -dependent and -independent mechanisms *in vitro*.¹⁴¹ Thus, IL-12 can primarily regulate T_{H1} cell differentiation, while suppressing the expansion of T_{H2} cell clones,¹³⁹ by early priming of undifferentiated T_H cells for IFN- γ secretion.¹⁴² Together with IL-18, IL-12 induces anti-CD40 activated B cells to produce IFN- γ , which inhibits IL-4 dependent IgE production.¹⁴³ Thus, IL-12 may direct

the development of T_{H1}-like T-cell responses against intracellular pathogens, whilst inhibiting the development of T_{H2}-like responses and IgE synthesis. IL-12 may also regulate airway eosinophilia.¹⁴⁴ IL-12 may play an important role in inhibiting inappropriate IgE synthesis and allergic inflammation as a result of allergen exposure.

Role in allergy and asthma.

A decreased number of IL-12 mRNA positive cells in airway mucosal biopsies from patients with asthma has been reported.¹⁴⁵ Corticosteroid treatment led to a reduction in the number of IL-12 mRNA expressing cells in steroid-sensitive asthmatics, but had no effect in steroid-resistant asthmatics.¹⁴⁵ IL-12 production from whole blood cultures from patients with allergic asthma was reduced when compared to those from nonatopic control subjects, together with a reduction in IL-12-dependent IFN- γ production.¹⁴⁶ Similarly, the percentage of peripheral blood eosinophils from asthmatic subjects expressing IL-12 mRNA was lower compared to nonatopic controls.¹²¹

IL-12 may play an important role in inhibiting inappropriate IgE synthesis and allergic inflammation as a result of allergen exposure. Thus, IL-12 treatment of mice during active sensitisation reduced antigen-induced influx of eosinophils in bronchoalveolar lavage fluid, inhibited IgE synthesis, and abolished antigen-induced bronchial hyperresponsiveness.¹⁴⁷ Once an inflammatory response is established, IL-12 inhibits antigen-induced bronchial hyperresponsiveness and inflammation.¹⁴⁸ These effects of IL-12 are largely mediated by IFN- γ .¹⁴⁹ In mice, IL-12 administered at the time of allergic sensitisation decreased specific IgE, tracheal ring responsiveness to acetylcholine and eosinophilia in bronchoalveolar lavage fluid after allergen challenge, together with IL-5 and IL-10 down-regulation; IL-12 administered after sensitisation did not alter specific IgE levels, had little effect on tracheal ring responsiveness and only a modest effect on the recruitment of eosinophils.¹⁵⁰ Similarly, in a murine model of house dust mite (Der p 1) sensitisation, IL-12 during the sensitisation period increased Der p 1-specific serum IgG2a while decreasing the levels of IgG1 and IgE antibodies following multiple allergen challenges, together with downregulation of IL-5 production, without affecting eosinophilia; IL-12 administration after active sensitisation down-regulated IL-5 production, increased IFN- γ production, and abolished recruitment of eosinophils.¹⁵¹ Thus, the effect of IL-12 was dependent on the timing of its administration in relation to active sensitisation.

Mucosal gene transfer of IL-12 to the lungs prevented the development of allergic sensitisation and airways hyper-responsiveness dependent on IFN- γ expression and suppressed established allergic disease, and reversed the suppression of local antiviral

cell mediated immunity responses to vaccinia virus resulting in rapid resolution of virus infection.¹⁵² Similarly, concurrent expression of IL-12 and GM-CSF in the airway led to inhibition of GM-CSF enhancement of ovalbumin-induced effects in the mouse, namely ovalbumin-specific IgE synthesis and airway eosinophilia, partly due to the expression of IFN- γ .¹⁵³ In another study, the suppressive effect of IL-12 on antigen-specific T_{H2}-like cell development, IgE up-regulation, airway hyperresponsiveness and eosinophilia in bronchoalveolar lavage fluid in an allergen-sensitised mouse model was only observed in combination with IL-18.¹⁵⁴ In mild allergic atopic asthmatics, IL-12 caused a reduction in allergen-induced blood eosinophilia, but did inhibit allergen-induced late phase responses.

The production of IL-12 and of IL-12-induced IFN- γ release is reduced in whole blood cultures from patients with allergic asthma compared to normal subjects.¹⁵⁵ There is a reduction of IL-12 mRNA expression in airway biopsies of patients with allergic asthma compared to normal subjects, but following oral corticosteroid treatment, the levels of IL-12 mRNA increased in corticosteroid-sensitive asthmatics, while no significant changes were observed in corticosteroid-resistant asthmatics.¹⁴⁵ This contrasts with the inhibitory effects of corticosteroids on IL-12 production in human monocytes *in vitro*.¹⁵⁶ Allergen immunotherapy results in an increase in IL-12 expression.³⁴ PGE₂, β_2 -agonists and corticosteroids inhibit IL-12 production from monocytes.¹⁵⁶⁻¹⁵⁸

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