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Review

The interleukin-12 family of cytokines: Therapeutic targets for inflammatory disease mediation

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Abstract

The interleukin (IL)-12 family of cytokines, including IL-12 and IL-23, are important mediators of immune-mediated inflammatory diseases such as psoriasis, multiple sclerosis, rheumatoid arthritis, and Crohn's disease. Interleukin-12 and IL-23 are heterodimeric proteins composed of the common subunit IL-12 p40, which interacts with the IL-12Rβ1 receptor, and the cytokine-specific subunits IL-12 p35 and IL-23 p19, respectively. The cytokines are proinflammatory factors linking innate and adaptive immune responses via the induction and differentiation of the T helper cell 1 pathway. Interleukin-12 and IL-23 target different subpopulations of T cells and antigen-presenting cells, as evidenced by their slightly different, but possibly clinically significant, characteristics and functions. Because both share the p40 subunit, the use of anti-IL-12 antibodies may not be as clinically effective as the use of anti-IL-12 p40 antibodies, since both IL-12 and IL-23 share the subunit, which compete, for the IL-12Rβ1 receptor. Also, while IL-12 is a key factor that drives T helper cell 1 responses and interferon-gamma production in the early phases of the immune responses, it may play a relatively minor immunoregulatory role in late-stage inflammation at the point when IL-23 strongly supports the inflammatory process. Thus, direct IL-23 blockade may be key in treating some inflammatory autoimmune diseases as we further define the roles and functions of IL-12 and IL-23. Research into

Abbreviations: AIA, autoimmune arthritis; APC, antigen-presenting cell; CD, Crohn's disease; CIA, collagen-induced arthritis; EAE, experimental autoimmune encephalomyelitis; EPI3, Epstein-Barr virus-induced gene 3; G-CSF, granulocyte-colony stimulating factor; IBD, inflammatory bowel disease; IFN-β, interferon-beta; IFN-γ, interferon-gamma; IL, interleukin; IMID, immune-mediated inflammatory disease; JAK, Janus kinase; mAb, monoclonal antibody; MS, multiple sclerosis; NK, natural killer; OA, osteoarthritis; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SCID, severe combined immunodeficiency disease; STAT, signal transducer and activator of transcription; T_H1, T helper cell 1; T_H2, T helper cell 2; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNF, tumor necrosis factor.

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the function and regulation of IL-12 and IL-23 is a promising area of study for inflammatory disease mediation, and inhibition of their actions may have clinical therapeutic applications.

Keywords: IL-12; IL-23; Psoriasis; Multiple sclerosis; Crohn's disease; Rheumatoid arthritis

1. Introduction

The success of anti-tumor necrosis factor (TNF) therapies in immune-mediated inflammatory diseases (IMIDs) has changed long-established dogma about the role of specific cytokines in disease pathogenesis. Previously, the cytokine cascade leading to chronic inflammation was notable for its redundancy; i.e., specific blockade of a single mediator of inflammation would likely not be a worthwhile strategy in human disease because numerous other cytokines would replace its function. Since the era of TNF blockade, the idea of redundancy among cytokines has been replaced by the prevailing concept that there is a hierarchy among cytokines; i.e., specific cytokines may have unique inflammatory functions within a disease and across numerous diseases that can be exploited in the design of therapeutic strategies. Tumor necrosis factor blockade has become an important contribution to the treatment of IMIDs [1–7] with limitations that include lack of efficacy and loss of response in some patients [8] and certain other reported risks and potential adverse effects [9–11]. Thus, significant unmet medical needs exist to identify cytokine targets in IMIDs that may provide safer and more effective means of therapeutic intervention.

Recent attention has turned to the role of the interleukin (IL)-12 family of cytokines in the pathogenesis and potential treatment of IMIDs [12,13]. Many proinflammatory factors of both the innate and adaptive immune responses that evolved in mammals to protect against infectious pathogens are commonly overexpressed or misregulated in autoimmune diseases. The mammalian IL-12 family of cytokines is one such group of factors [14,15]. Two members of the cytokine family, IL-12 and IL-23, have been implicated as key mediators of IMIDs such as psoriasis [16,17], multiple sclerosis (MS) [18–21], and Crohn's disease (CD) [5,22–24]. Thus, research into the function and regulation of IL-12 and IL-23 is a promising area of study for inflammatory disease mediation, and inhibition of their actions may have therapeutic applications.

A third member of the IL-12 family is the recently discovered cytokine IL-27 [25], which is formed by the dimerization of the Epstein-Barr virus-induced gene 3 protein (EBI3) and p28, a novel IL-12 p35–related polypeptide. Interleukin-27 appears to act as early mediator of naïve T cell proliferation, and is a potent inducer of interferon-gamma (IFN-γ) production, particularly in synergy with IL-12 and IL-18. The preponderance of experimental evidence thus far supports a role for IL-27 in T helper cell 1 (T_H1)-mediated immune responses [26–32], although some discrepancies remain to be elucidated. In an adjuvant-induced arthritis animal model, Goldberg et al. [32] revealed that IL-27 both directed the pro-T_H1 polarization of naïve T cells and affected the proliferative responses and cytokine production of antigen-specific effector/memory cells that have already been polarized. In this case, IL-27 influenced IFN-γ but not TNF production by T_H1 cells. However, its in vivo neutralization significantly reduced the ex vivo production of this cytokine which, in

turn, rapidly suppressed the arthritis. Conversely, Nieuwenhuis et al. [33] demonstrated that T_H2-mediated and not T_H1-mediated immune responses are impaired in EBI3-deficient animals. Therefore, due to the limited and contradictory data on this novel cytokine currently, the remainder of this review will focus on IL-12 and IL-23.

2. The characteristics of IL-12 and IL-23

Interleukin-12 and IL-23 have some important similarities and differences with respect to structure, receptor interactions, function, regulation, and targets. These are summarized in Table 1.

2.1. Structure and receptor interactions

Interleukin-12 and IL-23 are covalently linked heterodimeric cytokines composed of a common 40 kDa subunit (IL-12 p40) and a 35 kDa subunit (IL-12 p35) or 19 kDa subunit (IL-23 p19) [15,34]. Interleukin-12 p40 has homology with the cytokine IL-6 receptor alpha chain, and IL-12 p35 has homology with IL-6, suggesting an evolutionary link to the IL-6 cytokine family [15,35]. Based on gene sequence, IL-23 p19 is closely related to the IL-12 p35 subunit [36], as both subunits are capable of binding IL-12 p40 via disulfide covalent bonds.

Interleukin-12 and IL-23 signaling is mediated by a homologous pair of heterodimeric cell membrane receptors that are composed of a common protein, IL-12RB1: and alternate

Table 1
Similarities and differences between IL-12 and IL-23

	Similarities	Differences
Structure	Both: IL-12 p40 subunit	IL-12: IL-12 p35 subunit IL-23: IL-23 p19 subunit
Receptor interactions	Both: signaling by IL-12Rβ1 and IL-12Rβ2; use similar signal transduction components (JAK2, TYK2, STAT 1, 3, 4, 5)	IL-12: most prominent STAT protein is STAT 4 IL-23: most prominent STAT protein is STAT 3
Function	Both: stimulate activated T and natural killer cells	IL-12: induces naïve CD4+ T cells in a T_H1-specific manner; may inhibit IL-23 synthesis of IL-17 IL-23: late-phase factor in T_H1 response; distinct cell activation state that produces IL-17
Regulation	Both: secreted by primary APCs and phagocytes; proliferate naïve and CD4+ memory T cells	IL-12: promotes IFN-γ production and T_H1 differentiation; binds with a lesser affinity to IL-12Rβ1 than p40 homodimers IL-23: promotes TNF production in macrophages; perpetuates antigen-presenting cell activation
Targets	Both: anti-IL-12 p40 may be therapeutic	IL-12: anti-1L-12 may not be completely therapeutic in certain immune diseases IL-23: murine models resistant to EAE and CIA; unable to generate IL-17 producing T cells, which may be essential for end-stage autoimmune joint inflammation [65]

subunits, IL-12RB2 or IL-23R, respectively. Both receptors, which are expressed on activated T cells and natural killer cells [14,15], interact with members of the Janus kinase and signal transducer and activator of transcription (JAK-STAT) pathway to mediate signal transduction to the cell nucleus (Fig. 1) [15]. In fact, both cytokines use many of the same signal transduction components including JAK2, TYK2, and STAT 1, 3, 4, and 5 [37]. However, while both IL-12 and IL-23 have similar activities, their capacities to stimulate particular populations of memory T cells differ. This key difference is likely mediated by the use of alternate STAT transcription factor complexes. For example, the IL-12 signal transduction pathway activates primarily STAT4, while STAT3 is the principal downstream factor in the IL-23 pathway [38]. From a clinical standpoint, significant functional differences can be expected in the biological responses induced by IL-12 and IL-23, and thus, are the focus of ongoing research.

2.2. Function

Interleukin-12 and IL-23 are essential for the induction and maintenance of the T_H1 immune response, with some shared functions, likely arising from the common subunit IL-12 p40 and its interaction with the IL-12Rβ1 receptor subunit. Interleukin-12, and IL-23 to a lesser extent, stimulates activated T cells and natural killer cells to synthesize multiple proinflammatory factors, most importantly the T_H1-specific cytokine, IFN-γ [15,39]. In turn, IFN-γ potentiates several antigen-presenting cell functions, and drives the T_H1 immune response in infections and IMIDs [15,37,39]. The function and regulation of T_H1 immune responses have been extensively reviewed elsewhere [14,15,40]. Briefly, CD4+ T cells have been divided into functionally important types based on the cytokines they produce. Although this classification is oversimplified, it provides a clinically useful framework to

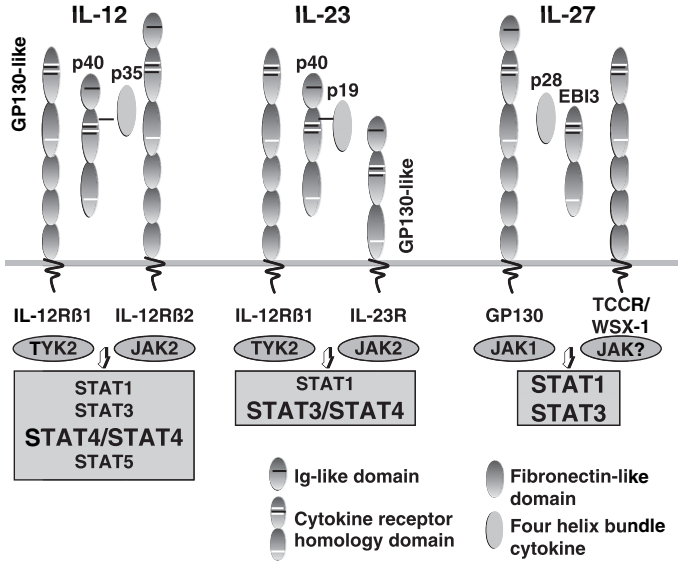

Fig. 1. The roles of IL-12, IL-23, and IL-27 in inflammatory disease mediation are represented in this schematic. (Adapted with permission from [15].)

understand T-cell responses in chronic inflammatory diseases. T_H1 cells—the hallmarks of cell-mediated immunity—produce the inflammatory cytokines IFN-γ and IL-2. T_H1 cells are necessary for the eradication of intracellular pathogens and the development of long-term immunity against infectious agents; however, if T_H1 responses persist unchecked, chronic inflammation can result. As will be discussed, inflammation in CD, MS, and psoriasis is characterized by expression of T_H1 cytokines.

Interleukin-12 and IL-23 differ with respect to target cell populations during the development of the T_H1 response. Interleukin-12 induces naïve CD4+ T cells in a T_H1-specific manner, and stimulates clonal expansion of early committed T_H1 cells [15]. In contrast, IL-23 is seemingly a late-phase factor in the T_H1 response by inducing the proliferation of memory CD4+ T cells [37,41,42]. Furthermore, IL-23 affects cytotoxic T lymphocyte function by enhancing sustained cytotoxic T lymphocyte responses to antigens such as viral DNA, suggesting a role for IL-23 in the maintenance of cytotoxic T lymphocyte responses [43]. Additionally, IL-23, but not IL-12, promotes immunogenic presentation of tolerogenic peptides by APCs [44], implicating a causal relationship in autoimmune disease states.

Interleukin-23 also differs from IL-12 by activating a distinct T cell population that produces IL-17 as a principle effector cytokine [45]. In contrast, IL-12 has only a marginal effect on IL-17 production, and may actually be an inhibitor of IL-23-mediated synthesis of IL-17 [45]. Interleukin-17 is a T cell–derived proinflammatory molecule that stimulates epithelial, endothelial, and fibroblastic cells to produce other inflammatory cytokines such as IL-6, IL-8, granulocyte-colony stimulating factor, and monocyte chemotactic protein-1 [46–53]. Interleukin-17 also synergizes with other cytokines including TNF and IL-1β to further induce chemokine expression [53,54], and is significantly elevated in rheumatoid arthritis (RA) synovium [55,56], during allograft rejection [57–60], and in other IMIDs [61–64]. In addition, the effect of IL-23 on IL-17 expression by T cells is particularly dramatic in animal models in which IFN-γ production is minimal [65]. In a study involving mice with collagen-induced arthritis (CIA), IL-17 played a key role in the development of the disease by activating autoantigen-specific cellular and humoral immune responses, whereby suppression of IL-17 prevented the priming of collagen-specific T cells and IgG2A production [66]. Thus, the incidence of CIA was strikingly reduced in IL-17 deficit mice.

The identification of IL-23 was preceded by the discovery of p19 subunit via a computational sequence screen for IL-6 like proteins [41]. Structurally related to IL-6, granulocyte-colony stimulating factor, and the p35 subunit [41], the constitutive expression of p19 in transgenic mice confers a striking phenotype characterized by runting, systemic inflammation, infertility, and premature death [35]. Histopathologic analyses demonstrate lymphocyte and macrophage infiltrates in numerous transgenic tissues, with associated elevated serum levels of TNF, IL-1, and other acute-phase proteins.

2.3. Regulation

In response to infection and injury, IL-12 and IL-23 are first secreted by primary APCs such as dendritic cells, and then by phagocytes such as activated macrophages [40]. The

regulation of these 2 cytokines is dependent on the coordinate expression of their respective subunits. In the absence of injury and infection, IL-12 p35 and IL-23 p19 are constitutively expressed at low levels in multiple cell types, while IL-12 p40 synthesis is restricted to cell types (macrophages and dendritic cells) that produce bioactive IL-12 and IL-23 heterodimers. With stress, the expression of all 3 cytokine subunits is induced, particularly IL-12 p40 [15,40,67–70]. Interleukin-12 p40 is highly induced by microbial stimuli such as lipopolysaccharide, CpG-rich bacterial DNA, lipoproteins via the toll-like receptor pathway and NF-κB signaling [15], and T cell-dependent cognate interactions via CD40-CD40 ligand. Interferon-gamma provides a potent priming signal for induction of IL-12 in macrophages and dendritic cells [15]. It synergizes with lipopolysaccharide and other microbial products to induce IL-12 p40 transcription. Transcription factors induced by IFN-γ, the IFN regulatory factors, are important for IL-12 p40 production.

Interleukin-12 p40 is produced in large excess over IL-12 and IL-23, and has been isolated as a both a secreted monomer and homodimer in cell culture and animals [15, 71–73]. In mice, the p40 homodimer binds to mouse IL-12Rβ1 with an affinity similar to that of IL-12 and competes with its heterodimeric counterparts for binding to IL-12R [73]. Thus, p40 may be a natural inhibitor of IL-12 in rodents. In contrast, the role of p40 homodimers in humans is far more questionable, as the homodimer has never been isolated from human specimens, and in vitro, p40 homodimers bind with a much lower affinity to human IL-12Rβ1 than does IL-12 [71].

3. Interleukin-12 and IL-23 in IMIDs

3.1. Psoriasis

Psoriasis is a T_H1-mediated disease of the skin characterized by the enhanced expression of IL-12 and IL-23 by dermal cells, APCs, and phagocytes in psoriatic lesions [74–76]. Ectopic IL-12 administration can induce inflammatory T cells that predispose psoriasiform lesions [16,74]. As described above, IL-23 acts on memory T cells to increase secretion of IL-17, whose overexpression is associated with psoriasis as well as RA and MS [45]. Interestingly, genotype studies of the p40 subunit gene have identified particular polymorphisms that are highly associated with the development of psoriasis, suggesting a causal relationship [77].

3.1.1. Preclinical therapeutic data

In a murine model of psoriasis, severe combined immunodeficiency disease mice, which lack endogenous T and B cells, developed more severe psoriatic lesions when exposed to IL-12 versus placebo 1 day after CD4+ memory T cell transfer [16]. The skin lesions induced by this method exhibited many of the histologic hallmarks observed in human psoriasis. Treatment with anti-IL-12 monoclonal antibodies abolished the development of psoriasis in the murine model, and was associated with a decreased expression of TNF and IFN-γ by lesion-infiltrating lymphocytes. Overall, these results suggest that IL-12 is able to stimulate pathogenic inflammatory T cells that are able to induce psoriaform lesions in mice. Interestingly, similar transfer of memory CD4+ T cells into severe combined

immunodeficiency disease mice results in T_H1-mediated colitis that can be attenuated by administration of anti-IL-12 p40 antibodies [78]. These observations highlight the importance of IL-12/IL-23 in chronic inflammatory disorders involving several organs, and emphasize a common immunopathogenesis and therapeutic approach.

3.1.2. Clinical therapeutic data

In a gene expression analysis from skin of patients with psoriasis vulgaris, researchers found that messenger RNA levels of IL-12 p40 in lesions were 12 times higher than in uninvolved skin [75]. In contrast, messenger RNA levels of IL-12 p35 were similar in both skin samples. Immunohistochemical analysis for IL-23 demonstrated expression primarily by those dermal cells with increased IL-12 p40 immunoreactivity. These observations suggest that IL-23 plays a more dominant role in psoriasis than does IL-12, although both cytokines contribute to the disease, possibly because they share the IL-12 p40 subunit, which interacts with the IL-12Rβ1 receptor.

In a subsequent phase 1 study of a single intravenous infusion of human IL-12 p40 antibody in patients with plaque psoriasis [34], dose-dependent associations with both the rate and extent of clinical response were observed across several experimental groups (Fig. 2). Twelve of 18 subjects (67%) achieved at least a 75% improvement in psoriasis area and severity index between 8 and 16 weeks after administration of the study agent. In addition, the antibody treatment was relatively well tolerated with no serious adverse effects reported after a 16-week follow-up period.

3.2. Multiple sclerosis

Multiple sclerosis is a T_H1-mediated disease of the central nervous system, with disease activity directly related to IL-12 and IFN-γ levels [20]. Elevated IL-12 in progressive MS is normalized by pulse cyclophosphamide-methylprednisolone therapy [20]. Significantly

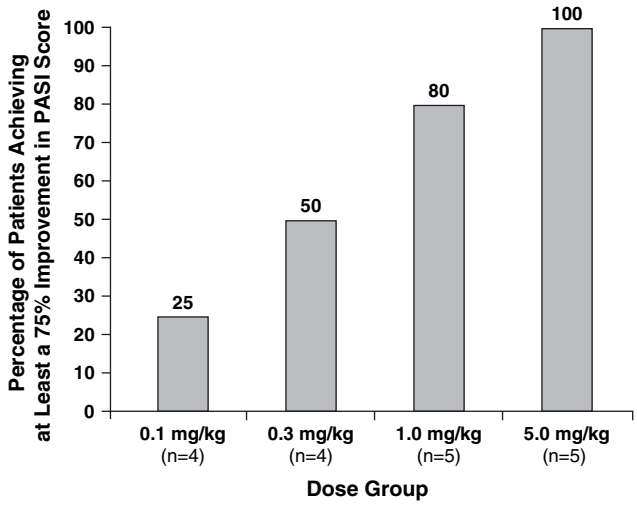

Fig. 2. Improvement in psoriasis area and severity index scores through week 16 [34].

increased production of IL-12 p40 and decreased production of the T_H2-specific cytokine IL-10 has been observed in secondary progressive disease stages compared with relapsing-remitting MS [21].

3.2.1. Preclinical therapeutic data

IL-12 has been characterized as an important factor for the differentiation of naïve T cells into T-helper type 1 CD4+ lymphocytes that secrete IFN-γ [79]. However, a study that involves the murine model of MS (experimental autoimmune encephalitis [EAE]) has revealed that, IL-23, and not IL-12, is required for the induction of EAE [80]. Thus, while IL-12 promotes the development of naïve T cells in this model, IL-23 mediates late-stage inflammation, such as the activation of inflammatory and central nervous system–resident macrophages and recruitment and/or reactivation of central nervous system T cells. Accordingly, transgenic synthesis of IL-12 p40 has been shown to suppress EAE [18], while only IL-23 p19 deficient and IL-12 p40 deficient mice, the latter lacking both IL-12 and IL-23, are highly resistant to EAE [80].

In vivo inhibition of IL-12 p40 using a novel antibody has beneficial effects in the myelin-induced EAE model in common marmosets (*Callithrix jacchus*), demonstrating that anti-IL-12 p40 treatment has a protective effect on the neurological dysfunction as well as on neuropathological changes normally observed in the brain and spinal cord of EAE-affected individuals [81]. Thus, IL-12 p40 is critical to the pathogenesis of EAE in multiple species. Conversely, IL-10, a dominant endogenous inhibitor of IL-12, is largely protective in these experimental surrogates for MS. Such data have suggested that an IL-12/IL-10 immuno-regulatory circuit is a key determinant of disease severity in EAE.

3.2.2. Clinical therapeutic data

In line with the above data obtained from animal studies, a clinical trial was conducted to examine the effects of IFN-β, used for the treatment of MS, on the IL-12/IL-10 axis [19]. The study showed that IFN-β therapy leads to inhibition of IL-12 and augmentation of IL-10 production in MS patients, significantly elevating the ratio of secreted IL-10 to IL-12. These effects were seen in patients with relapsing-remitting as well as progressive disease and indicate that IFN-β affects the IL-12/IL-10 axis in ways believed to be favorable to MS patients. Patient enrollment is under way for clinical studies of an anti-IL-12 p40 antibody in MS treatment [82].

3.3. Crohn's disease

Crohn's disease is a form of inflammatory bowel disease mediated by a T_H1 immune response as characterized by increased synthesis of IL-12 [83,84] by intestinal APCs, with concurrent induction of IL-12Rβ2 [85], STAT 4, and IFN-γ by intestinal lymphocytes and macrophages. Additionally, increased levels of IL-23 have been observed in diseased lesions from CD patients [86]. Conversely, IL-12 p35 and IL-12Rβ1 deficiency partially ameliorates colitis in murine inflammatory bowel disease models. Furthermore, IL-12 p40 deficiency significantly protects animals from developing colitis, possibly implicating roles for both IL-12 and IL-23 [78]. A genetic locus for susceptibility to 2,4,6-trinitrobenzene sulfonic

acid–induced colitis was mapped to the IL-12 p40 gene [87]. Interestingly, in transgenic mice that express the reporter gene luciferase under control of the IL-12 p40 promoter, constitutive IL-12 p40 promoter activation and protein production were restricted to dendritic cells residing in the lamina propria of the terminal ileum. Interleukin-12 p40 activation was correlated with uptake of enteric bacteria by ileal dendritic cells [88]. As the terminal ileum is the most common site of inflammatory involvement in CD, this study suggests that IL-12 p40 induction by enteric bacteria may explain this localization.

3.3.1. Preclinical data

Antibodies directed against IL-12 p40 can prevent and/or reverse colitis in multiple murine models of inflammatory bowel disease. For example, induction of colitis by the administration of the hapten 2,4,6-trinitrobenzene sulfonic acid can be suppressed by the administration of monoclonal anti-IL-12 antibodies both early and late in the disease process. Anti-IL-12 antibody treatment led to a remarkable improvement in both the clinical and histopathologic aspects of the disease and frequently eradicated the colitis completely [88]. Via an alternative treatment method, IL-12 p40 fused to an immunoglobulin (IgG2b) was found to act as a specific IL-12R antagonist, suppressing mucosal inflammation in 2,4,6-trinitrobenzene sulfonic acid–mediated murine colitis. Follow-up studies demonstrated that the IL-12 p40 fusion protein inhibited proinflammatory cytokine expression by intestinal mononuclear cells from CD patients [86].

3.3.2. Clinical therapeutic data

Based on the success of the above animal studies, a randomized double-blind clinical trial was carried out to examine the safety and efficacy of a human monoclonal anti-IL-12 p40 in the treatment of CD [5]. Patients with active CD were assigned to receive subcutaneous injections of anti-IL-12 antibody or placebo in various doses and dosing schedules. Those patients who received multiple weekly antibody injections had higher clinical responses than did placebo-treated patients. The experimental regimen was well tolerated with adverse event rates among patients receiving anti-IL-12 p40 similar to those among patients given placebo except for a higher rate of local injection site reactions in the former group. Molecular studies demonstrated decreased secretion of IL-12, IFN-γ, and TNF by intestinal mononuclear cells derived from treated patients versus control patients, correlating with clinical responses. Thus, it was concluded that treatment of CD with anti-IL-12 p40 antibodies was safe and may suppress disease activity via inhibition of the T_H1 response. The authors noted that, since the anti-IL-12 that was used in the study recognized the IL-12 p40 chain, IL-23 may also be involved in the observed responses.

3.4. Rheumatoid/autoimmune arthritis

The role of IL-12 is not as clear in RA and autoimmune arthritis as in the aforementioned IMIDs. In a synovial tissue analysis involving patients with arthritis, elevated levels of IL-12 and IFN-γ were found in the synovial fluid of RA-affected joints whereas production of IL-12 by osteoarthritis tissues was limited [89]. The inflamed joints of psoriatic arthritis patients appear to produce IL-12 locally [90]. Interleukin-12 levels were significantly higher

in patients with psoriatic arthritis than in patients with osteoarthritis, but lower than those in RA patients. Patients with juvenile chronic arthritis were also observed to have local overproduction of synovial IL-12 and marked elevation of serum IL-6, another cytokine that plays a critical role in the pathogenesis of autoimmune diseases [91,92]. These observations suggest that IL-12, produced mainly by macrophage-lineage cells, may be involved in IFN-γ-dominant cytokine production by infiltrating T cells in joints with chronic RA. Studies in gene-targeted mice indicate that IL-23 is an essential promoter of end-stage autoimmune joint inflammation and that IL-12 paradoxically mediates protection from autoimmune inflammation [65].

3.4.1. Preclinical therapeutic data

Anti-IL-12 antibodies can limit the severity of murine CIA. Mice with established arthritis were treated with anti-IL-12 p40 and/or anti-TNF antibodies for 10 days from the onset of disease [93]. Clinical assessment showed that the combined antibody treatment lessened disease severity to a greater extent than anti-TNF alone. Supporting these findings, a histological analysis demonstrated that joint damage was reduced in the mice that received combined anti-IL-12 p40 and anti-TNF treatment compared with the other treatment groups. This study suggests that, through the partial regulation of IL-12, TNF modulates the immune response as well as the inflammatory response in arthritis. The synergistic action of anti-TNF and anti-IL-12 p40 on CIA may provide a new therapeutic approach for treating RA.

In another study, mice with CIA received anti-IL-12 p40 antibodies twice weekly [94]. While the administration of anti-IL-12 p40 from immunization until the onset of clinical arthritis did not lower the incidence of arthritis, the severity of the disease was dramatically attenuated, both clinically and histopathologically. This regimen was associated with reduced IFN-γ levels and with diminished spontaneous ex vivo production of TNF, IL-6, and IL-10 by freshly isolated synovial cells. These findings suggest that IL-12 p40 plays a major role in the induction of murine CIA. However, as of yet, no human clinical studies using anti-IL-12 p40 antibodies in the treatment of RA or autoimmune arthritis have been attempted.

4. Discussion

4.1. Safety issues of IL-12/IL-23 blockade

Lessons from the human use of TNF blockade in treating IMID yield cautionary warnings for future trials targeting other proinflammatory mediators. Adverse events of TNF blockade have been described, including the development of infections, antinuclear antibodies and drug-induced lupus [10], inflammatory demyelinating disease of the central nervous system [9], and vasculitis [11].

Only limited safety information on IL-12 and IL-23 blockade in humans is available. The human IL-12 p40 antibody, used in the human clinical studies described above, demonstrated an acceptable safety profile, with no patients developing serious adverse events or adverse events requiring discontinuation or dose reduction [5,34]. However, these 2 studies included only 97 patients combined, followed for only approximately 18 weeks

after treatment. Thus, significantly more patients, followed over a longer period of time and receiving multiple treatment rounds, need to be studied for adequate safety assessment of this therapeutic strategy.

A limited spectrum of infections—particularly atypical mycobacterial and recurrent *Salmonella* infections but also *Candida*—has been associated with a genetic deficiency in IL-12R complexes in humans [95–97]. While this suggests that IL-12 plays a role in resistance to intracellular bacterial infections, IL-12 and IL-23 signaling is mediated by cell membrane receptors that share the IL-12Rβ1 receptor. Thus, while Verhagen et al. demonstrated that IL-12 acts as a partial agonist in the absence of that receptor, their results also revealed the existence of a novel IL-12Rβ1/STAT 4-independent pathway of L-12 and, possibly, IL-23 (by association with the same β1 receptor), responsiveness in activated human T cells involving MAP kinases. More research needs to be directed toward the clinical consequences of IL-23 blockade to discern the role of a STAT 4–independent pathway that may favor IL-23 activity in T cells.

Among clinical trials of IL-12 blockade, the limited spectrum of infections in genetically deficient humans is an interesting observation that may be predictive of a relatively low incidence of infectious complications. Opportunistic, intracellular, or serious infections have not been reported in clinical studies of anti-IL-12 p40 to date; however, the theoretical immunodeficiency warrants continued monitoring. Similarly, although no data exist on immunogenicity from anti-IL-12 therapy, the potential for its emergence should be an ongoing concern. In the CD study described above [5], injection site reactions (a possible consequence of immunogenicity) were the most frequently reported adverse event, and the only adverse event to occur significantly more often than in the placebo group ($P < .005$). In the psoriasis study [34], no infusion reactions were reported, although in this study, patients were given a single intravenous infusion of anti-IL-12 p40.

4.2. Summary and future questions

The IL-12 family of cytokines, including IL-12 and IL-23, plays an important role in bridging innate and adaptive immune responses via the induction and maintenance of T_H1-mediated inflammation. Consequently, it follows that regulation of IL-12 and IL-23 function affects the pathophysiology of IMIDs. These 2 cytokines share some functions, likely via their common subunit IL-12 p40, such as the ability to induce IFN-γ and to drive T_H1 differentiation. Therefore, anti-12 p40 can inhibit either cytokine. In contrast, IL-12 and IL-23 likely target different subpopulations of T cells and APCs as evidenced by the preferential action of IL-23 on memory T cells. On one hand, IL-12 is a key factor that drives T_H1 responses and IFN-γ production in the early phases of the immune responses. Conversely, IL-12 may play a more minor immunoregulatory role in late-stage inflammation at the point when IL-23 strongly supports the inflammatory process. Thus, direct IL-23 blockade may be key in treating some inflammatory autoimmune diseases as we further define the roles and functions of IL-12 and IL-23.

Now that it is recognized that the IL-12 family of cytokines plays a dominant role in IMIDs, we can proceed with further targeted explorations of the inflammatory response. To that end, inhibition of IL-12/IL-23 p40 in multiple immune-mediated inflammatory

Table 2
Anti-IL-12 agents in phase 2 clinical development

Agent (manufacturer)	Mechanism of action	Description	Uses under study
ABT-874 (Abbott)	Anti-IL-12	Human monoclonal antibody	• CD • MS
CNTO 1275 (Centocor)	Anti-IL-12 p40	Human monoclonal antibody	• Psoriasis • MS
STA-5326 (Synta)	Anti-IL-12	Small-molecule oral selective IL-12 inhibitor	• CD • Psoriasis

disorders is a growing field of research, as a number of anti-IL-12 agents are in clinical development in the United States (Table 2). This new prototype in immunology should be heartening news for the millions of people who suffer with autoimmune conditions, as it increases the possibility of developing targeted therapies that are more selective for specific immunologic defects, and potentially safer and more effective than existing alternatives.

More work is needed, of course, principally because the reported benefits of IL-12 inhibitors may actually be due to either specific inhibition of one or the other cytokine or the concurrent inhibition of both cytokines. Also, the differences between the 2 cytokines may raise more questions. What is the clinical impact of the observation that IL-23, but not IL-12, acts preferentially on memory CD4+ T cells? How much significance should be given to IL-23's ability to activate the secretion of IL-17, a proinflammatory cytokine associated with many autoimmune diseases, particularly in lieu of the fact that IL-12 only weakly affects IL-17 production? Such questions will guide us as we design future trials for the treatment of IL-12- and IL-23-mediated disorders.

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