The role of IL-12/23 in T–cell related chronic inflammation; implications of immunodeficiency and therapeutic blockade

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Short title: The role of IL-12/23 in chronic inflammation

The authors declare no conflicts of interest

Abstract

In this review, we discuss the divergent role of the two closely related cytokine, interleukin (IL)-12 and IL-23, in shaping immune responses. In light of current therapeutic developments using biologic agents to block these two pathways, a better understanding of the immunological function of these cytokines is pivotal.

Introduction:

The cytokines IL-12/23 are known to be pro-inflammatory and recognised to be involved in driving autoimmunity and inflammation. Antibodies blocking IL-12/23 have now been developed to treat patients with chronic inflammatory conditions such as seronegative spondyloarthropathy, psoriasis, inflammatory bowel disease, as well as multiple sclerosis. The anti-IL-12/23 drugs are very exciting for the clinician to study and use in these patient groups who have chronic, sometimes disabling conditions - either as a first line, or when other biologics such as anti-TNF therapies have failed. However, IL-12/23 have important biological functions, and it is recognised that their presence drives the body's response to bacterial and viral infections, as well as tumour control via their regulation of T cell function. We can therefore be excited, but also need to be cautious about the use of biologic therapies that block them. In the rare conditions, where there is a congenital deficit of IL-12/23, patients have presented with overwhelming infections. In this review, we cover the science behind the biologic role of IL-12/23, the evidence for biologic therapies targeting them in the clinical situation, as well as mention cases in the literature when patients have been found to have primary IL-12/23 deficiency.

IL-12/23 background

IL-12 is a pro-inflammatory cytokine that induces T helper type 1 (Th1) differentiation in CD4 T cells and acts as a third signal in the activation of CD8 T cells. IL-12 is made up of two covalently linked subunits p35 and p40, which together form the active heterodimer p70. The p40 subunit is shared with IL-23, a cytokine driving the development of IL-17 producing CD4 T cells. The second subunit p19 is unique to IL-23. While IL-12 has been studied in some detail, IL-23 has only been discovered recently (1), and less is known about its specific functions. However, it is becoming clear that IL-12 and IL-23 have distinct and often opposing functions. Biological activity and receptor binding is conferred by the shared p40 subunit. Both cytokines, IL-12 and IL-23 can be produced by activated dendritic cells and macrophages after sensing microbial products, however, expression of p35 and p19 is tightly regulated (2). IL-12 and IL-23 share a receptor chain, IL-12R-1, which then combines with IL-23R for IL-23 signalling (2, 3) or IL-12R-2 for IL-12 signalling (2, 4) (Figure 1).

Activation of T cells, the 3 signal hypothesis

T cells are vital in combating infection and inhibiting cancer growth. In order to be fully activated cytotoxic CD8 T cells need 3 signals according to the 3 signal hypothesis (5) (comprehensively reviewed in Goral 2011). Signal 1 is provided by recognition of peptide loaded MHC molecule through the T cell receptor, signal 2 is provided by co-stimulation through interactions of molecules expressed on the antigen-presenting cell with those on the T cell (e.g. CD28 with CD80/86). Finally, signal 3 is provided by pro-inflammatory cytokines (Figure 2). The best described cytokines to provide signal 3 are type I IFNs (IFN-γ) and IL-12 (5, 6). The IL-12 receptor is upregulated upon TCR stimulation on naïve T cells and expression is maintained on memory cells (7).

When naïve cytotoxic T cells are primed in the absence of signal 3 they only develop weak effector functions, failing to proliferate extensively, survive long term and form adequate memory responses (5, 8). T cell activation can be blocked through therapeutic intervention at each of the three signalling steps, (9).

Immune function of IL-12 on T cells

IL-12 regulates the expression of many genes important for effector T cell function, such as FasL and granzyme B. It also regulates the transcription factor T-bet, which in turn promotes IFNγ production (10) and stimulates Th1 responses in CD4 T cells. IL-12 stimulated CD8 T cells produce increased amounts of IFNγ, TNF and macrophage colony stimulating factor (GM-CSF) and show increased

persistence and function in T cell adoptive therapies (11). Furthermore, adjuvants activate the immune system by inducing dendritic cells to produce inflammatory cytokines such as IL-12 (12).

IL-12 counteracts T cell exhaustion

Repetitive stimulation of T cells for example by persistent antigens leads to a loss of effector functions, leading to a reduction in proliferation, cytotoxicity, cytokine production and ultimately viability, a process termed T cell exhaustion (13). Studies in patients with chronic hepatitis B and D have shown that T cell function was recovered by stimulation with IL-12 in culture (14, 15). These findings suggest that in chronic viral infections anti-viral T cells might benefit from IL-12 signalling.

Memory cells can respond to IL-12 in the absence of T cell receptor signalling with cytokine production, thereby supporting or aggravating an ongoing immune response (16, 17).

Additionally, IL-12 has also been described to have some regulatory properties in experimental autoimmune disease (18). Therefore, simultaneous blockade of IL-23/12 by p40 blocking agents (such as ustekinumab) might have disadvantages that need to be monitored closely to understand the pleiotropic effects of IL-12 signalling in vivo (19).

Immune function of IL-23 on T cells

In contrast to IL-12 signalling, naïve T cells cannot respond to IL-23, as they lack expression of the IL-23R chain (2), while memory and effector CD4 T cells can express the IL-23 receptor. Its upregulation on activated T cells is stimulated by autocrine IL-21 in response to IL-6, produced by dendritic cells during inflammation. However, IL-21 deficient mice still expressed IL-23R, suggesting alternative pathways exist (2). IL-23 has been implicated in maintaining inflammatory responses and chiefly drives IL-17A and F production (inducing Th17 rather than Th1 CD4 cells) (2). In the absence of IL-23, IL-17 producing cells can be induced by TGF-β and IL-6, however, these cells failed to induce disease in an autoimmune model, possibly due to containing a subpopulation of cells co-producing IL-17 and the immune suppressive cytokine IL-10, which could confer regulatory function (20).
It therefore seems that IL-23 is required for the pathogenic role of IL-17 producing T cells (21). So far, the effect of IL-23 has mainly been shown for CD4 effector memory cells and increased numbers of IL-23R+ CD4 T cells were shown to exist in autoimmune disease (AID). For example in synovial fluid up to 95% of CD4 T cells can be memory cells capable of responding to IL-23. The exact mechanism of action of IL-23 on CD8 T cells remains to be elucidated (22).

Clinical consequences of IL-12 deficiency

Valuable pathological inferences can be made from studying the clinical consequences of molecular defects in cytokine signalling. There are a number of case series in the literature, documenting patients with inherited defects in IL-12 signalling, many of which are in the context of consanguinity. The IL-12 and IFNγ pathways are inextricably linked; patients who have defective IL-12 production also have impaired IFNγ production (23). Genetic defects have now been described at a number of points in the IL-12/IFNγ signalling cascade. Such patients tend to present in infancy with persistent mycobacterial (known as Mendelian susceptibility to mycobacterial disease) and non-typhi salmonella infections and have a poor prognosis. Mycobacterial infection is often by otherwise poorly pathogenic non-tuberculous organisms or follows BCG vaccination. Of note, these patients do not seem to be as affected by infection with extra-cellular bacteria or respiratory viruses.

Genetic defects affecting the IL-12/IFN pathway can be classified as follows: mutations in the IL-12p40 subunit, the IL-12R (β1 or β2 chains), the IFN-γR (chains 1 and 2) and the downstream signalling molecule STAT1.

IL-12p40 deficiency

There appears to be a wide variation in the clinical consequences of IL-12p40 deficiency, varying from localised BCG infection to overwhelming mycobacterial or salmonella infection (24).

One report documents the case of a 3 year-old female with recurrent episodes of severe staphylococcal and pneumococcal sepsis whose peripheral blood mononuclear cells (PBMCs) failed to produce IL-12 despite appropriate stimulation (25). It was thought that the patient had either an abnormality in the activation pathway for IL-12 p40 gene expression or an abnormality in the gene itself. In another IL-12 p40-deficient child, disseminated *salmonella* and BCG infection were curable with chemotherapy and IFN-γ supplementation (26). The father of this child was heterozygous for this defect and had persistent non-typhi salmonella infection in infancy, eventually cured with prolonged antibiotics. Since p40 is the shared subunit, these patients would have also been IL-23 deficient, but the influence of this cytokine has not been studied in these cases (?).

IL-12R deficiency

The IL-12Rβ1 chain is common to both the IL-12 and IL-23 receptors and thus deficiency abolishes signalling of both Il-12 and IL-23. IL-12Rβ1 deficiency is inherited in an autosomal recessive fashion and is caused by mutations resulting in premature stop codons (27). Heterozygous carriers appear to be clinically healthy with normal IL-12 signalling and IFNγ production.

Fieschi *et al* (2003) conducted a comprehensive review of IL-12Rβ1- deficient patients and found that there was a surprising heterogeneity in terms of clinical outcome; furthermore, there was also evidence

of incomplete clinical penetrance as homozygous individuals identified through family screening had remained asymptomatic up to age 18 years (28).

The clinical course of infection in IL-12Rβ1 deficiency is variable. Two such patients who developed disseminated BCGosis were successfully treated with antituberculous chemotherapy; three further patients had curable *M. Avium* infection (29). In another family affected by IL-12Rβ1 deficiency, however, one child died of disseminated M. Avium despite chemotherapy, while his brother was cured by the addition of IFNγ (29).

A further patient has been described who developed disseminated BCGosis aged 4 months who was found to have a homozygous mutation of the IL-12β1 gene leading to absent IL-12β1 expression on activated T cells. The patient relapsed after initial successful chemotherapy aged 3 years. During futher antimycobacterial treatment he developed cutaneous leukocytoclastic vasculitis later shown to be secondary to infection with *salmonella enteriditis* (30).

Another report documents an 8-year old girl with recurrent bronchopneumonia (due to Haemophilus on one occasion) associated with very high IgE levels (31). She was found to have a complete absence of the IL-12Rβ2 chain due to a novel mutation. The patient's family history was notable for a high incidence of atopy in both lineages. This supports the theoretical notion that an impairment in type-1 immunity might upregulate the Th2-associated cytokines (IL-4, IL-5 and IL-13) which mediate allergic responses.

ROLE OF IL-12/23 IN CHRONIC INFLAMMATION

The ability of IL-12 to induce the differentiation of naïve T cells into IFN-γ-producing Th1 cells, and that of IL-23 to drive the expansion of IL-17-producing T-cell population, are suggestive of IL-12 and 23 involvement in autoimmunity and chronic inflammation. Animal model studies identified that mice deficient in the expression of p40 subunit of IL-12/23 were protected from developing arthritis, ocular or bowel inflammatory conditions after immunisation with specific antigens (32, 33). However, it is very likely that IL-12 and 23 also have divergent immune functions, as it was subsequently found that mice lacking IL-12 (p35) were highly susceptible to experimental autoimmune encephalomyelitis (EAE), whereas IL-12/23p40-deficient mice were completely resistant to developing this pathology (34). Further studies, showing the equivalence of two mice phenotypes (lacking the IL23p19 or IL-12/23p40 subunits), suggested that IL-23, rather than IL-12, is the major orchestrator of autoimmunity (35).

In addition to animal models, the presence of these two cytokines at the site of autoimmune inflammation in various human pathology provided additional rationale for their use as therapeutic targets.

Psoriasis and psoriatic arthritis (PsA)

The role of IL-12/23 in driving the excessive growth and aberrant differentiation of keratinocytes in psoriasis was proven by biopsy studies, which found high concentrations of IL-12/23p40, as well as IL-23p19 mRNA in psoriatic skin lesions compared to normal skin (36). In addition, IFN-γ, IL-17A, IL-17F and IL-22 were overexpressed in psoriasis plaques (37). However, in contrast to IL-23 that triggered abnormal differentiation of keratinocytes, IL-12 did not cause similar skin changes, suggesting a possible differential effect of the two cytokines (38).

The susceptibility to develop PsA was associated with single-nucleotide polymorphisms (SNPs) within IL-23R and IL-12B, the gene that encodes for subunit of ligand of IL-23R (39). Increased frequencies of IL-17 and IL-22 producing and IL23R expressing CD4+ T cells were also found in patients with PsA (40).

Inflammatory bowel disease (IBD)

There is evidence of elevated expression of the IL-12/23 p40 subunit both at mRNA and protein levels in bowel biopsies and serum in patients with IBD, and more significantly in patients with Crohn's disease (CD) (41). In addition, IL-12 Rβ2 had increased expression in CD biopsies and was upregulated by increased concentration of IL-12, and correlated with the activity of STAT4 proteins and IFN-γ expression (42). Moreover, the IL-12 polymorphisms were associated with both CD and ulcerative colitis (UC) susceptibility (43, 44). Furthermore, inhibition of IL-12/23 p40 subunit repressed Th1 immune responses, immune cell proliferation and promoted apoptosis in a mouse model of experimental colitis (45). All these biologic effects supported the hypothesis of therapeutic benefits associated with IL-12 inhibition in IBD.

IL-23 responsive innate lymphoid cells were identified in higher proportion in the bowel biopsies of patients with IBD (46). In addition, IL-23 was implicated in mucosal inflammation mediated by p19 subunit, whereas in the absence of IL-23, mice developed enhanced IL-12–driven inflammation (47). This suggested a potential mechanism of cross-regulation of both cytokines that might be relevant for therapeutic purposes.

Multiple sclerosis (MS)

IL-23 played a key role in perpetuating the immune responses in an animal model of EAE, as the IL-12/23p40 subunit expression by CNS-endogenous cells was critical for the development of the autoimmune inflammatory process (48).

THERAPEUTIC EFFECTS OF IL-12/23 BLOCKADE

There are several biologic agents targeting IL-12/23 and IL-23 inhibition that are currently under development or used in practice (Table 1). We detail below the most researched biologic treatments. Figure 3 details the differential blockade of the available biologic therapies.

IL-12/23 blockage in psoriasis and PsA

Ustekinumab (a human monoclonal targeting the IL-12/23 p40) is currently licensed for use in psoriasis based on the successful outcome of two large phase III randomised controled trials (RCTs) (49, 50). Ustekinumab is administered as subcutaneous injection of 45 mg or 90 mg at week 0, week 4, and every 12 weeks afterwards.

Briakinumab, another monoclonal antibody with similar target, was also effective in treating psoriasis (administered subcutaneously as monthly 200 mg injections for the first two doses, followed by 100 mg thereafter). However, this treatment was associated with a higher incidence of major adverse cardiac events (MACE), such as myocardial infarction, stroke or cardiovascular death, which led to the withdrawal of its registration application until further analysis or future studies become available (51).

The efficacy of Ustekinumab in treating moderate to severe PsA was confirmed in two large phase III clinical trials (52, 53). In addition to providing benefits in controlling the symptoms of arthritis, ustekinumab was also effective in improving enthesitis, and spinal inflammation. Despite not reaching statistical significance, IL-12/23 inhibition was also associated with clinical benefits in the treatment of dactylitis (54).

Future development of IL12/23 blockade in psoriasis and PsA

Despite the incontestable benefits of ustekinumab treatment in PsA and psoriasis, recent research suggested that the development of selective IL-23 blockage might have a theoretical advantage over IL-12 inhibition, as the inhibition of radiographic progression seems to be linked to the IL-23 - Th17 - IL-17 pathway of osteoclast activation (55). In addition, IL-23 deficient mouse model was protected against

developing arthritis, while IL-12 deficient mouse model developed severe synovitis (32), suggesting that IL-23 is the major contributor to PsA.

IL-12/23 blockage in rheumatoid arthritis

Phase II clinical trials of ustekinumab and guselkumab in RA did not meet their primary endpoint, and the clinical research program was discontinued.

IL-12/23 blockage in IBD

Ustekinumab is also effective in treating autoimmune inflammation associated with CD, irrespective of prior exposure to anti TNF therapy (56, 57). UNITI-1 [ClinicalTrials.gov identifier: NCT01369329] and UNITI-2 [ClinicalTrials.gov identifier: NCT01369342] are two large phase III RCTs in patients with CD, which are expected to be completed in November 2018. There is a suggestion that IL-12/23 inhibition might be more suitable in patients with late CD, as they had an increase in the peripheral Th17 cells and related cytokines, compared to patients with early disease (58). Briakinumab program in CD (NCT00562887) was terminated, as the primary endpoint of clinical remission at week 6 was not met in a phase II RCT (59).

Another anti IL-12/23 p40 subunit monoclonal antibody (ABT-874/J695) was also tested in a phase II RCT in patients with CD. The treatment was associated with a significant improvement of bowel inflammation after 7 weeks of uninterrupted administration at a dose of 3 mg/kg, but this response was not maintained at week 18 (primary endpoint). In the responder group, the treatment was associated with decrease in the Th1-mediated inflammatory cytokines in the colonic lamina propria (60). Because the study did not meet the primary endpoint, the research program was discontinued.

IL-12/23 blockage in MS

There is evidence of some benefit associated with treatment with briakinumab in relapsing-remitting multiple sclerosis (RRMS), consisting either in lower relapse rate at week 23 or decreased number of gadolinium-enhanced lesions on MRI, but the benefit was not significant enough to grant further development in MS. In contrast, ustekinumab was no associated with clinical benefit or imaging improvement in MS (61).

IL-12/23 blockage relevance for tumour growth

There is evidence of opposing effects of IL-12 and IL-23 inhibition effect on tumour overgrowth, which is relevant for the equilibrium phase of cancer immunoediting (62), as well as for the potential risk of

cancer in patients treated with ustekinumab for other indications. While, the IL-23 blockage in a sarcoma mouse model eliminated the residual tumour cells, the inhibition of both IL-12 and 23 (using an IL-12/23p40 neutralization antibody) enabled the progression of tumours (63). Further research aiming to establish the therapeutic role of these dual biologic inhibitory agents in cancer is required.

Table 1: Available biologic therapies for IL-12 and IL-23 blockage (as updated from https://clinicaltrials.gov/ct2/- August 2016).

Therapeutic agents / ***Diseases***	**IL-12/23p40 blockade**		**IL-23p19 blockade**				
	Ustekinumab	***Briakinumab***	***Guselkumab***	***Tildrakizumab***	***AMG-139***	***LY-3074828***	***BI-655066***
Psoriatic arthritis	**Licensed for use**	Phase II	Phase II				Phase II
Psoriasis	**Licensed for use**	Phase II*	Phase III	Phase III	Phase I	Phase I	Phase III
Ankylosing Spondylitis	Phase III	Phase II				Phase I	Phase II
Rheumatoid Arthritis	Phase II (ineffective)		Phase II (ineffective)				
Crohn's disease**	Phase III	Phase II (Ineffective)			Phase I		Phase II
Ulcerative colitis						Phase II	
Multiple sclerosis	Phase II (Ineffective)	Phase II*					

*Program discontinued in 2011

** The research program of ABT-874/J695 (another IL12p40 blocking agent) was discontinued in 2005.

Side-effects to IL-12/23 blockage

The combined analysis of phase II and III RCTs in psoriasis revealed a similar rate of adverse-events per 100 patient years in the ustekinumab treated groups compared with placebo (64). A meta-analysis of the phase II and III trials of briakinumab identified cases of opportunistic infections (*Candida)* (65), but there was no evidence of mycobacterial or *Salmonella* infections secondary to treatment with either ustekinumab or briakinumab. There are a few case reports of acute and re-activation of HBV infection (66, 67); however, the expert opinion is that the risk of chronic viral infection associated with the use of ustekinumab is very low (68). The rate of MACE, opportunistic infections and malignancy (non-melanoma skin cancers were the most common) was higher in the patients treated with briakinumab compared to ustekinumab. This could be explained by the more frequent dosing schedule of briakinumab and its higher affinity to IL-12/23 p40, both leading to higher drug exposure (49, 69).

Ustekinumab is the only IL-12/23 inhibitor licensed for use in moderate-severe psoriasis (since 2009) and psoriatic arthritis (since 2013). An application was recently made seeking approval for use of ustekinumab in CD.

Discussion:

Although, there is strong evidence for the contribution of IL-12 and 23 to autoimmunity and successful blockade of these cytokines in clinical practice, questions remain about the long-term efficacy of this therapeutic intervention and differential roles of IL-12 and 23.

Case reports of IL-12 deficiencies are characterised by increased risk of infections (especially mycobacterial disease) from early age, suggesting that a complete blockade of both cytokines could be associated with significant toxicity; however, there are no significant side effects documented from clinical trials of ustekinumab. In comparison, briakinumab, characterised by greater binding affinity and used at a higher equivalent dose in clinical trials, generated significant safety concerns, which prompted the discontinuation of its research program.

Although the neutralisation or absence of IL-23 in a murine model did not compromise the immune response to mycobacteria (70), humans with IL-23 deficiency are prone to low-virulence mycobacterial infections and nontyphoid salmonella infections (71). Interestingly, in a murine model, the presence of IL-23 can compensate partially for the absence of IL-12, which indicates a degree of immune redundancy characterising the function of the two cytokines (70).

Even if there are only a few cases of hepatitis B reactivation secondary to IL-12/23 blockade reported in the literature, there is evidence suggesting that IL-12 blockage might impair the immune system capacity to tackle hepatitis infection. In vitro stimulated human CD8 T cells from patients with chronic hepatitis B showed decreased PD-1 expression and survived better in the presence of IL-12 than IFNα; and IL-12 led to an induction of the transcription factor Tbet (14). In line with these findings, increased Tbet expression in murine T cells (LCMV) and human T cells in chronic hepatitis C, correlated with improved cell survival and function (72). As the majority of patients in clinical trials are screened for chronic hepatitis and excluded if tested positive, and there is a potential bias in reporting adverse events outside clinical trials, only the future will tell if these new biologic agents are associated with increased hepatitis infection/reactivation risk.

In addition, the differential effect of IL-12 and 23 in tumorigenesis control in animal models could raise questions about the optimal IL-12/23 blockage ratio needed to minimise the risk of cancer potentially associated with this therapy. If IL-12 blockage enabled tumour progression in a murine cancer model, IL-12 stimulated T cells were significantly more effective in controlling tumours than IFNα stimulated cells, and showed a lower expression of the co-inhibitory receptor PD-1 (73).

Apart from the problem of long-term safety, further research is needed to elucidate the most effective strategy to block selectively IL-12, IL-23 or both for different therapeutic purposes. P40 and p19 deficient mice are both resistant to EAE. However, p35 deficient mice show increased susceptibility to EAE (2), implying that IL-12 is not driving this autoimmune process. Deficiency of IL-12Rβ1 chain also made mice resistant to EAE. Despite these findings suggesting that IL-23 blockade might be beneficial in MS, ustekinumab was not associated with clinical improvement; however, briakinumab, despite sharing the same target, was effective (probably because of its higher binding affinity). Further research into selective IL-23 blockade might identify successful therapeutic strategies for MS.

Different effects of IL-12 and IL-23 were identified in CD as well. While systemic inflammation was driven by IL-23 p35 secretion, the intestinal inflammation was associated with IL-23 p19 and IL-17A mRNA, suggesting a differential role of IL-23 in the mucosal inflammation and potential benefit of selective IL-23 blockage (33, 74). Similarly, it was advocated that IL-23 inhibition in PsA is likely to be clinically more relevant than IL-12 inhibition, because of the effect of this pro-inflammatory cytokine on the Th17/ IL-17 pathway activation, which is relevant for the development of bone erosions.

In conclusion, further research into the mechanism of action of different IL-12/23 blocking agents is needed to characterize better the impact of these therapies on the homeostasis of the immune system and organ-specific inflammation associated with various autoimmune diseases. Long-term clinical data will further delineate if the increased infection risk characterising human IL12/23 immunodeficiency syndromes and the impaired tumorigenesis control identified in animal models will translate in relevant clinical events. Although it is likely that the IL-23 rather than IL-12 blockade might be more relevant for autoimmunity, head-to-head studies looking at the available biologic agents that selectively block the two cytokines are required for successfully translating this therapeutic strategy in clinical practice.

Key messages:

1. Biologic treatments targeting IL-12/23 blockade are effective in controlling the inflammation associated with several autoimmune diseases
2. Congenital IL-12 immunodeficiency has been described as associated with severe bacterial infections
3. Despite re-assuring toxicity profile of therapeutic IL-12/23 inhibition, clinicians should be aware of potential long-term infective complications associated with these new biologic agents

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