Interleukin 10 treatment for rheumatoid arthritis

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Interleukin (IL) 10 looms as a highly promising treatment for rheumatoid arthritis (RA) because of its capacity to inhibit cellular immunity and deactivate macrophages. In RA, activated CD4 T helper cells and macrophages are believed to be the primary driving force behind joint inflammation. Synovial macrophages play a critical part in stimulating synovial inflammation. They produce abundant quantities of pro-inflammatory cytokines such as IL1 and tumour necrosis factor (TNF) α. The body attempts to keep the inflammatory response in check by upregulating the synthesis of endogenous inhibitors such as IL1 receptor antagonist (ra), soluble TNF receptors (TNFR), and IL10. These inhibitors act in concert to dampen the inflammatory response. IL10 is relatively unique in its ability to downregulate the production of multiple pro-inflammatory cytokines, leading to the notion that IL10 would be an effective treatment for RA.

Biology of IL10

Monocytes and macrophages produce IL10 when they are activated with the bacterial endotoxin lipopolysaccharide (LPS). There is an initial burst of pro-inflammatory cytokines (TNFα, IL1, IL6, and GM-CSF) followed later by a rise in IL10 synthesis.[1] LPS stimulated IL10 production requires both the synthesis of TNFα and IL1 as well as cognate interactions between monocytes and T cells.[2 3] IL10 release from LPS stimulated monocytes may be increased by transforming growth factor (TGF) β, interferon (IFN) α, IFNβ,[4–6] histamine[7] and ligation of the Fcγ receptor I.[8] On the other hand, LPS stimulated IL10 production may be inhibited by IL4, IFNγ,[9 10] and ligation of CD23, the low affinity IgE receptor.[11] IL10 and IL12 appear to be coordinately regulated in many of these experimental systems such that IL12 upregulates the synthesis of IL10 that controls the extent of the response.[7 8]

T cells also produce IL10. This cytokine is a component of the overall T helper (Th) cell cytokine profile that has used to define specific Th cell subsets: Th0, Th1, and Th2. These subsets are readily distinguishable in mice in which Th1 cells secrete IL2, IFNγ, and TNFβ and Th2 cells synthesise IL4, IL5, IL6, IL10, and IL13.[12] Precursor Th0 cells produce IL2, IFNγ, IL4, and IL10.[12] In mice, the differentiation of the immune response depends on the relative amounts of IL12 and IL4. Monocyte derived IL12 polarises the immune system towards a Th1 cellular response, while IL4 preferentially activates Th2 cells to stimulate B cell mediated humoral immunity.[12] Human Th cells overlap in their patterns of cytokine production and are termed Th1-like and Th2-like. In humans, a Th cell phenotype in which high levels of IL10 are produced relative to IFNγ or IL2 appears to favour deactivation of an inflammatory response.[13]

IL10 exerts both stimulatory and inhibitory effects on a variety of cell types. The therapeutic potential of IL10 as an anti-inflammatory agent derives from its capacity to inhibit the monocyte/macrophage. IL10 has been shown in vitro to potently downregulate LPS induced production of TNFα, IL1, IL6, IL8, G-CSF, and GM-CSF.[1] It also inhibits LPS induced and hyaluronan induced synthesis of macrophage inflammatory protein (MIP) 1α and MIP1β, two members of the C-C family of chemokines.[14 15] IL10 also suppresses macrophage synthesis of reactive oxygen intermediates[16] and nitric oxide[17] and blocks cyclooxygenase-2 dependent synthesis of interstitial collagenase and gelatinase B.[18] Moreover, in monocyte cultures, IL10 has been shown to diminish cell surface expression of p75 TNF receptors and promote release of soluble p75 TNF receptors[19] and soluble IL1ra.[20] Taken together, IL10 produces diverse anti-inflammatory effects.

T cell function is also regulated by IL10. The net effects of IL10 are to weaken a Th1 cellular response and strengthen a Th2 humoral response. IL10 inhibits Th1 cell production of IFNγ. This inhibitory effect is indirect and probably results from several mechanisms. Firstly, IL10 reduces the expression of HLA-DR on the surface of antigen presenting cells (APCs), interfering with antigen mediated T cell activation.[1 21] IL10 also downregulates the expression of ICAM-1, CD80, and CD86 on the surface of APCs, which decreases co-stimulatory activity.[22] Finally, IL10 abrogates IL12 driven Th1 cellular responses by decreasing the transcription of the p40 subunit of the IL12 receptor.[23]

On the other hand, IL10 increases Th2 cell mediated humoral immunity by stimulating the growth and differentiation of B cells.[24–26] B cells activated in culture by IL10 and anti-CD40 monoclonal antibody proliferate, differentiate into antibody secreting cells, and switch to IgA, IgG1, and IgE isotypes.[27] IL10 also augments IgG4 production.[27] The life of a B cell can be extended by IL10 through induction of bcl-2 protein.[28]

IL10 mechanisms in animal models

Investigations in animal models have provided key insights into the biology of IL10. As IL10 inhibits Th1 cellular immunity, its presence in excessive amounts could theoretically pose an increased risk for infection. In mice, IL10 treatment inhibits the delayed type hypersensitivity reaction to *Leishmania major* antigen[29]

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and exacerbates *Candida albicans*[30] and *Listeria monocytogenes*[31] infections. IL10 functions have also been examined in transgenic mice. Transgenic T cells that overproduce IL10 can lose their ability to mediate a Th1 dependent pathological response.[29] Mice bearing transgenic T cells with a high output of IL10 still mount a competent Th1 response to *L major* infection,[32] but show impaired control of mycobacterial infection.[33] Transgenic mice whose APCs are engineered to overproduce IL10 also show certain defects in cellular and humoral immunity and greater susceptibility to infection with *L monocytogenes* and *L major*.[34] Overall, these data suggest that IL10 plays an important part in host defence against intracellular pathogens.

The mechanisms of IL10 have been studied in several different animal models of autoimmunity, including experimental allergic encephalomyelitis (EAE) in rodents, non-obese diabetic (NOD) mice, murine type II collagen induced arthritis (CIA), and IL10 transgenic mice. IL10 can both inhibit and promote autoimmune disease in these models. In EAE, IL10 seems to exert a predominately inhibitory effect on disease. Clinical recovery from EAE is accompanied by increased CNS synthesis of IL10.[35] Treatment with IL10 abrogates the subsequent development of EAE during the initiation phase of disease, but has no therapeutic benefit on established EAE.[36] The functions of IL10 are more obscure in the NOD mouse. Whereas systemic IL10 administration in the NOD mouse prevents the development of diabetes,[37] transgenic mice whose pancreatic cells are engineered to overproduce IL10 are characterised by accelerated disease.[38 39] These contrasting effects illustrate the potential importance of environment (for example, systemic versus tissue) on the clinical outcome of cytokine therapy.

IL10 is a mediator of murine CIA. The development of murine CIA is associated with increasing IL10 expression by synovial cells and chondrocytes.[40] IL10 treatment suppresses the clinical manifestations of both early and established CIA and reduces the histological signs of joint inflammation, synovial tissue expression of TNFα and IL1 mRNA, and the destruction of articular cartilage and bone.[41 42] Treatment of murine CIA with high doses of IL12 augments IL10 synthesis and decreases joint inflammation,[43] exemplifying the coordinated regulation of these two cytokines. These animal studies have provided further basis for the clinical development of IL10 as a possible treatment for RA.

IL10 in RA

The cellular sources of IL10 in the rheumatoid synovium are macrophages and, to a lesser extent, T cells.[44] IL10 properties have been extensively investigated in ex vivo cultures of synovial tissue containing a mixture of synovial cells (fibroblast-like and macrophage-like) and lymphocytes. These cultures have been shown to produce IL10 as well as the pro-inflammatory cytokines TNFα, IL1, IL6, IL8, and GM-CSF.[44 45] Most of the IL10 in these cultures derives from the macrophage-like cells. The pro-inflammatory cytokines themselves appear to trigger the synthesis of IL10 as evidenced by experiments in which adding TNFα or IL1 has been shown to augment IL10 production.[44] IL10 acts to curb the inflammatory response. Neutralisation of endogenous IL10 with anti-IL10 antibodies has been shown to increase the production of TNFα and IL1.[44] IL10 also inhibits the production of IL8, G-CSF, and GM-CSF in culture and IFNγ induced expression of HLA-DR, ICAM-1, and VCAM-1.[46] These results provide evidence for a tightly regulated cytokine network in which IL10 serves to inhibit synovial inflammation.

IL10 is also the product of synovial T cells. Most cloned T cells isolated from the rheumatoid joint display a T helper (Th) 1-like phenotype, and may produce IL10.[47] In fact, when stimulated with mitogens or growth factors, the majority of synovial T cell clones have been shown to produce both IL10 and IFNγ.[48] This pattern of cytokine production is compatible with the view that RA is a Th1 mediated pathological response.

IL10 may be immunostimulatory in the synovial microenvironment by promoting antibody production. In synoviocyte cultures, IL10 has been shown to upregulate the production of IgM rheumatoid factor (RF),[49] which may explain why IgM RF secreting B cells accumulate in the rheumatoid synovium.

Synovial fluid from patients with RA contains detectable levels of IL10 mRNA and protein.[50 51] The predominant source of IL10 in the synovial fluid is the mononuclear cell (MNC).[51 52] The addition of IL10 to synovial fluid cultures suppresses MNC production of TNFα, IL1, and GM-CSF, reduces MNC surface expression of HLA-DR, increases MNC surface expression of CD16 and CD64, and decreases spontaneous MNC proliferation.[51 52] IL10 also stimulates MNC expression of the surface p75 TNF receptor and the release of its soluble counterpart into the culture supernatant.[53]

IL10 may be an important enhancer of cartilage growth. Conditioned media from antigen stimulated synovial fluid MNCs have been shown to inhibit proteoglycan synthesis by cultured cartilage explants, an effect largely dependent on TNFα and IL1.[52] This inhibitory effect on proteoglycan synthesis is reversed by IL10.[52]

The median serum level of IL10 is increased in patients with RA compared with that of healthy controls.[50] Blood MNCs from patients with RA spontaneously produce IL10 in culture[54] and represent the main source of circulatory IL10. Serum IL10 levels do not correlate with clinical measures of disease activity, but they are positively correlated with serum RF titres.[50] In culture, IL10 inhibits blood MNC production of TNFα, IL1, and IL6[45] as well as stimulates MNC release of soluble p75 TNF receptor.[53]

Clinical applications of IL10 in RA

The first clinical studies of IL10 were undertaken to examine the safety and immunomodu-

latory properties of this cytokine in 17 healthy persons. Single intravenous infusions of 1, 10 and 25 μg/kg of IL10 were observed to produce transient neutrophilia, monocytosis, and lymphopenia.[55] IL10 infusions also inhibited mitogen induced proliferation of peripheral blood MNCs and, in whole blood cultures, decreased LPS stimulated production of TNFα and IL1.[55] These single doses of IL10 did not cause significant toxicity.

A phase I dose escalating, double blind, placebo controlled phase I trial has been performed in 72 patients with active RA.[56] In this study, eligible subjects were treated with 0.5, 1.0, 5, 10, or 20 μg/kg of recombinant human IL10 or placebo, which was administered daily by subcutaneous injection. The only significant toxicity observed was thrombocytopenia in subjects receiving the highest IL10 doses. Four subjects in the 20 μg/kg dose group developed an asymptomatic, transient drop in platelet count below 100 000/mm^3. The platelet counts rapidly normalised in each case after stopping the IL10 injections. In addition, IL10 treatment was associated with an increase in serum levels of soluble p55 and p75 TNFRs and IL1ra. Although the small sample sizes precluded any conclusions about clinical efficacy, a trend towards clinical improvement was noted in the 5 μg/kg dose group. Further trials are in progress to evaluate the clinical efficacy and safety of IL10 treatment for RA.

Concluding remarks

Advances in our understanding of the pathogenesis of RA have illuminated new therapeutic strategies that target cytokines involved in the inflammatory response. IL10 is a central participant in this inflammatory process. It functions primarily as an inhibitory cytokine to suppress Th1 mediated cellular responses and deactivate macrophages. Importantly, IL10 acts to diminish the production of pro-inflammatory cytokines such as TNFα and IL1, which are important mediators of joint inflammation in RA. The results from animal studies provide further support to the concept that IL10 administration could ameliorate the signs and symptoms of arthritis. Recombinant IL10 treatment is now being investigated in patients with RA. Its ultimate niche in the therapeutic armamentarium for RA remains to be seen, but cytokine modulation beyond the simple antagonism of TNFα and IL1 may be necessary to most effectively treat this diseased population.

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