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REVIEW ARTICLE

IL-12 as a therapeutic target for pharmacological modulation in immune-mediated and inflammatory diseases: regulation of T helper 1/T helper 2 responses

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1 Interleukin-12 (IL-12) is a pivotal cytokine in driving the immune system towards a T helper (Th)1 type response and preventing a Th2 type immune profile. Therefore, IL-12 is indispensable in the defense against certain, mainly intracellular pathogens, but overproduction of this cytokine is crucially involved in the etiology of several inflammatory and autoimmune diseases.

2 Hence, IL-12 is an ideal target for pharmacological intervention in the therapy of autoimmune and inflammatory diseases.

3 The production of IL-12 and a resultant Th1 type immune response can be suppressed with several pharmacological approaches including modulation of intracellular cyclic AMP levels, glucocorticoids and nuclear factor-κB inhibition. IL-12 responsiveness may be inhibited using anti-IL-12 antibodies, soluble IL-12 receptors or the IL-12 p40 homodimer.

4 Exploitation of these approaches may provide novel means for the experimental therapy of a variety of pathophysiological states.

Keywords: Inflammation; cytokines; autoimmune disease; interferon-γ; interleukin-2; interleukin-10; interleukin-4; transcription factors; catecholamine; nitric oxide

Abbreviations: GM-CSF, granulocyte-monocyte colony stimulating factor; HIV, human immunodeficiency virus; IB-MECA, N^6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; NK, natural killer; PDE, phosphodiesterase; PGE_2, prostaglandin E_2; TGF, transforming growth factor; Th, T helper; TNF, tumour necrosis factor

Introduction

Interleukin (IL)-12 is a heterodimeric cytokine that is secreted mainly by antigen-presenting cells and plays a key role in determining the nature of immune response to exogenous or endogenous antigens. IL-12 is comprised of two disulphide-linked protein subunits designated p35 and p40, which are encoded by two different genes (Trinchieri, 1995; Gately *et al*., 1998). Both subunits have to be produced within the same cell to obtain the biologically active dimer designated p70. The production of p40 exceeds the production of p70 by from 40 fold to more than 500 fold depending on the experimental system (Wysocka *et al*., 1995; Snijders *et al*., 1996; Haskó *et al*., 1998f). Five to forty per cent of this is secreted as a homodimer called $p(40)_2$. The $p(40)_2$ homodimer has been shown to exert antagonistic activity on the IL-12 receptor in both *in vitro* (Gilessen *et al*., 1995; Ling *et al*., 1995) and *in vivo* (Heinzel *et al*., 1997; Mattner *et al*., 1997; Rothe *et al*., 1997) systems. On the other hand, the $p(40)_2$ homodimer stimulates the differentiation of $CD8^+$ T cells with type 1 cytokine profile demonstrating agonistic properties (Gateley *et al*., 1998). The p35 subunit lacks any biological activity.

Cellular sources, inducers and cytokine regulators of IL-12

Although IL-12 was originally identified and purifed from supernatants of an Epstein-Barr virus-transformed human B lymphoblastoid cell line (Kobayashi *et al*., 1989), it is now clear that the main producers of IL-12 are cells of the innate immune system and professional antigen-presenting cells (Table 1). Cells of the monocyte/macrophage lineage including monocytes (D'Andrea *et al*., 1992), macrophages (Hsieh *et al*., 1993), Kupffer cells (Takahashi *et al*., 1996), mesangial cells (Bussolati *et al*., 1999) and glial cells (Park & Shin, 1996; Stalder *et al*., 1997) have all been shown to secrete IL-12. Other cell types of the innate system such as polymorphonuclear leukocytes (Cassatella *et al*., 1995) and mast cells (Smith *et al*., 1994), as well as keratinocytes (Muller *et al*., 1994) also express IL-12. Dendritic cells (Macatonia *et al*., 1995; Sousa *et al*., 1997) and Langerhans cells (Kang *et al*., 1996) are the most important professional antigen-presenting cells that produce IL-12.

The production of IL-12 both in macrophages and dendritic cells can be induced by two different mechanisms (Figure 1). The first pathway involves the interaction of the producer cells with various micro-organisms or microbial products. Live intracellular bacteria, such as *Listeria monocytogenes* (Tripp *et al*., 1993) and various *Mycobacteria* (Zhang *et al*., 1994) are potent triggers for IL-12 production. Intracellular parasites including *Leishmania major* (Reiner *et al*., 1994) and *Toxoplasma gondii* (Gazzinelli *et al*., 1993) and viruses (Biron, 1997) are also important inducers of IL-12. Bacterial lipopolysaccharide (LPS; Baron *et al*., 1993), killed *Mycobacterium tuberculosis* (D'Andrea *et al*., 1992), bacterial superantigens such as staphylococcus enteroxin B (Haskó *et al*., 1998f), and unmethylated CpG nucleotides (Klinman *et al*., 1996) have all been reported to evoke IL-12 production. The other main mechanism of IL-12 induction involves stimulation of CD40 on antigen-presenting cells wtih membrane bound or

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soluble CD40 ligand during T lymphocyte-antigen-presenting cell interactions (Shu *et al*., 1995; Figure 1).

The production of IL-12 is tightly regulated by other members of the cytokine cascade including both positive and negative regulatory signals (Figure 1). The most important positive regulators of IL-12 production are interferon (IFN)-γ (Ma *et al*., 1996), tumour necrosis factor (TNF)-α (Flesch *et al*., 1995) and granulocyte-monocyte colony stimulating factor (GM-CSF; Hayes *et al*., 1995; Randow *et al*., 1997). The potentially dangerous positive autoregulatory loop consisting of IL-12 and IFN-γ (see below) is offset by negative regulators of IL-12 production, such as IL-10 (D'Andrea *et al*., 1993; Berg *et al*., 1995), IL-4 (Snijders *et al*., 1996), IL-11 (Trepicchio *et al*., 1996), IL-13 (D'Andrea *et al*., 1995), transforming growth factor-β (TGF-β; D'Andrea *et al*., 1995) and IFN-α/β (Cousens *et al*., 1997). Interestingly, one of the mechanisms by which the human immunodeficiency virus (HIV) suppresses IL-12 production involves an indirect effect *via* induction of IL-10 by HIV-gp120 (Taoufik *et al*., 1997).

Table 1 Cell producers of IL-12

Cell group	*Cell type*	*Reference*
Monocytes/macrophages	Monocytes	D'Andrea *et al*., 1992
	Macrophages	Hsieh *et al*., 1993
	Kupffer cells	Takahashi *et al*., 1996
	Mesangial cells	Bussolati *et al*., 1999
	Microglia	Park *et al*., 1996
	Astrocytes	Stalder *et al*., 1997
Professional antigen-presenting cells	Dendritic cells	Macatonia *et al*., 1995
	Langerhans cells	Kang *et al*., 1996
Other cell types	Polymorphonuclear cells	Cassatella *et al*., 1995
	Mast cells	Smith *et al*., 1994
	Keratinocytes	Muller *et al*., 1994

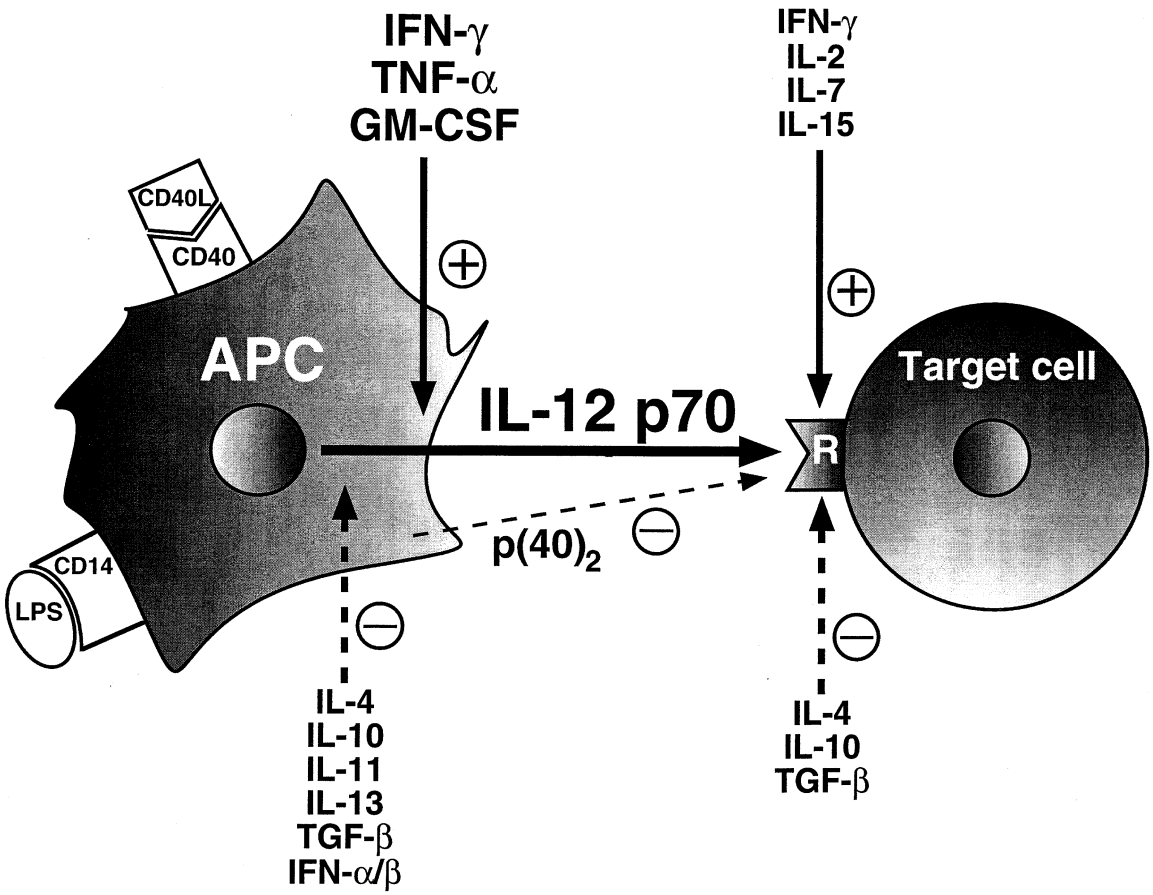

Figure 1 Schematic representation of the cytokine pathways involved in the modulation of IL-12 production and IL-12 receptor expression. The production of IL-12 in antigen presenting cells is induced by stimulation of CD40 by CD40 ligand or activation of CD14 by LPS. The production of IL-12 is subject to inhibition by IL-4, IL-10, IL-11, IL-13, TGF-β and IFN-α/β. IFN-γ, TNF-α and GM-CSF augment IL-12 release. IL-12 receptor expression is down-regulated by IL-4, IL-10 and TGF-β, and up-regulated by IFN-γ, IL-2, IL-7 and IL-15. Continuous lines represent stimulatory effects, while dotted lines represent inhibitory effects. The homodimer $p(40)_2$ is a natural antagonist of the IL-12 receptor on Th cells. Abbreviations: APC, antigen-presenting cell; CD40L, CD40 ligand; LPS, lipopolysaccharide; R, IL-12 receptor.

Cellular targets of IL-12 action

The main targets of IL-12 are T lymphocytes and natural killer (NK) cells (Trinchieri, 1995), but IL-12 also affects the function of B lymphocytes (Metzger *et al*., 1995) and hematopoietic progenitor cells (Jacobsen, 1995). IL-12 is an important link between innate and adaptive immunity, as it is secreted upon stimulation of antigen-presenting cells and activates IFN-γ production, proliferation, and cytolytic activity of NK cells and T lymphocytes (Trinchieri, 1995). In turn, IFN-γ has been shown to promote IL-12 production and macrophage activation, which provides the basis of an autoregulatory positive feedback loop resulting in a strong immune/inflammatory response directed against the antigen. Early in the immune response, IL-12 also plays a critical role in directing the development of T helper (Th)1 versus Th2 cell differentiation characterized by an increased production of IFN-γ and IL-2 (Th1 cytokines) and suppression of IL-4, IL-5 and IL-10 (Th2 cytokines) formation (Trinchieri, 1995). While a Th1 type immune response (cellular immunity) is crucial in the successful elimination of intracellular pathogens such as certain bacteria and viruses, and tumour cells, a Th2 response (humoral immunity) is critical for the removal of certain parasites, such as intestinal worms.

IL-12 receptors and signal transduction

IL-12 exerts its effects by binding to specific cell surface receptors on its target cells. The high affinity IL-12 receptor is formed by the coexpression of two subunits, the IL-12Rβ1 (Chua *et al*., 1994) and IL-12Rβ2 (Presky *et al*., 1996). While both the IL-12Rβ1 and IL-12Rβ2 are responsible for providing the binding energy, the IL-12Rβ2 is essential for signal transduction (Gately *et al*., 1998). The expression of IL-12Rβ2 appears to be confined to Th1 cells (Rogge *et al*., 1997; Szabo *et al*., 1997b), which may provide a selective therapeutic target for altering the Th1/Th2 balance in immunopathological conditions. Similar to the production of IL-12, the expression of both IL-12Rβ1 and IL-12Rβ2 is regulated by cytokines (Figure 1). While IL-2, IL-7, IL-15 and IFN-γ enhance IL-12 receptor expression, IL-4, IL-10 and TGF-β down-regulate IL-12 receptors and IL-12 responsiveness (Gollob *et al*., 1997; Rogge *et al*., 1997; Szabo *et al*., 1997b; Wu *et al*., 1997; Himmelreich *et al*., 1998).

IL-12 receptor stimulation in target cells activates the receptor-associated tyrosine kinases JAK2 and TYK2 and the transcription factors STAT3 and STAT4 (Bacon *et al*., 1995; Jacobson *et al*., 1995). Inhibition of tyrosine kinase activation may therefore provide a means for suppressing IL-12 responsiveness (see below).

Role of endogenous IL-12 in immunopathology

Autoimmune diseases are characterized by specific alterations in the expression of the above inflammatory mediators. In some of these diseases, a clear polarization of cytokine expression can be observed (Del Prete, 1998), either to the Th1 (multiple sclerosis, experimental allergic encephalomyelitis, autoimmune thyroid disease, and insulin-dependent diabetes mellitus) or to the Th2 direction (systemic lupus erythematosus, autoimmune hemolytic anaemia). In a Th1 cytokine response IFN-γ, IL-2, and TNF-α are the key players, while a Th2 profile is often associated with increased expression of IL-10, IL-4, IL-13 and TGF-β. Consequently, an approach to inhibit Th1 cytokines and potentiate Th2 cytokines would be desirable in Th1 diseases, and *vice versa* in Th2-mediated disorders. Because IL-12 is one of the central factors deciding the fate of the immune response concerning the polarity of this response (the presence of IL-12 shifts the balance towards a Th1 phenotype), this cytokine can be an ideal target for shaping the immune processes during autoimmune diseases. This hypothesis is corroborated by the fact that IL-12 has been shown to be directly and prominently involved in the induction of the pathophysiology of several autoimmune diseases including multiple sclerosis (Leonard *et al*., 1995), inflammatory bowel disease (Neurath *et al*., 1995), insulin dependent diabetes mellitus (Trembleau *et al*., 1995), glomerulonephritis (Kitching *et al*., 1999) and rheumatoid arthritis (Germann *et al*., 1995). The overproduction of IL-12 is also an important pathogenetic factor in inflammatory states such as septic shock (Wysocka *et al*., 1995) and the generalized Shwartzman reaction (Ozmen *et al*., 1994). Furthermore, a potential role for IL-12 was suggested in the promotion and maintenance of inflammation in atherosclerotic or psoriatic lesions (Uyemura *et al*., 1996; Yawalkar *et al*., 1998).

In contrast to the immunopathological role of over-expression of IL-12 in Th1 driven responses, IL-12 deficiency can contribute to an overactive Th2 type immune phenotype. This was best shown by the fact that IL-12 treatment reversed the airway hyperresponsiveness and decreased IL-4 and IL-5 expression in a murine model of asthma, a disease associated with a hyperreactive Th2 immune response (Gavett *et al*., 1995). IL-12 deficiency has been associated with tumour growth, while this cytokine has been successfully administered in patients with cancer (Lotze *et al*., 1996). Finally, treatment with IL-12 has been proposed for controlling viral infections such as chronic hepatitis or AIDS (Gately, 1997).

Anticytokine strategies based on immmunoneutralization of IL-12 and antagonism of IL-12 receptors

The recent success of anti-TNF-α antibodies (Elliott *et al*., 1994) and soluble TNF receptors (Moreland *et al*., 1997) in the short-term treatment of human rheumatoid arthritis suggest that strategies aiming at neutralizing other cytokines involved in the etiology of autoimmune diseases may be worth pursuing. The pivotal role of IL-12 in animal models of arthritis and other Th1-driven autoimmune diseases (see above) suggests that targeting this cytokine may be of therapeutical utility in these disease states. Besides similar approaches as in the case of anti-TNF-α therapies (anti-cytokine antibodies or soluble cytokine receptors), the use of the $p(40)_2$ homodimer could constitute a different approach, based on the recent data that this molecule is a physiological antagonist of the IL-12 receptor (see above). In fact, exogenous application of IL-12 $p(40)_2$ ameliorates the course of disease in animal models of insulin-dependent diabetes mellitus (Rothe *et al*., 1997) and endotoxaemia (Mattner *et al*., 1997).

Although such approaches have some promise, the long-term administration of these proteins during chronic auto-immune diseases can precipitate an immune response against these molecules resulting in the loss of efficacy and development of immune-complex disease. Another limitation of such drugs is that they should be administered parenterally. Therefore, it is important to identify small molecular weight compounds, which can regulate the production and/or activity of cytokines without the side effects of protein therapies. Recent studies have shown that the production and/or activity of IL-12 and consequently the balance between Th1 and Th2 responses can be modulated using small molecular weight

compounds, which can interfere with the synthesis or action of this cytokine.

Pharmacological modulation of IL-12 production

The induction of IL-12 production is a highly regulated process involving multiple pathways, which provides several targets for modulation by small molecular weight compounds (Figure 2). The most important targets can be classified as follows: the cyclic AMP-protein kinase system, the transcription factor nuclear factor κB (NF-κB), the glucocorticoid receptor, cell membrane ion channels and pumps, and nitric oxide synthase. There are several other compounds, which do not fall into any of these categories, and will be discussed separately.

Cyclic AMP modulating strategies

Considerable evidence suggests that alterations in intracellular cyclic nucleotide concentrations have a profound effect on cytokine production by immune/inflammatory cells (Pastores *et al*., 1996; Haskó & Szabó, 1998; Haskó *et al*., 1998b). Van der Pouw Kraan *et al*. (1995) showed for the first time using human monocytes that prostaglandin E_2 (PGE_2), an agent known to elevate intracellular cyclic AMP levels, potently suppresses IL-12 production. Other agents capable of increasing cyclic AMP levels, including the non-selective phosphodiesterase (PDE) inhibitor 3-isobutyl-1-methylxanthine and the cyclic AMP analogue N-2-O-dibutyryl-cyclic AMP mimicked the effect of PGE_2. In another *in vitro* study, PGE_2 was shown to inhibit IL-12 production in microglial cells (Levi *et al*., 1998). Recently, these results were confirmed *in vivo*, where intraperitoneal injection into mice of PGE_2 prevented the systemic production of IL-12 in response to *Escherichia coli* infection (Takano *et al*., 1998). Consistent with the role of IL-12 in inducing a strong protective Th1 type immune response against bacteria, the resolution of infection was hampered in the PGE_2-treated mice.

The predominant form of cyclic AMP PDE in monocytes/macrophages is the PDE IV isotype, but lower amounts of PDE III and PDE I can also be found in these cells (Verghese *et al*., 1995; Souness *et al*., 1996; Gantner *et al*., 1997; Kelly *et al*., 1998). Selective inhibition of these enzymes suppresses IL-12 production in various *in vivo* models. For example, treatment of endotoxemic mice with the PDE IV inhibitor rolipram or the PDE III blocker amrinone suppressed plasma IL-12 levels and consequently decreased mortality (Haskó *et al*., 1998e). Rolipram also suppressed IL-12 production, down-regulated the on-going Th1 response and ameliorated the course of collagen-induced arthritis, autoimmune diabetes, and experimental allergic encephalomyelitis in both rodent and primate models (Genain *et al*., 1995; Sommer *et al*., 1995; Ross *et al*., 1997; Liang *et al*., 1998). The non-selective phosphodiesterase inhibitor pentoxifylline was evaluated in human

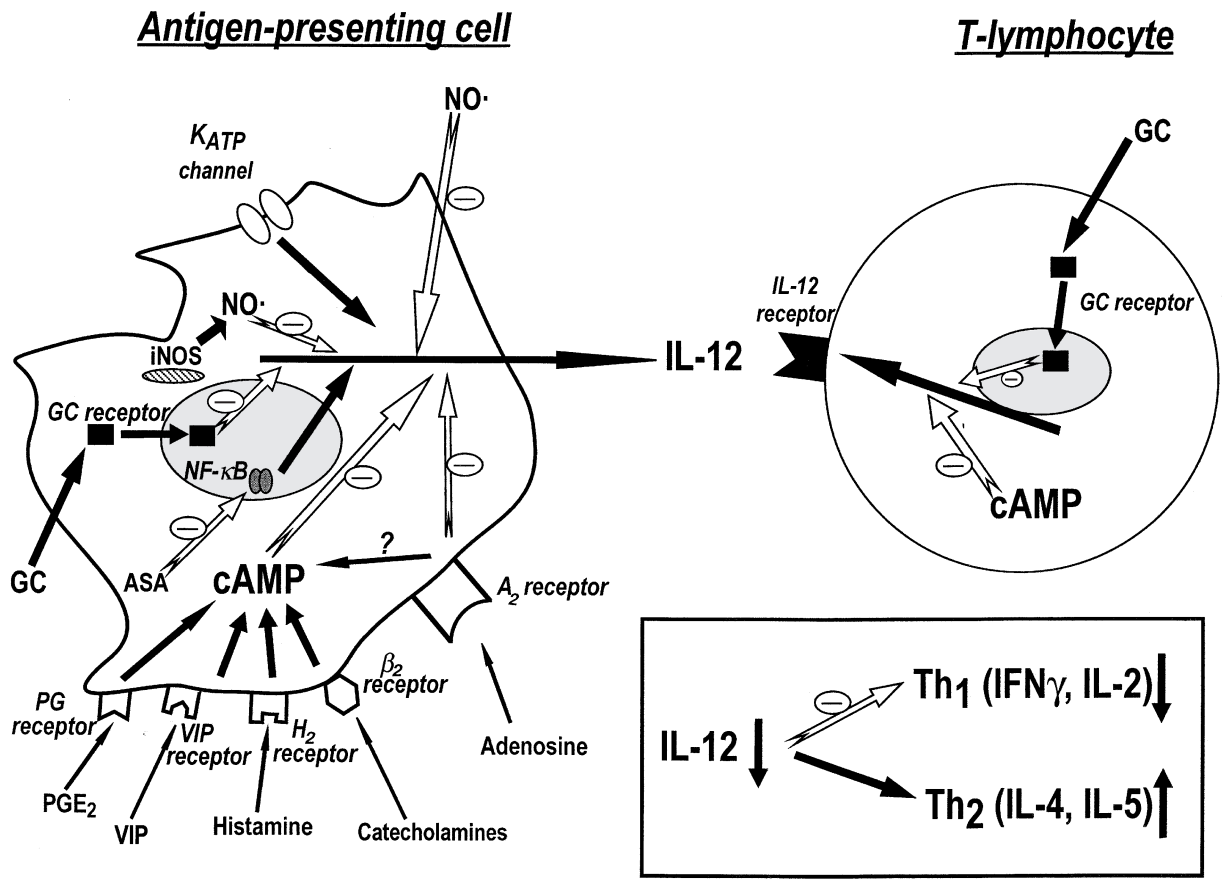

Figure 2 Schematic representation of the pathways involved in the modulation of IL-12 production and IL-12 receptor expression. IL-12 production can be inhibited by various approaches including elevation of intracellular cyclic AMP levels, activation of glucocorticoid receptors, inhibition of K_{ATP} channels, or inhibition of NF-κB. K_{ATP} channel activation enhances IL-12 production. The suppression of IL-12 shifts the immune response towards a T helper 2 profile, with decreased production of IFN-γ and IL-2, and augmented production of IL-4 and IL-5. Abbreviations: NO, nitric oxide; iNOS, inducible nitric oxide synthase; GC receptor, glucocorticoid receptor; ASA, acetyl salicylic acid; VIP, vasoactive intestinal peptide; NF-κB, nuclear factor κB; PG, prostaglandin.

patients with multiple sclerosis and was found to inhibit IL-12 production by peripheral blood mononuclear cells (Rieckmann *et al*., 1996). The suppressed IL-12 levels coincided with a deviation towards a Th2 type cytokine profile and six out of eight patients reported improved motor skills and less fatigue.

Besides PGE_2, other endogenous molecules which elevate cyclic AMP can also down-regulate IL-12 production. Most notably, the catecholamines adrenaline and noradrenaline are potent inhibitors of IL-12 release in human whole blood (Elenkov *et al*., 1996). These stress hormones act through β_2-adrenoceptors located on monocytes and dendritic cells and the mechanism of action involves transcriptional inhibition of both the p35 and p40 subunits and posttranslational inhibition of p35 (Panina-Bordignon *et al*., 1997). Again, the inhibition of IL-12 production coincided with a shift towards a Th2 type immune response. These observations might have important implications for the treatment of asthma, because the beneficial bronchodilatory effects of β_2-adrenoceptor agonists can, in the long term, be overshadowed by their ability to skew the immune response towards a harmful Th2 direction. The above *in vitro* findings were confirmed in endotoxemic mice, where isoproterenol, a non-selective agonist of β-adrenoceptors blunted the plasma IL-12 response (Haskó *et al*., 1998d). In contrast, the same study demonstrated that propanolol, a β-adrenoceptor antagonist augmented the release of this cytokine indicating that endogenously released catecholamines are involved in dampening the potentially harmful cytokine burst in acute systemic inflammation. Because septic shock is caused by an uncontrollable release of cytokines such as IL-12 and TNF-α, the potential immunomodulatory effects of β-adrenoceptor stimulation should also be considered during catecholamine treatment of septic states.

The purine adenosine is another endogenous agent capable of inhibiting both IL-12 production and Th1 development (Haskó *et al*., unpublished observation). Our results obtained with selective adenosine receptor agonists and antagonists suggest that the inhibition of IL-12 production is mainly dependent on A_{2a} receptor activation (Haskó *et al*., unpublished observation). On the other hand, the suppression of TNF-α by adenosine is primarily due to A_3 receptor activation (McWhinney *et al*., 1996; Sajjadi *et al*., 1996). Because both A_{2a} (Ralevic & Burnstock, 1998) and A_3 (Hines *et al*., 1999) receptors can be positively coupled to cyclic AMP, it can be suggested that the adenosine inhibition of both IL-12 and TNF-α is due to elevation of intracellular cyclic AMP levels. In agreement with the above *in vitro* data, the A_3 receptor agonist N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide (IB-MECA) suppresses IL-12 production in both arthritic and endotoxaemic mice, which correlates with its beneficial effect in both animal models (Haskó *et al*., 1998a; Szabó *et al*., 1998).

A whole host of endogenous molecules known to stimulate cyclic AMP, such as histamine, calcitonin gene-related peptide, vasoactive intestinal polypeptide and pituitary adenylate cyclase-activating peptide have been shown to inhibit IL-12 release in various *in vitro* experimental systems (Fox *et al*., 1997; Dewit *et al*., 1998; Van der Pouw Kraan *et al*., 1998; Xin & Sriram, 1998). Whether the endogenous production of these mediators affects IL-12 production, remains to be clarified.

Glucocorticoids

Glucocorticoids are used therapeutically as potent anti-inflammatory and immunosuppressive agents for a wide range of diseases, including autoimmune diseases, allergic states and other inflammatory illnesses. It has been known for a long time that these hormones are potent inhibitors of proinflammatory cytokine production, which is now considered as one of the central mechanisms of their anti-inflammatory action (Wilckens & De Rijk, 1997). Recently, it has been demonstrated that glucocorticoid hormones are important regulators of IL-12 production. Elenkov *et al*. (1996) showed for the first time that dexamethasone inhibits bioactive IL-12 production in LPS-stimulated human whole blood, an effect, which is effectively antagonized with a glucocorticoid receptor antagonist. Subsequently, it was shown that glucocorticoids inhibit IL-12 release from both monocytes and macrophages, as well as dendritic cells, and the reduced IL-12 production in all cases decreases the capacity of these cells to drive a Th1 response but enhances their ability to direct Th2 cytokine production (Blotta *et al*., 1997; DeKruyff *et al*., 1998; Vieira *et al*., 1998). Since dendritic cell IL-12 production is more important in the initiation of immune processes while macrophage-derived IL-12 is more significant in the maintenance of immune activation (Sousa *et al*., 1997), glucocorticoid suppression of IL-12 assures a continuous immunosuppressive effect. However, similarly to β_2-adrenoceptor agonists, chronic treatment of allergic diseases with corticosteroids may exacerbate the course of disease, because it promotes the overproduction of Th2 cytokines, which are already elevated and are causative factors in the pathogenesis of asthma and other allergic diseases. Recently, these *in vitro* findings were reproduced in an *in vivo* system, where treatment of mice with dexamethasone potently suppressed LPS-induced plasma IL-12 concentrations (Haskó *et al*., 1998d). Furthermore, endogenously secreted glucocorticoids also decrease IL-12 production, illustrated by the fact that inducibility of IL-12 exhibits diurnal rhythmicity in LPS-stimulated human whole blood; when plasma cortisol levels are low, the secretion of IL-12 is enhanced, but high plasma cortisol is paralleled with lower IL-12 release (Petrovsky *et al*., 1998). Interestingly, the HIV accessory gene product Vpr decreases IL-12 production and promotes the spread of infection by mimicking the effect of glucocorticoids *via* triggering the glucocorticoid receptor complex (Ayyavoo *et al*., 1997).

Targeting NF-κB

The transcription factor NF-κB plays a central role in coordinating immune/inflammatory processes. Recent studies have identified putative NF-κB sites in the promoter regions of both the IL-12 p35 and p40 genes (Murphy *et al*., 1995; Plevy *et al*., 1997). Recently, pharmacological evidence has been obtained that inhibition of this transcription factor system can decrease IL-12 production. For example, pyrrolidine dithiocarbamate, a selective blocker of NF-κB has been shown to inhibit LPS-induced IL-12 production both *in vivo* (Németh *et al*., 1998a) and *in vitro* (Haskó *et al*., unpublished observation). The *in vivo* inhibition of IL-12 by pyrrolidine dithiocarbamate exerts a protective effect against endotoxemic shock in mice (Németh *et al*., 1998a). This study also showed that pyrrolidine dithiocarbamate increases the production of IL-10, indicating that the inhibitory effect of this agent is selective for proinflammatory cytokines. Interestingly, the steroid hormone 1,25-dihydroxyvitamin D_3 suppressed IL-12 production not by binding to the vitamin D_3 receptor, but *via* inhibition of NF-κB binding to its consensus sequence on the IL-12 p40 gene (D'Ambrosio *et al*., 1998). 1,25-dihydroxyvitamin D_3 suppresses Th1 cytokine production and protects against diabetes in the IL-12 dependent non-obese diabetic mice (Casteels *et al*., 1998).

Similarly to 1,25-dihydroxyvitamin D_3, acetyl salicylic acid suppresses IL-12 production and Th1 development by a mechanism involving decreased NF-κB activation (Mazzeo *et al*., 1998). The Vpr-induced repression of IL-12 production (see above) also involves NF-κB (Ayyavoo *et al*., 1997). Although direct evidence has not yet been presented, it is conceivable that glucocorticoids, which are known to inhibit NF-κB (Dumont *et al*., 1998), may suppress IL-12 by interaction with this transcription factor system. Finally, retinoids inhibit IL-12 production by forming a transcriptionally inhibitory complex with NF-κB (Na *et al*., 1999).

Ion channels and pumps

The movement of ions across cell membranes mediates several cellular processes in the immune system and there is a large body of evidence indicating that altering the activity of ion channels and pumps can profoundly affect cytokine production (Haslberger *et al*., 1992; Hamon *et al*., 1997; Szabó *et al*., 1997a; Haskó *et al*., 1998c). IL-12 production is also subject to modulation by changes in ion movements. Blockade of dihydropyridine-sensitive calcium channels inhibits IL-12 production in human dendritic cells, which can be prevented by a calcium channel agonist (Poggi *et al*., 1998). These channels are also the molecular targets of HIV Tat, which blocks both calcium influx and IL-12 release in the dendritic cells. This mechanism might contribute to the immunosuppression seen during HIV infection. In LPS-treated mice, the calcium channel antagonists verapamil and diltiazem are unable to suppress plasma IL-12 levels, however, dantrolene, an agent known to prevent the release of calcium from intracellular stores inhibits IL-12 production (Németh *et al*., 1998b). Our group recently demonstrated that by modulating ATP-gated K^+ channels on immune cells, IL-12 production can be altered substantially: glibenclamide, a selective inhibitor of this channel, potently inhibits the release of this cytokine, while diazoxide, an opener of this channel, considerably increases IL-12 secretion (Haskó *et al*., unpublished observations). Consequently, in anti-CD3-stimulated mouse spleen cells, glibenclamide decreases the production of Th1 cytokines, but augments the production of the Th2 cytokine IL-4. Finally, inhibition of the Na/H antiporter by amiloride decreases IL-12 production, without altering the Th1/Th2 ratio: both IL-4 and IFN-γ production are inhibited by this agent in anti-CD3 antibody stimulated spleen cells (Haskó *et al*., unpublished observations).

Nitric oxide

The role of nitric oxide in the modulation of IL-12 expression is controversial. In the study of Rothe *et al*. (1996), addition of the nitric oxide synthase inhibitor N(G)-methyl-L-arginine suppressed INF-γ-induced IL-12 p40 mRNA formation, while nitric oxide generating compounds induced p40 mRNA. Furthermore, inhibition of nitric oxide synthase in mice with both N(^{G})-nitro-L-arginine-methylester and aminoguanidine decreased IL-12 production by dispersed lung cell cultures (Hogaboam *et al*., 1997; 1998). On the other hand, in J774 macrophages, N(^{G})-methyl-L-arginine markedly enhanced IL-12 protein secretion, while nitric oxide-generating compounds decreased it (Huang *et al*., 1998). Also, a number of recent studies showed that mice deficient in the inducible nitric oxide synthase produce enhanced amounts of IL-12 when compared to their heterozygous or wild-type counterparts (Huang *et al*., 1998; MacLean *et al*., 1998).

Immunosuppressive agents

Some of the most widely used immunosuppressive agents turned out to be potent inhibitors of IL-12 production. For example, tacrolimus (FK 506), a drug primarily used in organ transplantation, decreases IL-12 expression during primary skin responses (Homey *et al*., 1998). Thalidomide, which has beneficial effects in a host of inflammatory diseases, is a potent inhibitor of IL-12 production, and exerts its affect by a post-transcriptional mechanism (Moller *et al*., 1997). In contrast, cyclosporin A enhances IL-12 production in mouse spleen cells induced by CpG oligonucleotides (Redford *et al*., 1998), however, it remains to be determined whether this is the case when other inflammatory or immune stimuli are used. Other agents that are known to have immunosuppressive effects such as angiotensin converting enzyme inhibitors (Constantinescu *et al*., 1998) or sulfasalazine (Haskó *et al*., unpublished observation) are also capable of suppressing IL-12 production.

Modulation of IL-12 responsiveness

The expression of both subunits of the IL-12 receptor is up-regulated upon cellular activation. There is only very limited information available on the pharmacological modulation of the IL-12 receptor. In a recent study using human peripheral blood mononuclear cells and T lymphocytes, it has been shown that the expression of both chains of the IL-12 receptor is subject to suppression by dexamethasone or PGE_2 (Wu *et al*., 1998). Dibutyryl cyclic AMP, 8-Br-cyclic AMP or cholera toxin mimicks the effect of PGE_2 suggesting that it mediates its effects through enhancement of cyclic AMP (Wu *et al*., 1998; Braun *et al*., 1999). In contrast to the above agents, lisofylline inhibits IL-12 responsiveness, however it fails to suppress IL-12 production (Bright *et al*., 1998). It remains to be determined whether the action of lisofylline is due to an effect on the expression of IL-12 receptor or an interference with the intracellular events triggered by IL-12/IL-12R interaction. IL-12 responsiveness has been shown to be inhibited by protein kinase C and tyrosine kinase inhibition (Gerosa *et al*., 1993; Ye *et al*., 1995).

Conclusions

IL-12 plays an essential role in the protective immune responses against intracellular pathogens by directing the development of Th1 versus Th2 reactions. On the other hand, IL-12 driven Th1 reactions have deleterious consequences in certain autoimmune/inflammatory diseases. Therefore, the IL-12/IL-12 receptor system is an ideal target for pharmacological intervention in both immunodeficient states, when Th1 type immune responses are in demand or in 'hyperimmune' diseases, when an ongoing Th1-type immune response underlies the pathophysiological processes. Recent studies have identified a number of pharmacological approaches, which are able to influence IL-12 production and Th1 versus Th2 immune responses (Table 2). It is clear that most of these approaches are not specific for IL-12 and also influence the production of other cytokines and mediators. Because in most inflammatory states, there is a high degree of redundancy between the function of proinflammatory cytokines, the ability of an agent to inhibit several interrelated proinflammatory pathways may even be desirable. Furthermore, in some cases, the inhibition of IL-12 by a compound is paralleled by elevated IL-10 production, which may result in an even stronger anti-

Table 2 Pharmacological agents capable of decreasing Th1 and augmenting Th2 cytokine production

Pharmacological agent	*Cellular target*	*Reference*
Salbutamol	β_2-adrenoceptor, cyclic AMP	Panina-Bordignon *et al*., 1997
PGE_2	PGE_2 receptor, cyclic AMP	Wu *et al*., 1998
CGRP	CGRP receptor, cyclic AMP	Fox *et al*., 1997
Dexamethasone	glucocorticoid receptor on monocytes	Blotta *et al*., 1997
Dexamethasone	glucocorticoid receptor on macrophages	DeKruyff *et al*., 1998
Hydrocortisone	glucocorticoid receptor on dendritic cells	Vieira *et al*., 1998
Clobetasol	glucocorticoid receptor on dendritic cells	Vieira *et al*., 1998
Acetyl salicilic acid	NF-κB	Mazzeo *et al*., 1998
N(^{G})-methyl-L-arginine	nitric oxide synthase	Huang *et al*., 1998
Captopril and lisinopril	angiotensin converting enzyme	Constantinescu *et al*., 1998
Adenosine	adenosine receptor	Haskó *et al*., unpublished
Glibenclamide	K_{ATP} channel	Haskó *et al*., unpublished

inflammatory effect. On the other hand, it is conceivable that the non-selective blockade of proinflammatory pathways can be harmful in some pathophysiological states. For instance, when a chronic autoimmune process is complicated with an infectious disease, preservation of some immune functions may be crucial and selective pharmacological interventions to suppress the production of certain, but not all cytokines should be applied. In any case, exploitation of these approaches may provide novel means for the experimental therapy of a variety of pathophysiological states.

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