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Review Article

Inhibiting cytokines of the interleukin-12 family: recent advances and novel challenges

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Abstract

Interleukin-12 (IL-12) and the more recently discovered IL-23 and IL-27 constitute a unique family of structurally related, heterodimeric cytokines that regulate cell-mediated immune responses and T helper 1 (Th1)-type inflammatory reactions. Not surprisingly, the potentiality of treating conditions such as multiple sclerosis (MS) and rheumatoid arthritis (RA) through pharmacological interference with IL-12 pathways has received widespread attention. In this review we have examined over 50 substances with reported IL-12 inhibitory effects. We demonstrate that a majority of these belong to a limited number of major functional classes, each of which targets discrete events in the IL-12 biological pathway. Thus, most IL-12 inhibitory substances appear to work either through inhibition of transcription factor NF-κB activation, up-regulation of intracellular cAMP, blockage of posttranslational processing or interference with signal transduction pathways. In addition, cyclophilin-binding drugs, and generic inhibitors of nuclear histone deacetylases, and of ion channels, pumps and antiporters are emerging as potential leads to novel targets for interference with IL-12 production. Many inhibitors of NF-κB and of IL-12 signal transduction have been proven effective in limiting or preventing disease in experimental autoimmune encephalomyelitis (EAE) models of MS. The sharing of the p40 subunit, the IL-12Rβ1 and components of the signal transduction pathways between IL-12 and IL-23 raises the question as to whether the beneficial effects of various drugs previously ascribed to inhibition of IL-12 may, in fact, have been due to concurrent blockage of both cytokines, or of IL-23, rather than IL-12. Moreover, the homodimeric β_2-form of IL-12, though originally considered to display only antagonistic effects, is now emerging as a pronounced agonist in a variety of inflammatory processes. Reassessment of IL-12 inhibitory compounds is therefore needed to scrutinize their effects on IL-12 $\alpha\beta$, β_2 and IL-23 formation. This is likely to open exciting perspectives to the identification of drugs that target these cytokines either indiscriminately or selectively. The functional diversity of presently available inhibitors should facilitate an unprecedented flexibility in designing future trials for the treatment of IL-12- and IL-23-mediated disorders.

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Introduction

Inhibiting the action of interleukin-12 (IL-12) has been shown to suppress development and clinical progression of disease in a multitude of experimental models of autoimmunity and chronic inflammation (Caspi 1998). These observations have kicked off an ever-expanding quest for novel pharmacological inhibitors of IL-12 with potential relevance for treatment of Th1-type disorders such as multiple sclerosis, psoriasis, rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes and acute graft-versus-host disease. In this article, we have attempted to compile and classify numerous inhibitors that are known to suppress IL-12 production or signal transduction. We show that, according to their mode of action, most pharmacological inhibitors belong to one out of three major categories: inhibitors of NF-κB; compounds directly or indirectly raising intracellular cAMP or modulating related intracellular pathways; and those interfering with the IL-12 signal transduction pathway. In addition, we have included inhibitors of IL-12 that do not specifically belong to any of the 3 major categories and work through interfering with nuclear histone deacetylases; with cyclophilins; with ion pumps, channels and transporters, or through modification of posttranslational processing. For the purpose of this review, we did not consider inhibitors that solely act indirectly (e.g. through modifying the Th1–Th2 balance (e.g. by increasing IL-10 production)), and neither did we include

antibody-based drugs or endogenous polypeptides with intrinsic ability to suppress IL-12, such as chemokines. Whenever possible, we have attempted to correlate in-vitro with in-vivo data. In particular, many IL-12 inhibitors have been tested in animal models of experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis, and sometimes these studies were performed before the effects of these inhibitors on IL-12 production were known or understood.

The most important message emerging from this compilation may well reside in the conclusion that the huge number and functional diversity of presently available inhibitors should facilitate an unprecedented flexibility in designing future trials for treating IL-12- and IL-23-mediated diseases.

Structure, biology and signal transduction of interleukin-12, -23 and -27

The IL-12 family of cytokines: structure and regulation

IL-12 is the critical cytokine that regulates the differentiation of naïve $CD4^+$ T cells to T helper (Th) 1 cells that produce interferon-γ (IFN-γ). IL-12 is a heterodimer composed of two disulfide-linked subunits designated p40 (or β-chain) and p35 (or α-chain) (Gately et al 1991; Gubler et al 1991; Wolf et al 1991; Trinchieri & Scott 1995; Yoon et al 2000). Whereas p40 has homology with cytokine receptors, the p35 subunit is homologous to other cytokines, such as IL-6 and granulocyte-colony stimulating factor (G-CSF). The assembly of these subunits results in the biologically active p70 heterodimer. The p35 subunit is ubiquitously expressed, whereas p40 expression is more limited and highly regulated. The p40 promoter binds several transcription factors, such as NF-κB, IFN regulatory factor-1 (IRF-1) and Ets-family members (Murphy et al 1995; Ma et al 1996, 1997; Plevy et al 1997; Figure 1). Moreover, the IFN consensus binding protein (ICSBP) has also been shown to be important for IL-12 transcription (Wang et al 2000). IL-12 is produced by a variety of cells, but antigen-presenting cells (APCs) such as monocytes, macrophages and dendritic cells (DC) are the major producers. Binding of bacterial products from these organisms to Toll-like receptors results in IL-12 production. IL-12 participates in a positive feedback loop by promoting IFN-γ secretion that, in turn, potently primes monocytes and granulocytes for further IL-12 production (Kubin et al 1994; Yoshida et al 1994; Ma et al 1996).

The recent identification of a novel cytokine subunit, p19, which is homologous to IL-12p35, has turned IL-12 into a family of cytokines. Identified through database mining, p19 heterodimerizes with p40 to form the new cytokine, designated IL-23 (Oppmann et al 2000). p19 is also produced by APCs, and T cells and endothelial cells can produce it as well. IL-23 can induce IFN-γ in T cells but unlike IL-12, it strongly enhances memory T-cell proliferation.

IL-27 is the newest member of this family of heterodimeric cytokines (Pflanz et al 2002) and is the result of the dimerization of EBI3 (Epstein-Barr virus induced gene 3) with the novel protein p28. EBI3 is homologous to IL-12p40 (Devergne et al 1996, 1997), whereas p28 is related to IL-12p35. IL-27 is also produced by APCs. Similar to IL-12, it induces proliferation of naïve T cells and, in combination with IL-12, it promotes IFN-γ secretion and drives Th1 differentiation.

Receptors utilised by cytokines of the IL-12 family

The IL-12 receptor (IL-12R) is composed of two subunits, β1 and β2, structurally similar to the type I cytokine receptor super-family and homologous to gp130 (Chua et al 1994, 1995; Presky et al 1996). As is the case for other type-I cytokine receptors, co-expression of both subunits generates the high-affinity IL-12R. The β2-subunit contains on its cytoplasmic domain tyrosine residues that recruit signalling molecules, whereas IL-12Rβ1 is critical for ligand binding (Wu et al 2000). The IL-12R complex is expressed on T cells, natural killer (NK) cells and DCs (Grohmann et al 1998), with its expression on T cells being highly regu-

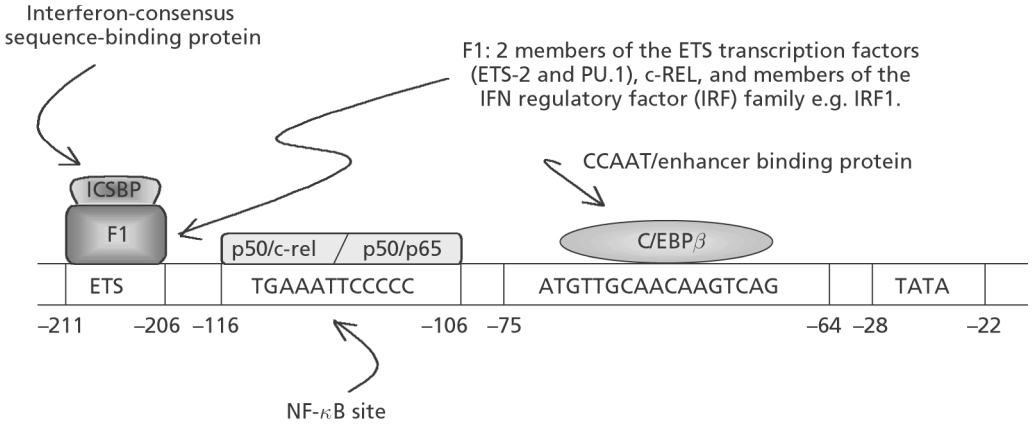

Figure 1 Regulation of interleukin-12 p40 gene expression. The ETS element is regarded as the major response region. This element interacts with a large nuclear complex (F1) and ICSBP (IFN-γ-induced nuclear factors). p50/c-rel and p50/p65 bind to the NF-κB site in response to LPS induction. More proximal to the TATA box, a third element is found, to which the C/EBPβ transcription factor binds.

lated. The control of IL-12Rβ2 expression is an important aspect in helper T cell regulation. IFN-γ up-regulates the transcription factor T-bet, which, in turn, maintains IL-12Rβ2 expression (Lighvani et al 2001; Afkarian et al 2002). In contrast, IL-4 down-regulates IL-12Rβ2 expression (Rogge et al 1997; Szabo et al 1997; Sinigaglia et al 1999), suggesting that the counter-regulation of IL-12 receptor expression by IFN-γ and IL-4 is an important factor governing Th1–Th2 differentiation.

IL-23 binds a receptor composed of IL-12Rβ1 and a second subunit, designated IL-23R, normally expressed at low levels on T cells, NK cells, monocytes and DCs (Parham et al 2002). The intracellular domain of the IL-23R contains seven tyrosine residues, which are probably important for downstream signalling events.

IL-27 binds the previously considered orphan receptor WSX-1/TCCR, another receptor with homology to the gp130 family mainly expressed in T cells (Chen et al 2000; Yoshida et al 2001; Pflanz et al 2002). Because of the similarity with the IL-12 and IL-23 receptors, it is predictable that a second IL-27R component will soon be discovered.

Biological effects of the IL-12 family of cytokines

IL-12 stimulates the production of IFN-γ in NK cells (Trinchieri 2003). IFN-γ thus produced stimulates bactericidal activity of phagocytic cells and enhances the innate immune response. Moreover, the development of naïve $CD4^+$ T cells to either Th1 or Th2 cells, a crucial event for effective acquired immunity, is critically regulated by IL-12, even if several other cytokines play a role in this process. Th1 cells produce IFN-γ and promote the cell-mediated immunity essential for the response against intracellular pathogens, viruses and bacteria (Dong & Flavell 2001; Glimcher 2001; Farrar et al 2002; O'Shea & Paul 2002; O'Shea et al 2002). IL-23 acts preferentially on memory $CD4^+$ T cells and its role is therefore more important for the anamnestic immune response. IL-27 synergizes with IL-12 to trigger IFN-γ production by T cells (Pflanz et al 2002) and preferentially acts on naïve $CD4^+$ T cells to induce their differentiation and expansion.

Signal transduction

Like other cytokine receptors IL-12R lacks intrinsic enzymatic activity (for overview of signal transduction see Figure 2). Signalling through the IL-12R involves activation of Janus kinases (JAKs) (O'Shea et al 2002). Jak2 and Tyk2 phosphorylate the IL-12R on tyrosines located in the intracellular domain (Zhou et al 1997). STATs (signal transducers and activators of transcription) are recruited to these phosphorylated tyrosines, and subsequently, phosphorylated STATs form transcriptionally active

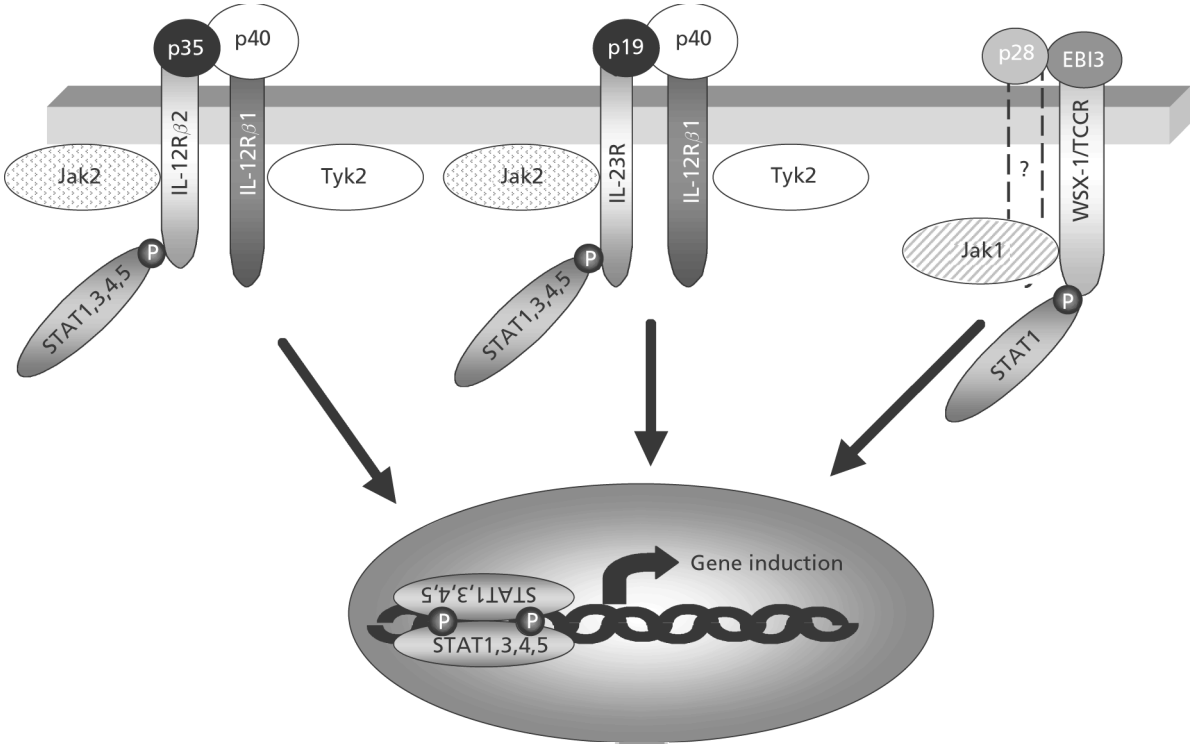

Figure 2 Signal transduction pathway of the IL-12 family of cytokines (Gadina et al 2003). IL-12 and IL-23 both utilize p40 and IL-12Rβ1. IL-27 is composed of a p35-related molecule called p28, and a p40-related soluble receptor, EBI3. IL-27 binds WSX-1/TCCR and another signal-transducing chain (still unknown). Upon cytokine binding to the receptors, the signal transduction leads to activation of the Jak/STAT pathway as indicated. IL-12 and IL-23 activate a similar pattern of Jaks and STATs. The signal tranduction pathway of IL-27 is still only partially characterized but involves Jak1 and STAT1.

hetero- or homo-dimers. STAT4 appears to be most critical for IL-12 signalling (Bacon et al 1995; Jacobson et al 1995). Jak2 and Tyk2 are also involved in the IL-23 signalling cascade and their activation also results in phosphorylation of STAT1, 2, 3, 4 and 5. The molecular events downstream of the IL-27R have not been completely elucidated yet. Jak1 and STAT1 have been shown to be recruited to the receptor (Takeda et al 2003) but the lack of a counterpart for the WSX-1 chain makes it difficult to draw any conclusion about the IL-27 signal transduction.

Role of IL-12 and IL-23 in inflammation: lessons from experimental animal models

Knocking out the p40 gene or using anti-IL-12 antibodies prevents Th1 cell-mediated disorders . . . but there is a caveat

The ability of IL-12 to strongly promote the development of Th1 cells makes it an ideal target for the treatment of Th1 cell-mediated diseases, such as autoimmune diseases and inflammatory bowel disease (IBD). Over the past 10 years many research groups have utilised a number of animal models to elucidate the role of endogenous IL-12 in pathogenesis. These models include experimental autoimmune encephalomyelitis (EAE), experimental autoimmune uveitis (EAU), collagen-induced arthritis (CIA), autoimmune nephritis, insulin-dependent diabetes mellitus (IDDM) and different models for IBD. In these models, the role of endogenous IL-12 has been addressed by using IL-12p40 knockout mice or by administering anti-IL-12 antibodies. IL-12p40 knockout mice have been found to be resistant to EAE (Becher et al 2002; Gran et al 2002; Cua et al 2003) and EAU (Tarrant et al 1998). In CIA, disease incidence was reduced in IL-12p40 knockout mice although some mice developed severe paw inflammation, indicating that IL-12 is not absolutely required in the etiopathogenesis of this disease (McIntyre et al 1996).

Autoimmune disease in MRL-Fas^{lpr} mice is characterized by fatal nephritis and systemic inflammation of skin, glands, lungs, and joints, and is a model of systemic lupus erythematosus. MRL-Fas^{lpr} mice deficient in IL-12p40 exhibited a significantly reduced systemic inflammation but only a delay in renal pathology (Kikawada et al 2003), indicating that IL-12 has a differential impact on pathology in multiple tissues undergoing autoimmune destruction. Endogenous IL-12 seems not to be required for spontaneous development of IDDM and insulitis in non-obese diabetic (NOD) mice, since IL-12p40-deficient NOD mice were found to develop IDDM and insulitis equally well as wild-type mice (Trembleau et al 1999). Remarkably, Th1 development in the peripheral organs, but not in the inflamed pancreas, was impaired in IL-12p40 knockout NOD mice. This is in contrast with findings in which NOD mice were treated with IL-12 antagonists; the pancreas-infiltrated T cells were skewed to a Th2 phenotype and the treated mice were protected from IDDM. In fact, in almost all other Th1-related animal models in which anti-IL-12 antibodies have been used, the treatment was found to abrogate Th1 development and to suppress the severity of the disease, provided that the antibodies were administered in the initial phase of the disease process.

EAE is an animal model for multiple sclerosis (MS) and is considered to be the prototype of Th1-associated autoimmune diseases. Neuroantigen-specific CD4$^+$ lymphocytes of the Th1 phenotype and inflammatory macrophages are thought to mediate the development of the inflammatory lesions and to trigger the demyelination of axons leading to progressive paralysis. In MS, increased IL-12 levels have been reported to correlate with disease activity and with active brain lesions detected by magnetic resonance imaging (Nicoletti et al 1996; Balashov et al 1997; Comabella et al 1998; Makhlouf et al 2001a). In mice, EAE can be induced by immunization with myelin components together with one or several adjuvants. Depending on the induction schedule and the mouse strain, an acute form of the disease or a chronic progressive and relapsing form of EAE develops. In both forms of EAE, monoclonal antibody against IL-12 has been shown to prevent or suppress the development and progression of the disease. The effects of anti-IL-12 antibody treatment were studied in chronic relapsing EAE in SJL/J and in Biozzi ABH mice (Heremans et al 1999). In Biozzi mice, relapses occur spontaneously with high frequency, while in SJL mice spontaneous relapses occurs infrequently but can be induced reproducibly by re-immunization. Anti-IL-12 treatment not only inhibited the first attack but also inhibited spontaneous or induced relapses, provided that the antibodies were given before the onset of the first symptoms. Anti-IL-12 antibody, given during remission of the first attack, inhibited the re-induced relapses in SJL/J mice but not spontaneous relapses in Biozzi ABH mice (Heremans et al 1999). These results indicate that endogenous IL-12 affects the active induction process, but may not be necessary for triggering spontaneous relapses.

CIA is an experimental autoimmune model for human rheumatoid arthritis. IFN-γ receptor knockout mice are highly susceptible to CIA in that they develop arthritis more rapidly and with a more severe disease score than their wild-type counterparts (Vermeire et al 1997; Matthys et al 1999). Not only did administration of an anti-IL-12 antibody significantly reduce the symptoms of arthritis, but it also abrogated the humoral and Th1 cytokine response to the autoantigen collagen type II. These effects were seen both in wild-type and in IFN-γ receptor knockout mice (Matthys et al 1998). Although IL-12 is a strong inducer of IFN-γ, these results reveal that endogenous IL-12 can promote Th1 and disease pathology through a pathway independent of IFN-γ production. Similar findings have been reported for autoimmune diabetes (Trembleau et al 2003).

While all of these findings suggest that endogenous IL-12 plays a critical role in the pathogenesis of Th1-related disorders, it is important to mention that the anti-IL-12 antibodies used in these studies are known to act by binding the p40 subunit of the heterodimer. As this subunit is also part of IL-23 (Oppmann et al 2000), it is likely that in-vivo administration of anti-IL-12 antibodies neutralizes both endogenously formed IL-12 and IL-23. This observation calls into question whether the observed effects in the various disease models referred to above are to be

attributed solely to blockage of IL-12. Indeed, similar concerns arise from studies based on the use of knockout mice deficient in p40, which, as a consequence, do not produce either cytokine.

Distinct roles for IL-12 and IL-23 in the pathogenesis of EAE and inflammation in the central nervous system (CNS)

The first indirect evidence for the importance of a p40-containing cytokine other than IL-12 came from studies using p35 gene-targeted mice. These mice lack bioactive IL-12 but have normal levels of IL-23. IL-12p35 knockout mice are at least as susceptible to induction of EAE as wild-type mice (Becher et al 2002; Gran et al 2002). The disease in the susceptible IL-12p35 knockout mice was associated with a down-regulation in autoantigen-specific Th1 responses (Becher at al 2002), or with a deviation to Th2 (Gran et al 2002). This confirmed the importance of bioactive IL-12 in orchestrating Th1 type immune responses, but also illustrated that EAE can develop in Th2-associated conditions. Together with the finding that p40 knockout mice are resistant to EAE, these data suggested that other p40-containing cytokines, such as IL-23, are required for establishment of the disease. Proof for the involvement of IL-23 in the pathogenesis of EAE came from Cua et al (2003). These authors used p19-deficient mice, which lack IL-23 but not IL-12, and compared their susceptibility for EAE with p35-deficient mice (lacking IL-12 but not IL-23) and p40-deficient mice (lacking both IL-12 and IL-23). Similar to p40 knockout mice, p19 knockout mice were completely resistant to EAE. The p35 knockout mice were highly susceptible for EAE, a finding that is in confirmation with the earlier reports cited above (Becher et al 2002; Gran et al 2002). Cua et al (2003) further demonstrated that administration of IL-23 into the CNS of p19 knockout mice just before the disease onset can abrogate the resistance to EAE. In p40 knockout mice, IL-12 alone was not sufficient to provoke the disease. However, when the IL-12 treatment (at an early time point in the disease) was followed by administration of IL-23 into the CNS, EAE comparable with that seen in wild-type mice was induced. Administration of IL-23 alone in p40 knockout mice induced only a weak and delayed form of EAE. Additional experiments led the authors to conclude that IL-12 is necessary for the development of Th1 cells during the induction phase, whereas IL-23 is necessary for the subsequent CNS inflammation by activation of CNS-resident macrophages. In the mouse, the biological activity of IL-23 is distinct from IL-12: it does not induce significant amounts of IFN-γ, but unlike IL-12 it does induce proliferation of memory T cells (Brombacher et al 2003).

Taken together, the use of mice deficient in p40, p35 and p19 has revealed distinct roles for IL-12 and IL-23 in EAE. IL-23 cross-reactivity with anti-IL-12 antibodies questions the validity of effects previously attributed solely to IL-12 in a variety of other Th1-related disease models. Specific targeting of the p35 and p19 subunit in these models of disease will be important to clarify the individual contribution of each cytokine to overall pathogenesis. Equally, the sharing of the p40 subunit, the IL-12Rβ1 and components of the signal transduction pathways between IL-12 and IL-23 implies that the beneficial effects of various drugs previously ascribed to inhibition of IL-12 may, in fact, have been due to blockage of IL-23-associated, rather than IL-12-associated, pathogenesis. Hence, re-evaluation of these inhibitors is mandatory to differentiate between these possibilities.

Inhibitors of IL-12 (Figure 3)

Inhibitors of NF-κB

1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is the biologically active metabolite of vitamin D_3 and is required for calcium and phosphorus homoeostasis and control of bone remodelling (De Luca & Schnoes 1983). Moreover, $1,25(OH)_2D_3$ has multiple effects on differentiation and function of haematopoietic cells, and was reported to inhibit activation of T cells (Bhalla et al 1984) and to induce tolerogenic dendritic cells (Adorini et al 2003). $1,25(OH)_2D_3$ inhibits IL-12 production by activated macrophages and dendritic cells by a mechanism involving down-regulation of NF-κB activation and binding to the κB sequence in the β-chain promotor (Lemire 1995; D'Ambrosio et al 1998). Interestingly, administration of $1,25(OH)_2D_3$ or the non-hypercalcaemic analogue Ro 63-2023 prevented myelin oligodendrocyte glycoprotein (MOG)-induced chronic-relapsing experimental allergic encephalomyelitis (CR-EAE) in a Biozzi AB/H mouse model of multiple sclerosis (Mattner et al 2000). This inhibition was associated with a reduction of inflammatory infiltrates, demyelination and axonal loss in the affected areas of the CNS. The recent finding that IL-23 rather than IL-12 is crucial in the regulation of CNS inflammation (Cua et al 2003) does not necessarily conflict with the former findings (Mattner et al 2000), as endogenous IL-23 production may inadvertently have been blocked through the inhibitory effect of $1,25(OH)_2D_3$ on transcription of the shared β-chain (Lemire 1995; D'Ambrosio et al 1998). Also, dehydroepiandrosterone (DHEA), a C_{19} adrenal steroid, down-regulates transcription of the β-chain by an NF-κB-dependent mechanism (Du et al 2001). In-vivo administration of DHEA reduced severity and incidence of acute EAE in SJL/L mice (Du et al 2001). Retinoids are naturally occurring or synthetic compounds that display vitamin-A-like properties, and were found to inhibit IL-12 production in lipopolysaccharide (LPS)-stimulated macrophages, presumably through formation of an inhibitory complex between NF-κB and the retinoid X receptor (Na et al 1999; Kang et al 2000a). To a certain extent, this mechanistic pathway could explain the previously observed beneficial effects of retinoid treatment on disease course in a model of CR-EAE (Racke et al 1995).

Curcumin (diferuloylmethane) is a natural pigment, isolated from the rhizomes of the South Asian plant *Curcuma longa* (Srimal & Dhawan 1973), that exhibits a spectrum of antioxidant, antitumour and anti-inflammatory activity. The anti-inflammatory activity of curcumin is associated with prevention of activation of the transcription factors NF-κB, AP-1 and c-Jun kinase (Kafar & Roy 1994; Surh et al 2001). Kang et al (1999a, b) demonstrated that curcumin

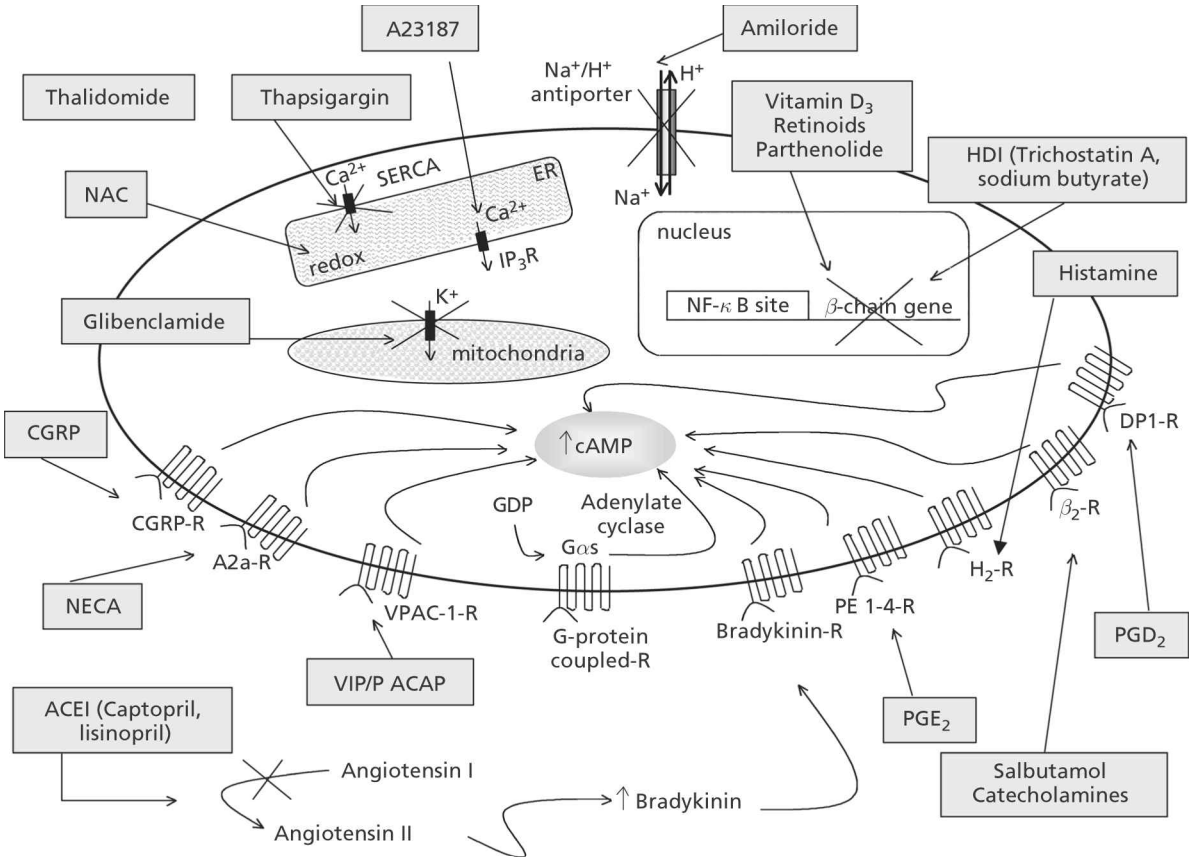

Figure 3 Overview of components of the cellular IL-12 production machinery targeted by inhibitory drugs and agents. The nuclear transcription stage is affected by inhibitors of nuclear HDAC and NF-κB, and is also modulated by increased cAMP synthesis brought about by the interaction of a number of specific ligands with their G-protein-coupled receptors. At the protein level, the assembly or endoplasmic reticulum-to-Golgi trafficking of IL-12 can be prevented by drugs perturbing the function of the endoplasmic reticulum.

inhibits IL-12 production from macrophages with the repressive effect mapping to the κB sequence in the β-chain promoter. Treatment of SJL/J mice with curcumin reduced duration, clinical score and demyelination and inflammation in EAE (Natarajan & Bright 2002a). This effect was associated with inhibition of IL-12 production from MBP-immune spleen cells in response to neural antigen. Remarkably, Natarajan & Bright (2002a) showed that curcumin also blocks IL-12-induced tyrosine phosphorylation of Jak-2, Tyk-2, STAT3 and STAT4. Therefore, the pronounced disease-limiting effect of curcumin in EAE may be due to interference with two separate events in the IL-12 pathway (i.e. inhibition of IL-12 production by monocytes and microglia, and disabling of the responsiveness of activated T cells to IL-12).

Sesquiterpene lactones have been identified in several plant families. Parthenolide, a predominant sesquiterpene lactone found in the Mexican-Indian species *Tanacetum parthenium*, is used as a herbal anti-inflammatory remedy. Part of this property is thought to be due to the ability of this compound to inhibit both NF-κB activation and MAP kinases (Hwang et al 1996; Hehner et al 1998). Parthenolide appeared to block IL-12 production in macrophages through the NF-κB-sensitive site in the β-chain promoter (Kang et al 2001). Also, in LPS-induced dendritic cells, parthenolide abrogated production of IL-12 (Uchi et al 2002).

Kang et al (1999c, d; 2000b) have identified several other compounds that block IL-12 production by inhibition of NF-κB binding to the β-promoter, including sulfasalazine, tanshinones from *Salvia miltiorrhiza* and chloromethyl ketone serine protease inhibitors. Of these, sulfasalazine had been tested previously in EAE, and had been found to lead to a clinically more protracted disease and to increase the number of autoreactive T-cells (Correale et al 1991), indicating that either the inhibitory effect of this drug on IL-12 (or IL-23) production may not be available in-vivo or that other (as yet undefined) disease-promoting effects may overshadow repressed IL-12 production.

Gold compounds are used in the treatment of rheumatoid arthritis. The work of Jeon et al (2000) revealed that the anti-inflammatory effect of the lipophilic gold compound auranofin occurs through inhibition of NF-κB activation. They found that auranofin suppressed LPS-induced NF-κB binding activity, degradation of IκB proteins and activation of multi-subunit IκB kinase. In a

separate study, auranofin was found to inhibit IL-12 production in macrophages and dendritic cells (Kim et al 2001). Though these authors did not investigate the intracellular mechanisms responsible for this effect, the former study (Jeon et al 2000) may support the notion that auranofin-induced IL-12 repression occurs predominantly through inhibition of the NF-κB pathway.

Taken together, low-molecular-weight compounds such as $1,25(OH)_2D_3$, DHEA, curcumin and retinoids, that act on NF-κB activation and translocation and block IL-12/IL-23 β-chain transcription, may constitute promising approaches to down-regulation of both IL-12 and IL-23-dependent inflammation (Racke et al 1995; Mattner et al 2000; Du et al 2001; Natarajan & Bright 2002a).

Modulating intracellular cAMP, and related pathways

Prostaglandin E_2 (PGE_2) potently inhibits LPS-induced IL-12 production by macrophages by a mechanism involving activation of adenylate cyclase and subsequent generation of increased levels of intracellular cAMP (van der Pouw Kraan et al 1995). Since β_2-agonists are also known to elevate intracellular cAMP (Barnes 1995), Panina-Bordignon et al (1997) investigated the effect of salbutamol, a bronchodilator indicated in asthma, and other β_2-agonists, on IL-12 production. These β_2-agonists were found to inhibit transcription of the α- as well as the β-chains of IL-12 in both CD40L-stimulated dendritic cells and LPS-stimulated monocytes, an effect that could be reproduced by in-vivo administration of a therapeutic dose of salbutamol. That immunomodulation through β_2-agonists may be of value in the treatment of inflammatory conditions is demonstrated by the finding that salbutamol potently suppressed established CIA by a mechanism involving reduced production of both IL-12 and TNF-α (Malfait et al 1999), and decreased IL-12 production in patients with secondary progressive MS (Makhlouf et al 2001b). Similarly, β_2-adrenergic re-programming of IL-12- or IL-23-mediated Th1 responses may have contributed to the disease amelioration seen in a Lewis rat CR-EAE model of MS upon treatment with the β_2-agonist terbutaline (Wiegmann et al 1995). In addition, the β-agonist isoproterenol was found to suppress CR-EAE in Lewis rats (Chelmicka-Schorr et al 1989; Wiegmann et al 1995), an effect that could be associated with inhibition of IL-12 (or IL-23) production mediated by this compound (Haskó et al 1998).

A number of studies have extended this concept by documenting suppressive effects of corticosteroids and catecholamines on IL-12 production. Dexamethasone (Elenkov et al 1996; DeKruyff et al 1998; Visser et al 1998), methylprednisolone (Vanderheyde et al 1999), budesonide (Larsson & Linden 1998), dopamine interacting with β-adrenoceptors (Haskó et al 2002a), histamine interacting with the H_2 receptor (van der Pouw Kraan et al 1998; Mazzoni et al 2001), and adrenaline (epinephrine) and noradrenaline (norepinephrine) (Elenkov et al 1996) were all reported to strongly inhibit production of IL-12 in appropriately stimulated dendritic or monocytic cells. While IL-10 production was found to be relatively insensitive to glucocorticoids (Elenkov et al 1996; Visser et al 1998), PGE_2 and β-agonists tend to increase production of IL-10 by monocytes (van der Pouw Kraan et al 1995; Elenkov et al 1996; Haskó et al 1998, 2002a). Inhibition of IL-12 production and concomitant induction of Th2 development by histamine or PGE_2 is thus thought to contribute to disorders such as atopic dermatitis, allergic asthma and hyper-IgE syndrome (van der Pouw Kraan et al 1995, 1998). It is of relevance to note that, because of its inhibitory effect on IL-12 synthesis and Th1 differentiation, corticosteroid therapy may ultimately negatively affect the long-term course of allergic diseases (DeKruyff et al 1998). On the other hand, down-regulation of IL-12- (or IL-23-) dependent CNS inflammation by glucocorticosteroids may provide an explanatory basis for the previously observed correlation between endogenously produced corticosterone and spontaneous recovery of Lewis rats from EAE (MacPhee et al 1989). A similar rationale could probably be adopted to explain why the histamine H_2 agonist dimaprit significantly reduced clinical signs, blood–brain leakage and CNS inflammation in both C57BL/6 and iNOS-deficient mice models of EAE (Emerson et al 2002).

The opposite effects of PGE_2 on IL-12 versus IL-10 synthesis point toward the possibility of adopting pharmacological inhibition of cyclooxygenase-2 (COX-2) as a therapeutic means to restore the Th1 cytokine balance in disorders associated with low IL-12 production. COX-2 is the enzyme at the rate-limiting step of prostanoid synthesis, and is over-expressed in various malignancies, especially lung and colon carcinomas. IL-12 displays anti-metastatic and antineoplastic effects (Brunda et al 1993). In a murine Lewis lung carcinoma model, COX-2 inhibitors increased tumour infiltration of lymphocytes and limited tumour growth. This process was associated with restored IL-12 and suppressed IL-10 synthesis (Stolina et al 2000). Similarly, the COX-2 inhibitor rofecoxib attenuated growth and metastasis of colorectal carcinoma in mice by a mechanism involving decreased PGE_2 and IL-10 and increased IL-12 synthesis (Yao et al 2003).

Apart from the EP_{1-4}, H_2 and β_2-receptors discussed above, at least four other G-protein-coupled receptors are currently considered as potential pharmacological targets for inhibiting the production of IL-12 through intracellular pathways that, at least partially, rely on stimulation of cAMP production. Binding of vasoactive intestinal peptide (VIP) or pituitary adenylate cyclase-activating peptide (PACAP) to the VPAC-1 receptor inhibited production of IL-12 through both cAMP-dependent and -independent pathways (Delgado & Ganea 1999). The therapeutic value of VIP for curing IL-12-mediated disorders was demonstrated by experiments showing that administration of VIP potently reduced incidence and severity of CIA (Delgado et al 2001). The second receptor is the adenosine A2a receptor, stimulation of which, with the non-specific analogue 5′-*N*-ethylcarboxamidoadeno sine (NECA) or the A2a-specific agonist CGS-21680, suppressed production of IL-12 by stimulated whole blood or primary monocytes in a cAMP-dependent manner (Link et al 2000). Third, the binding of calcitonin gene-related peptide (CGRP) to its receptor attenuates IL-12 transcription in LPS-stimulated macrophages (Fox et al 1997; Torii et al 1997). Liu et al (2000) showed that cAMP phosphodiesterase inhibitors

such as rolipram potentiated, and the inhibitor of cAMP-dependent protein kinase H89 reversed, CGRP-mediated IL-12 suppression in LPS-stimulated macrophages. Phosphodiesterase inhibitors were also reported to block transcription of IL-12 by microglia (Suzumura et al 2003), suggestive for a therapeutic potential in the treatment of MS. Fourth, binding of prostaglandin D_2 to its DP1 cell surface receptor on LPS- or CD40-stimulated dendritic cells activates a cAMP-dependent inhibition of IL-12 production (Faveeuw et al 2003). Remarkably, these authors showed that the inhibition of IL-12 production is, in part, DP1-independent, and that the latter is associated with a blockade of NF-κB binding activity (Faveeuw et al 2003).

It is worthwhile mentioning another regulator of cAMP with potential relevance to inhibition of IL-12 – cholera toxin. Cholera toxin is known to directly activate the α-subunit of the heterotrimeric G-protein complex by ADP ribosylation. Braun et al (1999) demonstrated that cholera toxin directly inhibits IL-12 production by monocytes and dendritic cells. In a model of LPS-induced shock, pretreatment of mice with cholera toxin down-regulated endogenous IL-12 β-chain levels (Braun et al 1999; Procopio et al 1999). Interestingly, the cholera toxin subunit B, which is devoid of ADP ribosylating activity, was also found to inhibit IL-12 production by an as yet unknown pathway (Boirivant et al 2001). Oral administration of cholera toxin subunit B inhibited trinitrobenzene sulfonic acid-induced colitis in mice, a model of Crohn's disease. This was correlated with increased apoptosis of lamina propria cells due to deprivation of IL-12 (Boirivant et al 2001).

The inhibitors of angiotensin converting enzyme (ACE) captopril and lisinopril were reported to suppress production of IL-12 by LPS-stimulated monocytes (Constaninescu et al 1998). Though the underlying mechanism remains as yet elusive, a potential pathway could involve ACE inhibitor-mediated prevention of bradykinin breakdown resulting in increased occupation of bradykinin G-protein-coupled receptors on macrophages (Sato et al 1996) and ensuing cAMP-dependent inhibition of IL-12 transcription. This could be brought about either directly or through formation of PGE_2 (Bareis et al 1983). It is of interest to note that cross-talk between β-adrenergic and bradykinin B_2 receptors superactivates accumulation of intracellular cAMP (Hanke et al 2001). In EAE, administration of captopril diminished severity scores and lymphocyte reactivity to MBP (Constantinescu et al 1995).

Finally, pentoxyfylline is a methylxanthine derivative that prevents breakdown of intracellular cAMP via non-specific inhibition of phophodiesterases, thus increasing intracellular cAMP levels. It was reported to inhibit production of the IL-12 α-chain, though its effect on that of the β-chain is less clear and may be dependent on co-stimulatory signals (Moller et al 1997a; Marcinkiewicz et al 2000). In an open trial in rheumatoid arthritis, combination therapy with pentoxifylline and thalidomide did not modify endogenous production of IL-12 (Huizinga et al 1996). In contrast, pentoxifylline decreased TNF-α and IL-12 levels, and restored IL-4 and IL-10 production in MS patients (Rieckmann et al 1996).

In conclusion, modulation of intracellular cAMP by either phosphodiesterase inhibitors, or cholera toxin, or by the recruitment of ligands as diverse as β-agonists, VIP/PACAP, adenosine A2a agonists, dopamine, histamine, PGD_2 and PGE_2 to their respective G-protein-coupled receptors all seem to converge in a suppression of IL-12 production. Even this list is not exhaustive as, among others, also the chemokine receptors CCR5 and CCR2, the adenosine A3 receptor and the complement factor C5a receptor CD88 are known to modulate IL-12 production upon interaction with their ligands (reviewed by Braun & Kelsall 2001).

Cyclophilin-mediated inhibition of IL-12 production

Binding and complexation of the drug ciclosporin to cyclophilin mediates immunosuppression by inhibiting the serine/threonine phosphatase activity of calcineurin (Braun et al 1995). Ciclosporin was found to inhibit IL-12 production in an allogeneic mixed lymphocyte reaction (Bonnotte et al 1996). Addition of exogenous IL-12 restored ciclosporin-inhibited alloreactive cytotoxic T lymphocyte generation, indicating that blockade of IL-12 production contributes to the immunosuppressive effect of ciclosporin (Bonnotte et al 1996). The chemically unrelated immunosuppressant tacrolimus (FK506) is known to bind to a second class of cyclophilins (Braun et al 1995). Tacrolimus is utilized in bone marrow transplantation to prevent graft-versus-host disease. It appeared to suppress production of Th1 cytokines including IL-12 in LPS-stimulated myeloid dendritic cells (Szabo et al 2001) and in heart allografts (Egi et al 2002). Dendritic cells derived from $CD34^+$ haematopoietic progenitor cells cultured in the presence of tacrolimus showed significantly lower IL-12 production, and were capable of inducing IL-4 production in a $CD4^+$ T cell line (Shimuzu et al 2000). More recently, sanglifehrin A (SfA), a novel cyclophilin-binding immunosuppressant, was found to abrogate IL-12 production by human dendritic cells (Steinschulte et al 2003). SfA potently suppressed transcription of the α- and β-chain, and of the IL-23-specific p19 subunit. Interestingly, competition experiments with ciclosporin indicated that the inhibition of IL-12 production was independent of cyclophilin A-blockade (Steinschulte et al 2003). In view of these findings, application of cyclophilin-binding compounds may open perspectives to shifting the Th1–Th2 cytokine balance toward a more dominant Th2 profile in some chronic inflammatory conditions.

Inhibitors of nuclear histone deacetylases (HDAC)

Suberoylanilide hydroxamic acid (SAHA), trichostatin A and butyrate are HDAC inhibitors, which exert an anti-tumour effect by increasing expression of genes involved in the cell cycle, tumour suppression and apoptosis. SAHA was recently found to reduce transcription in LPS-stimulated monocytes of a number of pro-inflammatory cytokines including IL-12, TNF-α, IL-1β and IFN-γ (Leoni et al 2002). Interestingly, the anti-inflammatory effect of SAHA was observed at lower concentrations than those required to suppress tumour growth in-vitro and in-vivo (Leoni et al 2002). Trichostatin A suppressed LPS-induced accumulation of IL-12 α- and β-chain

mRNA in SV40-tranformed lung epithelial cells (Iwata et al 2002). These effects may be partially independent from HDAC inhibition and could be due to hyperacetylation of nonhistone proteins such as the Rel A subunit of NF-κB (Chen et al 2001). Similarly, the intestinal metabolite butyrate strongly inhibited transcription of the IL-12 α- and β-chains but increased IL-10 secretion in *Staphylococcus aureus*-stimulated monocytes (Säemann et al 2000). In addition, butyrate reduced release of IL-12 from LPS/PHA-stimulated whole blood cultures (Nancey et al 2002). Thus, butyrate may play an important role in homoeostasis between colonic bacteria and intestinal mucosa by modulation of the local Th1–Th2 cytokine balance. This unique property could likely be exploited in the treatment of Crohn's disease, which is characterized by excessive release of IL-12 by lamina propria mononuclear cells (Monteleone et al 1997).

Ion channels, pumps and antiporters

Calcium-channel blockers such as diltiazem and verapamil inhibit inward displacement of calcium ions through the slow channels on cell membranes, and are indicated for the treatment of angina. These drugs also exert, alone or in combination with ciclosporin, immunosuppressive effects that could potentially be exploited in organ transplantation efforts (Chitwood & Heim-Duthoy 1993). Th1 cytokines are known to initiate allogeneic immune responses underlying allograft rejection. D'Ambrosio et al (2001) found that diltiazem inhibited IL-12 production in cultures of LPS- or CD40L-stimulated dendritic cells, while this treatment did not affect IL-1β, IL-6 or TNF-α production. In addition, diltiazem appeared to prevent maturation of dendritic cells (Bachetoni et al 2002). Calcium-channel blockers have also been shown to exert protective effects in a canine model of *E. coli* endotoxic shock (Bosson et al 1985), pointing to yet another potential target for implementation of these drugs. Though intraperitoneal pretreatment of mice with verapamil or diltiazem had no effect, dantrolene, an inhibitor of Ca^{2+} release from cytoplasmic stores, suppressed plasma levels of LPS-induced IL-12 (Németh et al 1998). Nevertheless, a further indication for a potential role of extracellular Ca^{2+} influxes in regulation of IL-12 expression comes from the work of Sutterwala et al (1997). These authors demonstrated that ligation of Fcγ, complement or scavenger receptors generates a calcium influx that leads to specific suppression of transcription of the IL-12 β-chain in LPS-stimulated macrophages, an effect that could be mimicked by the calcium ionophores ionomycin and A23187. Ligation of phagocytic receptors on macrophages may be exploited by pathogens to prevent generation of an IL-12-dependent anti-infectious immune response (Sutterwala et al 1997).

Both inhibition of the Na^+/H^+ antiporters by amiloride (Németh et al 2001) and of ATP-binding cassette transporters by glibenclamide (Haskó et al 2002b) were also reported to suppress IL-12 β chain production. The effect of the former was post-translational, as IL-12 β chain mRNA levels induced by IFN-γ and LPS in the macrophage cell line J774 in the presence of amiloride were not affected (Németh et al 2001). Finally, extracellular ATP was reported to down-regulate production of IL-12 by mature dendritic cells, probably by a mechanism involving the family of ATP-gated P2X ion-channel receptors (la Sala et al 2001). Also, activation of the ATP-specific receptor $P2Y_{11}$ can inhibit IL-12 synthesis, through a mechanism involving elevation of intracellular cAMP (Wilkin et al 2002). Thus, whichever transduction pathway is utilized, both purinergic receptor sub-families P2X and P2Y are emerging as potential targets for intervention with endogenous IL-12 production. At present, however, it remains unclear whether drugs targeting ion channels, pumps or antiporters can be therapeutically exploited to suppress IL-12 production in-vivo, as solid evidence from experimental animal models is not (yet) available.

Posttranslational inhibition of IL-12 production

Thalidomide is well known for its effects on the generation and release of TNF-α (reviewed by Meierhofer et al 2001). Secretion of IL-12 was potently suppressed by thalidomide in LPS-stimulated human peripheral blood mononuclear cells and monocytes in a concentration-dependent manner (Moller et al 1997b). This inhibition was additive to that induced by suboptimal doses of dexamethasone. Since thalidomide did not significantly alter transcription of α- and β-chain mRNAs, it was suggested that its inhibitory effects occur, at least in part, through posttranscriptional mechanisms (Moller et al 1997b). One such involves enhancement of mRNA degradation, and was identified previously as one of the chief mechanisms by which thalidomide suppresses TNF-α production (Moreira et al 1993).

Members of the tetracycline family are commonly utilized as antibiotics. Recent findings suggest that their spectrum of activity includes anti-inflammatory effects that are unrelated to their antimicrobial properties. D'Agostino et al (2001) evaluated the effect of chemically modified tetracyclines (CMTs) on the production of inflammatory mediators such as nitric oxide and IL-12 by the LPS-stimulated macrophage cell line J774. They found that two CMTs, CMT-1 and -8, strongly increased accumulation of IL-12 mRNA. Nevertheless, they were not able to demonstrate IL-12 by ELISA in the supernatants of these cells. Thus, CMT-mediated disabling of as yet unkown components of the IL-12 secretory pathway probably overrides its reinforcing effect on LPS-induced IL-12 transcription.

The folding and assembly of β_2- and $\alpha\beta$-dimers of IL-12 occurs in the endoplasmic reticulum, and is likely to be regulated through interaction with a number of chaperones and folding catalysts, as is mostly the case for multi-subunit proteins. The calcium-dependent chaperones calreticulin and GRP94 were found to interact transiently and non-covalently with the α- and β-chain, respectively, during transit in recombinant HEK293 cell lines (Alloza et al 2004). Addition of calcium sequestering compounds such as thapsigargin and A23187, or tunicamcyin, to these cell lines totally blocked IL-12 $\alpha\beta$-heterodimer or β_2-homodimer secretion, while secretion of the monomeric α- and β-chains was virtually unhampered (Alloza & Vandenbroeck 2002). In cell-free experiments, protein disulfide isomerase (PDI), a foldase that normally resides in the endoplasmic

reticulum, facilitated oxidative assembly of the β-chain into disulfide-linked β_2-homodimers (Martens et al 2000). Thus, the finding that the thiol antioxidants *N*-acetylcysteine and reduced glutathione (GSH) inhibited heterodimer secretion by IFN-γ- and *S. aureus* Cowan strain I-stimulated human THP-1 cells (Mazzeo et al 2002), may relate to an impediment in the oxidative folding pathway and oligomer assembly of IL-12 that is induced by shifting the redox potential of the endoplasmic reticulum toward a more reduced status. Indeed, *N*-acetylcysteine did not inhibit transcription of the α- or β-chain, and neither did it affect NF-κB activation (Mazzeo et al 2002).

Inhibition of the IL-12 and IL-23 signal transduction pathways

Binding of IL-12 to its receptor induces tyrosine phosphorylation and activation of Jak-2, Tyk-2, STAT3 and STAT4. This initiates an intracellular signalling cascade that ultimately results in transcription of many IL-12 responsive genes (Gubler & Preskey 1996; Zhou et al 1997; O'Shea et al 2002). Thus, pharmacological targeting of components of the Jak-STAT pathway has recently emerged as a very promising approach to blocking disease-associated effects induced by IL-12. Since IL-23 activates largely the same Jak-STAT molecules as IL-12 (Parham et al 2002), many of the compounds reported in the context of IL-12 inhibition might also be useful for blocking the IL-23 transduction pathway.

Tyrphostin B42, a potent inhibitor of protein kinases, was found to inhibit IL-12-induced tyrosine phosphorylation and activation of Jak-2 (Bright et al 1999). When applied in-vivo, tyrphostin B42 decreased proliferation of neural Ag-specific T cells and reduced the incidence and severity of active and passive EAE (Bright et al 1999). The 3-hydroxy-3-methylglut aryl coenzyme A (HMG-CoA) reductase inhibitor atorvastatin was found to inhibit or reverse CR-EAE (Youssef et al 2002). Atorvastatin activates STAT6, and inhibits STAT4, phosphorylation, thus promoting differentiation of naive Th0 cells into Th2 cells, which protected mice from EAE induction in adoptive transfer experiments (Youssef et al 2002). The anti-inflammatory compound lysofylline blocks IL-12-dependent Th1 differentiation and signalling by a mechanism involving both inhibition of IL-12-induced STAT4 tyrosine phosphorylation and prevention of repression of the transcription factor GATA-3, which is known to be crucial for Th2 cytokine gene expression in $CD4^+$ T cells (Coon et al 1999). Lysofylline was found to completely block demyelination and lymphocyte infiltration in the CNS in EAE (Bright et al 1998). This inhibitory effect correlated with the inhibition of Th1 differentation in-vivo.

The extraordinary therapeutic potential of compounds interfering with the IL-12/IL-23 Jak-STAT signal transduction pathway, to prevent CNS inflammation and demyelination, is documented by two other studies. The peroxisome proliferator-activated receptor-gamma (PPARγ) agonists ciglitazone or 15-deoxy$\Delta^{12,14}$ prostaglandin J_2 potently decreased duration, clinical severity and inflammatory demyelination in models of active and passive EAE (Natarajan & Bright 2002b). This was associated with a decrease in IL-12 production and inhibition of activation of IL-12-induced Jak-STAT signalling and Th1 differentiation. Second, the compound curcumin is also known to block the IL-12-induced activation of the Jak-STAT pathway, and has proven beneficial in EAE (Natarajan & Bright 2002a; discussed above).

Concluding remarks

In recent years we have witnessed an explosion in the number and diversity of substances reported to display IL-12 inhibitory effects. However structurally different these substances are, in this review we have demonstrated that a majority of these inhibitors can be categorized in a limited number of functional classes, each of which seem to interfere with discrete events in the IL-12 metabolic pathway. Some of these were previously known to display generic anti-inflammatory effects (e.g. auranofin, curcumin, parthenolide, etc.). The discovery that these effects are at least partially due to blockage of IL-12 or IL-23 production, or both, may open new targets for implementation of such drugs. From a variety of experimental animal models, inhibitors of NF-κB and of IL-12 signal transduction seem to emerge as the most powerful ones for preventing or curing chronic inflammatory disorders. It should be noted, however, that inhibitors targeting posttranslational events, cyclophilins, ion channels and nuclear HDACs have generally not yet been studied in as much detail or in appropriate experimental animal models. Any conclusions on the relative potency, specificity and efficacy of the different classes of inhibitors would thus, for now, be premature. Not surprisingly, only very limited information is available on the effects of the inhibitors discussed above on IL-23. Since IL-23 and IL-12 have their p40 subunit, the IL-12Rβ1 receptor subunit and components of their signal transduction pathways in common, it can be readily assumed that many inhibitors, especially those blocking activation of NF-κB and Jak-STAT pathways, act by concurrent inhibition of both cytokines. To this should be added that the homodimeric β_2-form of IL-12, while originally considered to merely constitute an IL-12 antagonist, is now emerging as a pronounced agonist in diverse processes including recruitment of macrophages (for review see Brombacher et al 2003).

Further research into the molecular biology of IL-23, IL-12 and β_2, as well as reassessment of these inhibitors, should clarify which, if any, is the major pro-inflammatory cytokine to be targeted in specific inflammatory conditions. This, in turn, is likely to open exciting perspectives to the identification of drugs that inhibit these cytokines either indiscriminately or selectively. It goes without saying that our rapidly increasing knowledge on the family of dimeric IL-12-like cytokines, combined with the availability of an extensive arsenal of modulators, will offer almost endless possibilities in forthcoming attempts aiming at disease-specific intervention with endogenous production of these cytokines.

References

Adorini, L., Pennar, G., Giarratana, N., Uskokovic, M. (2003) Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting allograft rejection and autoimmune diseases. *J. Cell. Biochem.* **88**: 227–233

Afkarian, M., Sedy, J. R., Yang, J., Jacobson, N. G., Cereb, N., Yang, S. Y., Murphy, T. L., Murphy, K. M. (2002) T-bet is a STAT1-induced regulator of IL-12R expression in naive $CD4^+$ T cells. *Nat. Immunol.* **3**: 549–557

Alloza, I., Vandenbroeck, K. (2002) Ca^{2+} and *N*-glycosylation-dependent regulation of the folding, dimerization and secretion of IL-12 $\alpha\beta$ and β_2 chains. *J. Interferon Cytokine Res.* **22** (Suppl. 1): S115

Alloza, I., Martens, E., Hawthorne, S., Vandenbroeck, K. (2004) Crosslinking approach to affinity capture of protein complexes from chaotrope-solubilised cell lysates, *Anal. Biochem.* **324**: 137–142

Bachetoni, A., D'Ambrosio, A., Mariani, P., Cortesini, R., Quintieri, F. (2002) Diltiazem impairs maturation and functions of human dendritic cells. *Hum. Immunol.* **64**: 524–533

Bacon, C. M., Petricoin, E. F., Ortaldo, J. R., Rees, R. C., Larner, A. C., Johnston, J. A., O'Shea, J. J. (1995) Interleukin 12 induces tyrosine phosphorylation and activation of STAT4 in human lymphocytes. *Proc. Natl Acad. Sci. USA* **92**: 7307–7311

Balashov, K. E., Smith, D. R., Khoury, S. J., Hafler, D. A., Weiner, H. L. (1997) Increased interleukin 12 production in progressive multiple sclerosis: induction by activated CD4(+) T cells via CD40 ligand. *Proc. Natl Acad. Sci USA* **94**: 599–603

Bareis, D. L., Manganiello, V. C., Hirata, F., Vaughan, M., Axelrod, J. (1983) Bradykinin stimulates phospholipid methylation, calcium influx, prostaglandin formation, and cAMP accumulation in human fibroblasts. *Proc. Natl Acad. Sci. USA* **80**: 2514–2518

Barnes, P. J. (1995) Beta-adrenergic receptors and their regulation. *Am. J. Respir. Crit. Care Med.* **152**: 838–860

Becher, B., Durell, B. G., Noelle, R. J. (2002) Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. *J. Clin. Invest.* **110**: 493–497

Bhalla, A. K., Amento, E. P., Serog, B., Glimcher, L. H. (1984) 1,25-Dihydroxyvitamin D_3 inhibits antigen-induced T cell activation. *J. Immunol.* **133**: 1748–1754

Boirivant, M., Fuss, I. J., Ferroni, L., De Pascale, M., Strober, W. (2001) Oral administration of recombinant cholera toxin subunit B inhibits IL-12-mediated murine experimental (trinitrobenzene sulfonic acid) colitis. *J. Immunol.* **166**: 3522–3532

Bonnotte, B., Pardoux, C., Bourhis, J. H., Caignard, A., Burdiles, A. M., Chehimi, J., MamiChouaib, F., Chouaib, S. (1996) Inhibition of the human allogeneic mixed lymphocyte response by cyclosporin A: relationship with the IL-12 pathway. *Tissue Antigens* **48**: 265–270

Bosson, S., Kuenzig, M., Schwartz, S. I. (1985) Verapamil improves cardiac function and increases survival in canine *E. coli* endotoxin shock. *Circ. Shock* **16**: 307–316

Braun, M. C., Kelsall, B. L. (2001) Regulation of interleukin-12 production by G-protein-coupled receptors. *Microbes Infect.* **3**: 99–107

Braun, W., Kallen, J., Mikol, V., Walkinshaw, M. D., Wuthrich, K. (1995) 3-Dimensional structure and actions of immunosuppressants and their immunophilins. *FASEB J.* **9**: 63–72

Braun, M. C., He, J., Wu, C. Y., Kelsall, B. L. (1999) Cholera toxin suppresses interleukin (IL)-12 production and IL-12 receptor β1 and β2 chain expression. *J. Exp. Med.* **189**: 541–552

Bright, J. J., Du, C., Coon, M., Sriram, S., Klaus, S. K. (1998) Prevention of experimental allergic encephalomyelitis via inhibition of IL-12 signaling and IL-12-mediated Th1 differentiation: an effect of the novel anti-inflammatory drug lisofylline. *J. Immunol.* **161**: 7015

Bright, J. J., Du, C., Sriram, S. (1999) Tyrphostin B42 inhibits IL-12-induced tyrosine phosphorylation and activation of Janus kinase-2 and prevents experimental allergic encephalomyelitis. *J. Immunol.* **162**: 6255–6262

Brombacher, F., Kastelein, R. A., Alber, G. (2003) Novel IL-12 family members shed light on the orchestration of Th1 responses. *Trends Immunol.* **24**: 207–212

Brunda, M. J., Luistro, L., Warrier, R. R., Wright, R. B., Hubbard, B. R., Murphy, M., Wolf, S. F., Gateley, M. K. (1993) Antitumor and antimetastatic activity of interleukin 12 against tumors. *J. Exp. Med.* **178**: 1223

Caspi, R. R. (1998) Il-12 in autoimmunity. *Clin. Immunol. Immunopathol.* **88**: 4–13

Chelmicka-Schorr, E., Kwasniewski, M. N., Thomas, B. E., Arnason, B. G. (1989) The beta-adrenergic agonist isoproterenol suppresses experimental allergic encephalomyelitis in Lewis rats. *J. Neuroimmunol.* **25**: 203–207

Chen, Q., Ghilardi, N., Wang, H., Baker, T., Xie, M. H., Gurney, A., Grewal, I. S., de Sauvage, F. J. (2000) Development of Th1-type immune responses requires the type I cytokine receptor TCCR. *Nature* **407**: 916–920

Chen, L. F., Fischle, W., Verdin, E., Greene, W. C. (2001) Duration of NF-κB action regulated by reversible acetylation. *Science* **293**: 1653–1657

Chitwood, K. K., Heim-Duthoy, K. L. (1993) Immunosuppressive properties of calcium channel blockers. *Pharmacotherapy* **13**: 447–454

Chua, A. O., Chizzonite, R., Desai, B. B., Truitt, T. P., Nunes, P., Minetti, L. J., Warrier, R. R., Presky, D. H., Levine, J. F., Gately, M. K. (1994) Expression cloning of a human IL-12 receptor component. A new member of the cytokine receptor superfamily with strong homology to gp130. *J. Immunol.* **153**: 128–136

Chua, A. O., Wilkinson, V. L., Presky, D. H., Gubler, U. (1995) Cloning and characterization of a mouse IL-12 receptor-beta component. *J. Immunol.* **155**: 4286–4294

Comabella, M., Balashov, K., Issazadeh, S., Smith, D., Weiner, H. L., Khoury, S. J. (1998) Elevated interleukin-12 in progressive multiple sclerosis correlates with disease activity and is normalized by pulse cyclophosphamide therapy. *J. Clin. Invest.* **102**: 671–678

Constantinescu, C. S., Ventura, E., Hilliard, B., Rostami, A. (1995) Effects of the angiotensin-converting enzyme-inhibitor captopril on experimental autoimmune encephalomyelitis. *Immunopharmacol. Immunotoxicol.* **17**: 471–491

Constantinescu, C. S., Goodman, D. B. P., Ventura, E. S. (1998) Captopril and lisinopril suppress production of interleukin-12 by human peripheral blood mononuclear cells. *Immunol. Lett.* **62**: 25–31

Coon, M. E., Diegel, M., Leshinsky, N., Klaus, S. J. (1999) Selective pharmacologic inhibition of murine and human IL-12-dependent Th1 differentiation and IL-12 signaling. *J. Immunol.* **163**: 6567–6574

Correale, J., Olsson, T., Bjork, J., Smedegard, G., Hojeberg, B., Link, H. (1991) Sulfasalazine aggravates experimental autoimmune encephalomyelitis and causes an increase in the number of autoreactive T-cells. *J. Neuroimmunol.* **34**: 109–120

Cua, D. J., Sherlock, J., Chen, Y., Murphy, C. A., Joyce, B., Seymour, B., Lucian, L., To, W., Kwan, S., Churakova, T., Zurawski, S., Wiekowski, M., Lira, S. A., Gorman, D., Kastelein, R. A., Sedgwick, J. D. (2003) Interleukin-23 rather

than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* **421**: 744–748

D'Agostino, P., Ferlazzo, V., Milano, S., La Rosa, M., Di Bella, G., Caruso, R., Barbera, C., Grimaudo, S., Tolomeo, M., Feo, S., Cillari, E. (2001) Anti-inflammatory effects of chemically modified tetracyclines by the inhibition of nitric oxide and interleukin-12 synthesis in J774 cell line. *Int. Immunopharmacol.* **1**: 1765–1776

D'Ambrosio, D., Cippitelli, M., Cocciolo, M. G., Mazzeo, D., Di Lucia, P., Lang, R., Sinigaglia, F., Panina-Bordignon, P. (1998) Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-κB downregulation in transcriptional repression of the p40 gene. *J. Clin. Invest.* **101**: 252–262

D'Ambrosio, D., Bachetoni, A., Giacomini, E., Quaranta, M., Quintieri, F. (2001) Diltiazem downregulates IL-12 production by human dendritic cells. *Transplant. Proc.* **33**: 236–239

DeKruyff, R. H., Fang, Y., Umetsu, D. T. (1998) Corticosteroids enhance the capacity of macrophages to induce Th2 cytokine synthesis in $CD4^+$ lymphocytes by inhibiting IL-12 production. *J. Immunol.* **160**: 2231–2237

Delgado, M., Ganea, D. (1999) Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit interleukin-12 production by regulating nuclear factor κB and Ets activation. *J. Biol. Chem.* **274**: 31930–31940

Delgado, M., Abad, C., Martinez, C., Laceta, J., Gomariz, R. P. (2001) Vasoactive intestinal peptide prevents experimental arthritis by downregulating both autoimmune and inflammatory components of the disease. *Nat. Med.* **7**: 563–568

De Luca, H. F., Schnoes, H. K. (1983) Vitamin D: recent advances. *Annu. Rev. Biochem.* **52**: 411–439

Devergne, O., Hummel, M., Koeppen, H., Le Beau, M. M., Nathanson, E. C., Kieff, E., Birkenbach, M. (1996) A novel interleukin-12 p40-related protein induced by latent Epstein-Barr virus infection in B lymphocytes. *J. Virol.* **70**: 1143–1153

Devergne, O., Birkenbach, M., Kieff, E. (1997) Epstein-Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin. *Proc. Natl Acad. Sci. USA* **94**: 12041–12046

Dong, C., Flavell, R. A. (2001) Th1 and Th2 cells. *Curr. Opin. Hematol.* **8**: 47–51

Du, C., Khalil, M. W., Sriram, S. (2001) Administration of dehydroepiandrosterone suppresses experimental allergic encephalomyelitis in SJL/L mice. *J. Immunol.* **167**: 7094–7101

Egi, H., Hayamizu, K., Kitayama, T., Ohmori, I., Okajima, M., Asahara, T. (2002) Downregulation of both interleukin-12 and interleukin-2 in heart allografts by pretransplant host treatment with granulocyte colony-stimulating factor and tacrolimus. *Cytokine* **18**: 164–167

Elenkov, I. J., Papanicolaou, D. A., Wilder, R. L., Chrousos, G. P. (1996) Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. *Proc. Assoc. Am. Physicians* **108**: 374–381

Emerson, M. R., Orentas, D. M., Lynch, S. G., LeVine, S. M. (2002) Activation of histamine H-2 receptors ameliorates experimental allergic encephalomyelitis. *Neuroreport* **13**: 1407–1410

Farrar, J. D., Asnagli, H., Murphy, K. M. (2002) T helper subset development: roles of instruction, selection, and transcription. *J. Clin. Invest.* **109**: 431–435

Faveeuw, C., Gosset, P., Bureau, F., Angeli, V., Hirai, H., Maruyama, S., Narumiya, S., Capron, M., Trottein, F. (2003) Prostaglandin D_2 inhibits the production of interleukin-12 in murine dendritic cells through multiple signaling pathways. *Eur. J. Immunol.* **33**: 889–898

Fox, F. E., Kubin, M., Cassin, M., Niu, Z. T., Hosoi, J., Torii, H., Granstein, R. D., Trinchieri, G., Rook, A. H. (1997) Calcitonin gene-related peptide inhibits proliferation and antigen presentation by human peripheral blood mononuclear cells: effects on B7, interleukin-10 and interleukin-12. *J. Invest. Dermatol.* **108**: 43–48

Gadina, M., Ferguson, P. R., Johnston, J. A. (2003) New interleukins: are there any more? *Curr. Opin. Infect. Dis.* **16**: 211–217

Gately, M. K., Desai, B. B., Wolitzky, A. G., Quinn, P. M., Dwyer, C. M., Podlaski, F. J., Familletti, P. C., Sinigaglia, F., Chizonnite, R., Gubler, U. (1991) Regulation of human lymphocyte proliferation by a heterodimeric cytokine, IL-12 (cytotoxic lymphocyte maturation factor). *J. Immunol.* **147**: 874–882

Glimcher, L. H. (2001) Lineage commitment in lymphocytes: controlling the immune response. *J. Clin. Invest.* **108**: s25–s30

Gran, B., Zhang, G. X., Yu, S., Li, J., Chen, X. H., Ventura, E. S., Kamoun, M., Rostami, A. (2002) IL-12p35-deficient mice are susceptible to experimental autoimmune encephalomyelitis: evidence for redundancy in the IL-12 system in the induction of central nervous system autoimmune demyelination. *J. Immunol.* **169**: 7104–7110

Grohmann, U., Belladonna, M. L., Bianchi, R., Orabona, C., Ayroldi, E., Fioretti, M. C., Puccetti, P. (1998) IL-12 acts directly on DC to promote nuclear localization of NF-kappaB and primes DC for IL-12 production. *Immunity* **9**: 315–323

Gubler, U., Preskey, D. H. (1996) Molecular biology of interleukin-12 receptors. *Ann. NY Acad. Sci. USA* **795**: 36

Gubler, U., Chua, A. O., Schoenhaut, D. S., Dwyer, C. M., McComas, W., Motyka, R., Nabavi, N., Wolitzky, A. G., Quinn, P. M., Familletti, P. C. (1991) Coexpression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation factor. *Proc. Natl Acad. Sci. USA* **88**: 4143–4147

Hanke, S., Nürnberg, B., Groll, D. H., Liebmann, C. (2001) Cross talk between β-adrenergic and bradykinin B_2 receptors results in cooperative regulation of cyclic AMP accumulation and protein kinase activity. *Mol. Cell. Biol.* **24**: 8452–8460

Haskó, G., Szabó, C., Németh, Z. H., Salzman, A. L., Vizi, E. S. (1998) Stimulation of β-adrenoreceptors inhibits endotoxin-induced IL-12 production in normal and IL-10 deficient mice. *J. Neuroimmunol.* **88**: 57–61

Haskó, G., Szabó, C., Németh, Z. H., Deitch, E. A. (2002a) Dopamine suppresses IL-12 p40 production by lipopolysaccharide-stimulated macrophages via a β-adrenoceptor-mediated mechanism. *J. Neuroimmunol.* **122**: 34–39

Haskó, G., Deitch, E. A., Németh, Z. H., Kuhel, D. G., Szabó, C. (2002b) Inhibitors of ATP-binding cassette transporters suppress interleukin-12 p40 production and major histocompatibility complex II up-regulation in macrophages. *J. Pharm. Exp. Ther.* **301**: 103–110

Hehner, S. P., Heinrich, M., Bork, P. M., Vogt, M., Ratter, F., Lehmann, V., Schulze-Osthoff, K., Droge, W., Schmitz, M. L. (1998) Sesquiterpene lactones specifically inhibit activation of NF-κB by preventing the degradation of IκB-α and IκB-β. *J. Biol. Chem.* **273**: 1288–1297

Heremans, H., Dillen, C., Groenen, M., Matthys, P., Billiau, A. (1999) Role of endogenous interleukin-12 (IL-12) in induced and spontaneous relapses of experimental autoimmune encephalomyelitis in mice. *Eur. Cytokine Netw.* **10**: 171–179

Huizinga, T. W., Dijkmans, B. A. C., van der Velde, E. A., van de Pouw Kraan, T. C. T. M., Verweij, C. L., Breedveld, F. C. (1996) An open study of pentoxyfylline and thalidomide as adjuvant therapy in the treatment of rheumatoid arthritis. *Ann. Rheum. Dis.* **55**: 833–836

Hwang, D., Fischer, N. H., Jang, B. C., Tak, H., Kim, J. K., Lee, W. (1996) Inhibition of the expression of inducible cyclooxygenase and proinflammatory cytokines by sesquiterpene lactones in

macrophages correlates with the inhibition of MAP kinases. *Biochem. Biophys. Res. Commun.* **226**: 810–818

Iwata, K., Tomita, K., Sano, H., Fujii, Y., Yamasaki, A., Shimizu, E. (2002) Trichostatin A, a histone deacetylase inhibitor, down-regulatess interleukin-12 transcription in SV-40 transformed lung epithelial cells. *Cell. Immunol.* **218**: 26–33

Jacobson, N. G., Szabo, S. J., Weber-Nordt, R. M., Zhong, Z., Schreiber, R. D., Darnell, J. E., Murphy, K. M. (1995) Interleukin 12 signaling in T helper type 1 (Th1) cells involves tyrosine phosphorylation of signal transducer and activator of transcription Stat3 and Stat4. *J. Exp. Med.* **181**: 1755–1762

Jeon, K. I., Jeong, J. Y., Jue, D. M. (2000) Thiol-reactive metal compounds inhibit NF-κB activation by blocking IκB kinase. *J. Immunol.* **164**: 5981–5989

Kafar, S. S., Roy, D. (1994) Curcumin inhibits TPA induced expression of *c-fos, c-jun* and *c-myc* proto-oncogenes messenger RNAs in mouse skin. *Cancer Lett.* **87**: 85

Kang, B. Y., Chung, S. W., Chung, W.-J., Im, S.-Y., Hwang, S. Y., Kim, T. S. (1999a) Inhibition of interleukin-12 production in lipopolysaccharide-activated macrophages by curcumin. *Eur. J. Pharmacol.* **384**: 191–195

Kang, B. Y., Song, Y. J., Kim, K. M., Choe, Y. K., Hwang, S. Y., Kim, T. S. (1999b) Curcumin inhibits Th1 cytokine profile in CD4(+) T cells by suppressing interleukin-12 production in macrophages. *Br. J. Pharmacol.* **128**: 380–384

Kang, B. Y., Chung, S. W., Im, S. Y., Hwang, S. Y., Kim, T. S. (1999c) Chloromethyl ketones inhibit interleukin-12 production in mouse macrophages stimulated with lipopolysaccharide. *Immunol. Lett.* **70**: 135–138

Kang, B. Y., Chung, S. W., Im, S. Y., Choe, Y. K., Kim, T. S. (1999d) Sulfasalazine prevents T-helper 1 immune response by suppressing interleukin-12 production inn macrophages. *Imunology* **98**: 98–103

Kang, B. Y., Chung, S. W., Kim, S. H., Kang, S. N., Choe, Y. K., Kim, T. S. (2000a) Retinoid-mediated inhibition of interleukin-12 production in mouse macrophages suppresses Th1 cytokine profile in CD4+ T cells. *Br. J. Pharmacol.* **130**: 581–586

Kang, B. Y., Chung, S. W., Kim, S. H., Ryu, S. Y., Kim, T. S. (2000b) Inhibition of interleukin-12 and interferon-gamma production in immune cells by tanshinones from *Salvia miltiorhizza*. *Immunopharmacology* **49**: 355–361

Kang, B. Y., Chung, S. W., Kim, T. S. (2001) Inhibition of interleukin-12 production in lipolysaccharide-activated mouse macrophages by parthenolide, a predominant sesquiterpene lactone in Tanacetum parthenium: involvement of nuclear factor-κB. *Immunol. Lett.* **77**: 159–163

Kikawada, E., Lenda, D. M., Kelley, V. R. (2003) IL-12 deficiency in MRL-Fas^{lpr} mice delays nephritis and intrarenal IFN-γ expression, and diminishes systemic pathology. *J. Immunol.* **170**: 3915–3925

Kim, T. S., Kang, B. Y., Lee, H. M., Choe, Y. K., Hwang, S. Y. (2001) Inhibition of interleukin-12 production by auranofin, an anti-rheumatic gold compound, deviates CD4$^+$ T cells from the Th1 to the Th2 pathway. *Br. J. Pharmacol.* **134**: 571–578

Kubin, M., Chow, J. M., Trinchieri, G. (1994) Differential regulation of interleukin-12 (IL-12), tumor necrosis factor alpha, and IL-1 beta production in human myeloid leukemia cell lines and peripheral blood mononuclear cells. *Blood* **83**: 1847–1855

la Sala, A., Ferrari, D., Corinti, S., Cavani, A., Di Virgilio, F., Girolomoni, G. (2001) Extracellular ATP induces a distorted maturation of dendritic cells and inhibits their capacity to initiate Th1 responses. *J. Immunol.* **166**: 1611–1617

Larsson, S., Linden, M. (1998) Effects of a corticosteroid, budesonide, on production of bioactive IL-12 by human monocytes. *Cytokine* **10**: 786–789

Lemire, J. M. (1995) Immunomodulatory actions of 1,25-dihydroxyvitamin D_3. *J. Steroid Biochem. Mol. Biol.* **53**: 599–602

Leoni, F., Zaliani, A., Bertolini, G., Porro, G., Pagani, P., Pozzi, P., Dona, G., Fossati, G., Sozzani, S., Azam, T., Bufler, P., Fantuzzi, G., Goncharov, I., Kim, S.-H., Pomerantz, B. J., Reznikov, L. L., Siegmund, B., Dinarello, C. A., Mascagni, P. (2002) The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits anti-inflammatory properties via suppression of cytokines. *Proc. Natl Acad. Sci. USA* **99**: 2995–3000

Lighvani, A. A., Frucht, D. M., Jankovic, D., Yamane, H., Aliberti, J., Hissong, B. D., Nguyen, B. V., Gadina, M., Sher, A., Paul, W. E., O'Shea, J. J. (2001) T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. *Proc. Natl Acad. Sci. USA* **98**: 15137–15142

Link, A. L., Kino, T., Worth, J. A., McGuire, J. L., Crane, M. L., Chrousos, G. P., Wilder, R. L., Elenkov, I. J. (2000) Ligand-activation of the adenosine A2a receptor inhibits IL-12 production by human monocytes. *J. Immunol.* **164**: 436–442

Liu, J., Chen, M., Wang, X. (2000) Calcitonin gene-related peptide inhibits lipopolysaccharide-induced interleukin-12 release from peritoneal macrophages, mediated by the cAMP pathway. *Immunology* **101**: 61–67

Ma, X., Chow, J. M., Gri, G., Carra, G., Gerosa, F., Wolf, S. F., Dzialo, R., Trinchieri, G. (1996) The interleukin 12 p40 gene promoter is primed by interferon gamma in monocytic cells. *J. Exp. Med.* **183**: 147–157

Ma, X., Neurath, M., Gri, G., Trinchieri, G. (1997) Identification and characterization of a novel Ets-2-related nuclear complex implicated in the activation of the human interleukin-12 p40 gene promoter. *J. Biol. Chem.* **272**: 10389–10395

MacPhee, I. A., Antoni, F. A., Mason, D. W. (1989) Spontaneous recovery of rats from experimental allergic encephalomyelitis is dependent on regulation of the immune system by endogenous adrenal corticosteroids. *J. Exp. Med.* **169**: 431–445

Makhlouf, K., Weiner, H. L., Khoury, S. J. (2001a) Increased percentage of IL-12+ monocytes in the blood correlates with the presence of active MRI lesions in MS. *J. Neuroimmunol.* **119**: 145–149

Makhlouf, K., Comabella, M., Imitola, J., Weiner, H. L., Khoury, S. J. (2001b) Oral salbutamol decreases IL-12 in patients with secondary progressive multiple sclerosis. *J. Neuroimmunol.* **117**: 156–165

Malfait, A. M., Malik, A. S., Marinova-Mutafchievva, T., Butler, D. M., Maini, R. N., Feldmann, M. (1999) The β2-adrenergic agonist salbutamol is a potent suppressor of established collagen-induced arthritis: mechanism of action. *J. Immunol.* **162**: 6278–6283

Marcinkiewicz, J., Grabowska, A., Lauterbach, R., Bobek, M. (2000) Differential effects of pentoxifylline, a non-specific phosphodiesterase inhibitor, on the production of IL-10, IL-12 p40 and p35 subunits by murine peritoneal macrophages. *Immunopharmacology* **49**: 335–343

Martens, E., Alloza, I., Scott, C. J., Billiau, A., Vandenbroeck, K. (2000) Protein disulfide isomerase-mediated cell-free assembly of recombinant interleukin-12 p40 homodimers. *Eur. J. Biochem.* **267**: 6679–6683

Matthys, P., Vermeire, K., Mitera, T., Heremans, H., Huang, S., Billiau, A. (1998) Anti-IL-12 antibody prevents development and progression of collagen-induced arthritis in IFN-γ receptor-deficient mice. *Eur. J. Immunol.* **28**: 2143–2151

Matthys, P., Vermeire, K., Mitera, T., Heremans, H., Huang, S., Schols, D., De Wolf-Peeters, C., Billiau, A. (1999) Enhanced autoimmune arthritis in IFN-γ receptor-deficient mice is

conditioned by mycobacteria in Freund's adjuvant and by increased expansion of Mac-1(+) myeloid cells. *J. Immunol.* **163**: 3503–3510

Mattner, F., Smiroldo, S., Galbiati, F., Muller, M., Di Lucia, P., Poliani, P. L., Martino, G., Panino-Bordignon, P., Adorini, L. (2000) Inhibition of Th1 development and treatment of chronic-relapsing experimental allergic encephalomyelitis by a non-hypercalcemic analogue of 1,25-dihydroxyvitamin D_3. *Eur. J. Immunol.* **30**: 498–508

Mazzeo, D., Sacco, S., Di Lucia, P., Penna, G., Adorini, L., Panina-Bordignon, P., Ghezzi, P. (2002) Thiol antioxidants inhibit the formation of the interleukin-12 heterodimer: a novel mechanism for the inhibition of IL-12 production. *Cytokine* **17**: 285–293

Mazzoni, A., Young, H. A., Spitzer, J. H., Visintin, A., Segal, D. M. (2001) Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. *J. Clin. Invest.* **108**: 1865–1873

McIntyre, K. W., Shuster, D. J., Gillooly, K. M., Warrier, R. R., Connaughton, S. E., Hall, L. B., Arp, L. H., Gately, M. K., Magram, J. (1996) Reduced incidence and severity of collagen-induced arthritis in interleukin-12-deficient mice. *Eur. J. Immunol.* **26**: 2933–2938

Meierhofer, C., Dunzendorfer, S., Wiermann, C. J. (2001) Theoretical basis for the activity of thalidomide. *Biodrugs* **15**: 681–703

Moller, D. R., Wysocka, M., Greenlee, B. M., Ma, X., Wahl, L., Trinchieri, G., Karps, C. L. (1997a) Inhibition of human interleukin-12 production by pentoxifylline. *Immunology* **91**: 197–203

Moller, D. R., Wysocka, M., Greenlee, B. M., Ma, X., Wahl, L., Flockhart, D. A., Trinchieri, G., Karp, C. L. (1997b) Inhibition of IL-12 production by thalidomide. *J. Immunol.* **159**: 5157–5161

Monteleone, G., Biancone, L., Marasco, R., Morrone, G., Marasco, O., Luzza, F., Pallone, F. (1997) Interleukin 12 is expressed and released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology* **112**: 1169–1178

Moreira, A. L., Sampaio, E. P., Zmuidzinas, A., Frindt, P., Smith, K. A., Kaplan, G. (1993) Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing messenger RNA degradation. *J. Exp. Med.* **177**: 1675–1680

Murphy, T. L., Cleveland, M. G., Kulesza, P., Magram, J., Murphy, K. M. (1995) Regulation of interleukin 12 p40 expression through an NF-kappa B half-site. *Mol. Cell. Biol.* **15**: 5258–5267

Na, S.-Y., Kang, B. Y., Chung, S. W., Han, S.-J., Ma, X., Trinchieri, G., Im, S.-Y., Lee, J. W., Kim, T. S. (1999) Retinoids inhibit interleukin-12 production in macrophages through physical associations of retinoid X receptor and NFκB. *J. Biol. Chem.* **274**: 7674–7680

Nancey, S., Bienvenu, J., Coffin, B., Andre, F., Descos, L., Flourié, B. (2002) Butyrate strongly inhibits in vitro stimulated release of cytokines in blood. *Digest. Dis. Sci.* **47**: 921–928

Natarajan, C., Bright, J. J. (2002a) Curcumin inhibits experimental allergic encephalomyelitis by blocking IL-12 signaling through Janus kinase-STA pathway in T lymphocytes. *J. Immunol.* **169**: 6506–6513

Natarajan, C., Bright, J. J. (2002b) Peroxisome proliferator-activated receptor-gamma agonists inhibit experimental allergic encephalomyelitis by blocking IL-12 production, IL-12 signaling and Th1 differentiation. *Genes Immun.* **3**: 59–70

Németh, Z. H., Haskó, G., Szabó, C., Salzman, A. L., Vizi, E. S. (1998) Calcium channel blockers and dantrolene differentially regulate the production of interleukin-12 and interferon-γ in endotoxemic mice. *Brain Res. Bull.* **46**: 257–261

Németh, Z. H., Mabley, J. H., Deitch, E. A., Szabó, C., Haskó, G. (2001) Inhibition of the Na^+/H^+ antiporter suppresses IL-12 p40 production by mouse macrophages. *Biochim. Biophys. Acta* **1539**: 233–242

Nicoletti, F., Patti, F., Cocuzza, C., Zaccone, P., Nicoletti, A., DiMarco, R., Reggio, A. (1996) Elevated serum levels of interleukin-12 in chronic progressive multiple sclerosis. *J. Neuroimmunol.* **70**: 87–90

Oppmann, B., Lesley, R., Blom, B., Timans, J. C., Xu, Y., Hunte, B., Vega, F., Yu, N., Wang, J., Singh, K., Zonin, F., Vaisberg, E., Churakova, T., Liu, M., Gorman, D., Wagner, J., Zurawski, S., Liu, Y., Abrams, J. S., Moore, K. W., Rennick, D., de Waal-Malefyt, R., Hannum, C., Bazan, J. F., Kastelein, R. A. (2000) Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* **13**: 715–725

O'Shea, J. J., Paul, W. E. (2002) Regulation of T(H)1 differentiation – controlling the controllers. *Nat. Immunol.* **3**: 506–508

O'Shea, J. J., Gadina, M., Schreiber, R. D. (2002) Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* **109** (Suppl.): S121–S131

Panina-Bordignon, P., Mazzeo, D., Di Lucia, P., D'Ambrosio, D., Lang, R., Fabbri, L., Self, C., Sinigaglia, F. (1997) β_2-agonists prevent Th1 development by selective inhibition of interleukin-12. *J. Clin. Invest.* **100**: 1513–1519

Parham, C., Chirica, M., Timans, J., Vaisberg, E., Travis, M., Cheung, J., Pflanz, R., Zang, R., Singh, K. P., Vega, F., To, W., Wagner, J., O'Farrell, A.-M., McClanahan, T., Zurawaski, S., Hannum, C., Gorman, D., Rennick, D. M., Kastelein, R. A., de Waal Malefyt, R., Moore, K. W. (2002) A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rβ1 and a novel cytokine receptor subunit, IL-23R. *J. Immunol.* **168**: 5699–5708

Pflanz, S., Timans, J. C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E., Blumenschein, W. M., Mattson, J. D., Wagner, J. L., To, W., Zurawski, S., McClanahan, T. K., Gorman, D. M., Bazan, J. F., de Waal Malefyt, R., Rennick, D., Kastelein, R. A. (2002) IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. *Immunity* **16**: 779–790

Plevy, S. E., Gemberling, J. H., Hsu, S., Dorner, A. J., Smale, S. T. (1997) Multiple control elements mediate activation of the murine and human interleukin 12 p40 promoters: evidence of functional synergy between C/EBP and Rel proteins. *Mol. Cell. Biol.* **17**: 4572–4588

Presky, D. H., Yang, H., Minetti, L. J., Chua, A. O., Nabavi, N., Wu, C. Y., Gately, M. K., Gubler, U. (1996) A functional interleukin 12 receptor complex is composed of two beta-type cytokine receptor subunits. *Proc. Natl Acad. Sci. USA* **93**: 14002–14007

Procopio, D. O., Teixeira, M. M., Camargo, M. M., Travassos, L. R., Ferguson, M. A. J., Almeida, I. C., Gazzinelli, R. T. (1999) Differential inhibitory mechanism of cyclic AMP on TNF-α and IL-12 synthesis by macrophages exposed to microbial stimuli. *Br. J. Pharmacol.* **127**: 1195–1205

Racke, M. K., Burnett, D., Pak, S. H., Albert, P. S., Cannella, B., Raine, C. S., McFarlin, D. E., Scott, D. E. (1995) Retinoid treatment of experimental allergic encephalomyelitis – IL-4 production correlates with improved disease course. *J. Immunol.* **154**: 450–458

Rieckmann, P., Weber, F., Gunther, A., Martin, S., Bitsch, A., Broocks, A., Kitze, B., Weber, T., Borner, T., Poser, S. (1996) Pentoxifylline, a phosphodiesterase inhibitor, induces immune deviation in patients with multiple sclerosis. *J. Neuroimmunol.* **64**: 193–200

Rogge, L., Barberis-Maino, L., Biffi, M., Passini, N., Presky, D. H., Gubler, U., Sinigaglia, F. (1997) Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J. Exp. Med.* **185**: 825–831

Säemann, M. D., Böhmig, G. A., Österreicher, C. H., Burtscher, H., Parolini, O., Diakos, C., Stöckl, J., Hörl, W. H., Zlabinger, G. J. (2000) Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. *FASEB J.* **14**: 2380–2382

Sato, E., Koyama, H., Kubo, K., Sekiguchi, M. (1996) Bradykinin stimulates alveolar macrophages to release neutrophil, monocyte, and eosinophil activity. *J. Immunol.* **157**: 3122–3129

Shimuzu, K., Fujii, S., Fujimoto, K., Kawa, K., Yamada, A., Kawano, F. (2000) Tacrolimus (FK506) treatment of $CD34^+$ hematopoietic progenitor cells promote the development of dendritic cells that drive $CD4^+$ T cells toward Th2 responses. *J. Leukocyte Biol.* **68**: 633–640

Sinigaglia, F., D'Ambrosio, D., Panina-Bordignon, P., Rogge, L. (1999) Regulation of the IL-12/IL-12R axis: a critical step in T-helper cell differentiation and effector function. *Immunol. Rev.* **170**: 65–72

Srimal, R. C., Dhawan, B. N. (1973) Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. *J. Pharm. Pharmacol.* **25**: 447

Steinschulte, C., Taner, T., Thomson, A. W., Bein, G., Hackstein, H. (2003) Cutting edge: Sanglifehrin A, a novel cyclophilin-binding immunosuppressant, blocks bioactive IL-12 production by human dendritic cells. *J. Immunol.* **171**: 542–546

Stolina, M., Sharma, S., Lin, Y., Dohadwala, M., Gardner, B., Luo, J., Kronenberg, M., Miller, P. W., Portanova, J., Lee, J. C., Dubinett, S. M. (2000) Specific inhibition of cyclooxygenase 2 restores antitumor reactivity by altering the balance of IL-10 and IL-12 synthesis. *J. Immunol.* **164**: 361–370

Surh, Y., Chun, K., Cha, H., Han, S. S., Keum, Y., Park, K., Lee, S. S. (2001) Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-κB activation. *Mutat. Res.* **480**: 243

Sutterwala, F. S., Noel, G. J., Clynes, R., Mosser, D. M. (1997) Selective suppression of interleukin-12 induction after macrophage receptor ligation. *J. Exp. Med.* **185**: 1977–1985

Suzumura, A., Ito, A., Mizuno, T. (2003) Phosphodiesterase inhibitors suppress IL-12 production with microglia and T helper 1 development. *Mult. Scler.* **9**: 574–578

Szabo, S. J., Dighe, A. S., Gubler, U., Murphy, K. M. (1997) Regulation of the interleukin (IL)-12R beta 2 subunit expression in developing T helper 1 (Th1) and Th2 cells. *J. Exp. Med.* **185**: 817–824

Szabo, G., Gavala, C., Mandrekar, P. (2001) Tacrolimus and cyclosporine A inhibit allostimulatory capacity and cytokine production of human myeloid dendritic cells. *J. Invest. Med.* **49**: 442–449

Takeda, A., Hamano, S., Yamanaka, A., Hanada, T., Ishibashi, T., Mak, T. W., Yoshimura, A., Yoshida, H. (2003) Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. *J. Immunol.* **170**: 4886–4890

Tarrant, T. K., Silver, P. B., Chan, C. C., Wiggert, B., Caspi, R. R. (1998) Endogenous IL-12 is required for induction and expression of experimental autoimmune uveitis. *J. Immunol.* **161**: 122–127

Torii, H., Hosoi, J., Beissert, S., Xu, S., Fox, F. E., Asahina, A., Takashima, A., Rook, A. H., Granstein, R. D. (1997) Regulation of cytokine gene expression in macrophages and the Langerhans cell-like line XS52 by calcitonin gene-related peptide. *J. Leukoc. Biol.* **61**: 216–223

Trembleau, S., Penna, G., Gregori, S., Chapman, H. D., Serreze, D. V., Magram, J., Adorini, L. (1999) Pancreas-infiltrating Th1 cells and diabetes develop in IL-12-deficient nonobese diabetic mice. *J. Immunol.* **163**: 2960–2968

Trembleau, S., Penna, G., Gregori, S., Giarratana, N., Adorini, L. (2003) IL-12 administration accelerates autoimmune diabetes in both wild-type and IFN-γ-deficient nonobese diabetic mice, revealing pathogenic and protective effects of IL-12-induced IFN-γ. *J. Immunol.* **170**: 5491–5501

Trinchieri, G. (2003) Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat. Rev. Immunol.* **3**: 133–146

Trinchieri, G., Scott, P. (1995) Interleukin-12: a proinflammatory cytokine with immunoregulatory functions. *Res. Immunol.* **146**: 423–431

Uchi, H., Arrighi, J.-F., Aubry, J.-P., Furue, M., Hauser, C. (2002) The sesquiterpene lactone parthenolide inhibits LPS- but not TNF-α-induced maturation of human monocyte-derived dendritic cells by inhibition of the p38 mitogen-activated protein kinase pathway. *J. Allergy Clin. Immunol.* **110**: 269–276

van der Pouw Kraan, T. C. T. M., Boeije, L. C. M., Smeenk, R. J. T., Wijdenes, J., Aarden, L. A. (1995) Prostaglandin-E2 is a potent inhibitor of human IL-12 production. *J. Exp. Med.* **181**: 775–779

van der Pouw Kraan, T. C. T. M., Snijders, A., Boeije, L. C. M., de Groot, E. R., Alewijnse, A. E., Leurs, R., Aarden, L. A. (1998) Histamine inhibits the production of interleukin-12 through interaction with H2 receptors. *J. Clin. Invest.* **102**: 1866–1873

Vanderheyde, N., Verhasselt, V., Goldman, M., Willems, F. (1999) Inhibition of human dendritic cell functions by methylprednisolone. *Transplantation* **67**: 1342–1347

Vermeire, K., Heremans, H., Vandeputte, M., Huang, S., Matthys, P. (1997) Accelerated collagen-induced arthritis in IFN-gamma receptor-deficient mice. *J. Immunol.* **158**: 5507–5513

Visser, J., van Boxel-Dezaire, A., Methorst, D., Brunt, T., de Kloet, E. R., Nagelkerken, L. (1998) Differential regulation of interleukin-10 (IL-10) and IL-12 by glucocorticoids in vitro. *Blood* **91**: 4255–4264

Wang, I. M., Contursi, C., Masumi, A., Ma, X., Trinchieri, G., Ozato, K. (2000) An IFN-gamma-inducible transcription factor, IFN consensus sequence binding protein (ICSBP), stimulates IL-12 p40 expression in macrophages. *J. Immunol.* **165**: 271–279

Wiegmann, K., Muthyala, S., Kim, D. H., Arnason, B. G. W., Chelmickaschorr, E. (1995) Beta-adrenergic agonists suppress chronic-relapsing experimental allergic encephalomyelitis (CREAE) in Lewis rats. *J. Neuroimmunol.* **56**: 201–206

Wilkin, F., Stordeur, P., Goldman, M., Boeynaems, J. M., Robaye, B. (2002) Extracellular adenine nucleotides modulate cytokine production by human monocyte-derived dendritic cells: dual effect on IL-12 and stimulation of IL-10. *Eur. J. Immunol.* **32**: 2409–2417

Wolf, S. F., Temple, P. A., Kobayashi, M., Young, D., Dicig, M., Lowe, L., Dzialo, R., Fitz, L., Ferenz, C., Hewick, R. M. (1991) Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. *J. Immunol.* **146**: 3074–3081

Wu, C., Wang, X., Gadina, M., O'Shea, J. J., Presky, D. H., Magram, J. (2000) IL-12 receptor beta 2 (IL-12R beta 2)-deficient mice are defective in IL-12-mediated signaling despite the presence of high affinity IL-12 binding sites. *J. Immunol.* **165**: 6221–6228

Yao, M., Kargman, S., Lam, E. C., Kelly, C. R., Zheng, Y. X., Luk, P., Kwong, E., Evans, J. F., Wolfe, M. M. (2003)

Inhibition of cyclooxygenase-2 by rofecoxib attenuates the growth and metastatic potential of colorectal carcinoma in mice. *Cancer Res.* **63**: 586–592

Yoon, C., Johnston, S. C., Tang, J., Stahl, M., Tobin, J. F., Somers, W. S. (2000) Charged residues dominate a unique interlocking topography in the heterodimeric cytokine interleukin-12. *EMBO J.* **19**: 3530–3541

Yoshida, A., Koide, Y., Uchijima, M., Yoshida, T. O. (1994) IFN-gamma induces IL-12 mRNA expression by a murine macrophage cell line, J774. *Biochem. Biophys. Res. Commun.* **198**: 857–861

Yoshida, H., Hamano, S., Senaldi, G., Covey, T., Faggioni, R., Mu, S., Xia, M., Wakeham, A. C., Nishina, H., Potter, J., Saris, C. J., Mak, T. W. (2001) WSX-1 is required for the initiation of Th1 responses and resistance to *L. major* infection. *Immunity* **15**: 569–578

Youssef, S., Stüve, O., Patarroyo, J. C., Ruiz, P. J., Radosevich, J. L., Mi Hur, E., Bravo, M., Mitchell, D. J., Sobel, R. A., Steinman, L., Zamvil, S. S. (2002) The HMG-CoA reductase inhibitor atorvastatin promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature* **420**: 78–84

Zhou, J., Presky, D. H., Wu, C. Y., Gubler, U. (1997) Differential associations between the cytoplasmic regions of the interleukin-12 receptor subunits beta1 and beta2 and JAK kinases. *J. Biol. Chem.* **272**: 6073–6077