Interleukin 10 and arthritis

It is now almost 10 yr since interleukin 10 (IL-10) was first described as a murine Th2 cell product, which inhibited cytokine synthesis [especially interferon gamma (IFN-\(\gamma\))] by Th1 cells [1]. Studies since then have indicated that it also inhibits many aspects of immune function, and that its synthesis is not restricted to Th2 cells as it is also produced by CD4\(^+\) Th0 and Th1 cells, CD8\(^+\) T cells, B cells, keratinocytes, various tumour cell lines and in particular monocytes/macrophages (reviewed) [2]. Based on its potent immunoregulatory activities, attention has not unsurprisingly focused upon the potential therapeutic use of this cytokine to treat chronic inflammatory diseases such as rheumatoid arthritis (RA). In this short editorial, I will discuss those studies which have investigated IL-10 in arthritis, preliminary results from clinical trials using IL-10, and recent studies investigating whether IL-10 polymorphisms are associated with arthritic disease.

IL-10 functions as an effective immunoregulatory molecule based on two important functions: inhibition of cytokine synthesis and downregulation of antigen-presenting cell function [3–5]. This latter activity is largely due to the ability of IL-10 to downregulate both constitutive and induced expression of HLA-DR, HLA-DP and HLA-DQ [6], and co-stimulatory molecules such as B7-1 and 2 [5, 7–9]. It is generally thought that the inhibitory effect of IL-10 upon T cells is indirect via its ability to inhibit cytokine synthesis from monocytes [7]. The range of macrophage-derived cytokines which IL-10 inhibits is truly impressive and includes IL-1, IL-6, IL-12, IFN-\(\gamma\), tumour necrosis factor alpha (TNF-\(\alpha\)), granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF), but also chemokines such as IL-8 and macrophage inflammatory protein (MIP)-1\(\alpha\) [4, 10] at both protein and mRNA levels. The mechanism by which IL-10 inhibits cytokine synthesis is somewhat contradictory. Some studies have indicated that IL-10 inhibits TNF synthesis through blockade of gene transcription [11], mediated possibly via effects on NF-\(\kappa\)B [12]. Other reports suggest the effect of IL-10 to be mainly post-transcriptional [13]. It is likely, however, that all of these observations are correct, and that IL-10’s inhibitory effects are at multiple levels. This is reflected in the observation that IL-10 will inhibit TNF production in either resting or pre-activated cells [14]. IL-10 can also induce the production of cytokine inhibitors. These include the IL-1 receptor antagonist (IL-1ra) [15] and the release of both soluble TNF receptors: p55 and p75 in monocytes and in RA mononuclear membrane culture [16]. These results collectively indicate that IL-10 exerts strong anti-inflammatory activities and thus has been termed a ‘macrophage deactivating factor’ [17].

Investigations into the role of IL-10 in arthritis started in the early 1990s. The presence of IL-10 was found in RA peripheral blood [18] synovial joints by reverse transcriptase-polymerase chain reaction (RT-PCR), immunostaining, and by assay of 24 h culture supernatants of dissociated joint cell cultures [19, 20]. It was also found that the endogenous IL-10 in these RA synovial cell cultures was functional, since inhibition of its activity using a neutralizing monoclonal antibody enhanced TNF-\(\alpha\) and IL-1 production, and that addition of recombinant IL-10 inhibited TNF-\(\alpha\) and IL-1 production by ~ 50% in cultures [19]. In a similar study, but using synovial tissue organ cultures [15], it was observed that exogenous IL-10 also inhibited IL-1\(\beta\), although IL-4 was more potent, and additionally that IL-4 (but not IL-10) induced the production of the native inhibitor of IL-1, IL-1ra. In addition, IL-10 seems to ameliorate the degradation of cartilage and bone by inhibiting the synthesis of matrix metalloproteinases (MMPs) such as collagenase in stimulated human monocytes, and by increasing the synthesis of the natural MMP inhibitor TIMP-1 (tissue inhibitor of metalloproteinase) [21].

These observations on human RA tissue, and the finding that collagen-induced arthritic (CIA) mice receiving neutralizing anti-IL-10 antibodies developed accelerated more severe disease [22], prompted therapeutic studies in animals with established disease. Studies in our group demonstrated that recombinant murine IL-10 was therapeutically active in collagen type II-induced arthritis in mice when delivered by a single daily i.p. injection and significantly inhibited clinical disease progression at a dose of 5 \(\mu\)g/day [23]. Additional studies have also demonstrated the therapeutic potential of IL-10 \textit{in vivo}. A pre-clinical 48 day course of IL-10 treatment at a dose of 100 ng/day was found to suppress significantly the clinical severity of collagen-induced arthritis in DBA/1 mice [24]. Importantly, no augmentation of the anti-collagen antibody response was observed in treated mice. In another study, treatment of established collagen-induced arthritis with recombinant IL-10 mildly suppressed clinical disease; however, the clinical benefit was markedly augmented by combination treatment with IL-4 [25]. This combination of IL-10 and IL-4 was also effective in streptococcal cell wall arthritis [26], and in both models accelerated onset of disease was observed following treatment with neutralizing anti-IL-10 antibodies, suggesting a dominant role for IL-10 in the natural suppression of arthritis in this model system. More recently, suppression of arthritis in CIA has been achieved using adenoviral vectors to deliver viral IL-10 to the mice [27, 28]. Viral IL-10 has the advantage (and possibly disadvantage) of not binding to T lymphocytes (presumably...
due to receptor expression) and thus will not exhibit the full spectrum of biological functions as human/mouse IL-10.

Since IL-10 is effective in animal models of arthritis, there clearly has been interest to establish whether IL-10 is also effective in human RA. The safety issues regarding IL-10 have been examined, and IL-10 administered to healthy volunteers in a single i.v. bolus injection demonstrated no clinical adverse event [29]. In vitro studies on isolated peripheral blood mononuclear cells from those IL-10-treated volunteers showed a transient reduction in phytohaemagglutinin-stimulated T-cell proliferation and a marked inhibition of endotoxin-induced IL-1β and TNF-α production, whereas their respective antagonists remained unaffected.

In light of this background, a recent trial of recombinant human IL-10 in subjects with active RA was performed [30]. The primary objective of this trial was to assess the safety and tolerability of rIL-10 and, secondarily, to evaluate its effect on disease activity and on cytokine physiology. In this multicentre, randomized, double-blind, placebo-controlled, multiple-dose study, after a 4 week wash-out period of disease-modifying anti-rheumatic drugs (DMARDs), 72 subjects with active RA received rIL-10 at doses of 0.5, 1.0, 5, 10, 20 µg/kg or placebo by daily s.c. injections for 4 weeks. rIL-10 was clinically well tolerated and no anti-IL-10 antibodies were detected in any subjects at any time during the study. During treatment, there was a reduction in mean platelets in subjects treated with the highest rIL-10 doses, although overall rIL-10 was well tolerated and safe. Despite the beneficial effects of IL-10 in murine CIA, the clinical effects of IL-10 in this trial of human RA were marginal with a beneficial trend towards improvement in RA disease activity at 4 weeks (>20% ACR criteria) only observed in the 5 µg/kg rIL-10 group. However, subjects receiving doses of rIL-10 required less use of DMARDs during the 8 weeks following completion of therapy. Circulating levels of soluble TNF-α (p55 and p75) as well as IL-1ra showed a significant increase (P < 0.001 for the former and P < 0.01 for the latter) at the highest rIL-10 doses, indicating that the IL-10 was having a biological role in vivo.

Two important issues have arisen from this trial. The first is that despite good indications from animal studies, pre-clinical trials may not always translate well to the clinic. Secondly, whilst IL-10 has many immunoregulatory functions, it also has activities which could be considered as immunostimulatory. For example, circulating B cells require IL-10 to reach terminal maturation in order to produce IgM-rheumatoid factor (IgM-RF), and in RA serum, IL-10 levels correlate with IgM-RF titres [20, 31], indicating that IL-10 may stimulate humoral immune responses, worsening disease activity correlated with high RF production. Interestingly, whilst IL-10 treatment in DA rats with established disease reduced paw swelling, higher levels of autoantibody production to collagen type II were observed. However, the thrombocytopenia observed in the clinical trial at the higher dose of IL-10 was totally unpredicted. Trials are currently under way focusing on IL-10 dosage within the area of efficacy, and plans are being made for combination trials with methotrexate. Of interest in this regard are recent studies from our group investigating the role of IL-11 in RA [32]. IL-11 is a member of the IL-6 supergene family which was reported to inhibit lipopolysaccharide (LPS)-stimulated TNF production in mouse macrophages [33]. We found abundant IL-11 in RA synovial joint cell cultures, and blocking its activity resulted in a modest, but significant, 2-fold increase in TNF production. What was unexpected, however, was the effect of neutralizing both endogenous IL-10 and IL-11; this resulted in a 22-fold increase in TNF levels. This suggests that the regulation of pro-inflammatory cytokines is highly complex, and that interactions and synergies between different molecules are a key component in this process. Combining IL-10 and IL-11 in a clinical trial is not necessarily the answer, but undoubtedly future therapeutics will wish to capitalize on the maximum benefits a drug gives, with the minimum side-effects achieved using sub-optimal amounts of two different therapeutics.

In common with other cytokines which have been associated with arthritic disease, the IL-10 gene has been subject to polymorphic analysis. The IL-10 gene is highly polymorphic with two microsatellites (dinucleotide repeats) at 1.4 kb (IL-10.G) and 4.1 kb (IL-10.R). In addition, there are three single-nucleotide repeats at −1082, −819 and −592. Differences in IL-10 secretion have been assigned to the −1082 polymorphism [34] and a more recent study [35] indicated that IL-10 secretion from LPS-stimulated whole blood cultures varied according to the IL-10 locus, as assigned by allelic haplotypes at the 4.1 kb microsatellite. The obvious question thus emerges: are certain IL-10 polymorphisms associated with disease? Two separate reports indicated that there was no difference in allele or haplotype frequency between controls and RA patients with respect to the −1082, −819 and −592 polymorphisms [36, 37], whereas a recent report comparing the microsatellite alleles in RA and controls indicated that the IL-10.R2 microsatellite allele was overrepresented in RA. Interestingly, this allele is associated with increased IL-10 synthesis, suggesting that rather than IL-10 being protective, it may have pro-inflammatory properties such as RF induction titres [20, 31]. However, as genetic studies are complex, and published reports do not always agree, this observation [38] needs to be confirmed before firm conclusions can be made.

In conclusion, IL-10 is a very potent immunoregulatory cytokine, which, despite encouraging results in therapeutic trials in animal models, still needs to ‘deliver’ in the clinic. The lack of good ‘clear-cut’ results in clinical trials is not restricted to RA, as phase I trials in Crohn’s disease have had limited success [39]. It remains to be seen whether in the future IL-10, either alone or
in combination with other DMARDs, will be an efficacious therapy.

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References


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The interactions between endocrinology and immunology have recently been further highlighted by the publication of a special edition of the journal *Lupus* devoted to prolactin in systemic lupus erythematosus (SLE) [1]. This issue, edited by Sara Walker and Luis Jara, two major students of this subject, brings together a number of papers linking hyperprolactinaemia with alterations in the immune response and with clinical fluctuations in human and murine lupus.

Such is the strength of the association between hyperprolactinaemia and lupus activity noted in some studies, that therapeutic trials of bromocriptine in lupus are already appearing.

To date, the majority of reports of hyperprolactinaemia in lupus are anecdotal. Nevertheless, their number is increasing and at the recent 5th International Conference on SLE, no less than 17 studies relating to the association were presented [2].

Prolactin, a functional cytokine, is secreted by a number of extra-pituitary sites, including the brain and lymphocytes. It influences the proliferation and differentiation of a variety of immune cells, and it is thought to play a role in maintaining immune competence, especially in T-cell responses. Receptors for prolactin are found on T and B cells, amongst others. Thymic epithelial cells have a particularly high expression of prolactin receptors.

Interferon regulatory factor-1 (IRF), an important regulator of T- and B-cell differentiation, has been shown to be sensitive to stimulation by prolactin.

Prolactin is also a stimulator of macrophages, increasing (in mice) production of macrophage interleukin-1 and nitric oxide, and stimulating phagocytic activity. Conversely, hyperprolactinaemia has been shown to be associated with macrophage depletion.

A number of patients with autoimmune diseases have been reported with hyperprolactinaemia and sera from some women with hyperprolactinaemia have demonstrated a number of circulating autoantibodies, including antibodies to DNA, anticyclic lipoprotein antibodies and antiendothelial antibodies.

A number of studies have now reported the presence of hyperprolactinaemia in lupus and the summary of these reports is analysed in the editorial by Walker and colleagues introducing the *Lupus* supplement.

These observations raise interesting questions regarding the relative roles of the hormones prolactin and oestrogen in SLE. Certainly, in animal experiments, notably in the New Zealand lupus mouse model, the production of hyperprolactinaemia has been shown to accelerate the production of antibodies to DNA, to result in hypergammaglobulinaemia, and to produce earlier renal disease and premature mortality. Conversely, the giving of daily injections of bromocriptine to produce hypoprolactinaemia delayed the appearance of anti-DNA antibodies and prolonged survival.

The nine papers in the current issue of *Lupus* record a variety of observations, including association of circulating antibodies (Krause et al.), studies of the hypothalamic–pituitary response in SLE (Rovensky et al.) and confirmation of the stimulation by prolactin of the autoimmune disease activity in the female New Zealand mouse lupus model (Elbourne et al.). This latter study suggested that a normal level of serum prolactin is necessary for oestrogen to act as a stimulant in the murine model of SLE.

Clinical studies demonstrated hyperprolactinaemia in 28% of SLE patients (Jimenez et al.) and a possible association with renal disease (Miranda et al.). In a trial of bromocriptine (2.5 mg daily) in 66 lupus patients from Jara’s group in Mexico, significant improvement in the lupus activity index was found in the treated group compared with controls (Alvarez-Nemegyei et al.).
It is well known that hyperprolactinaemia is a common finding, associated with stress, pregnancy, breast feeding, renal failure and a variety of medications. Those taking part in these studies were well aware of these caveats. Although the association of hyperprolactinaemia with altered immune responses and lupus activity is a relatively novel finding, it does, if substantiated, have therapeutic implications.

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References