New strategies to control inflammatory synovitis: interleukin 15 and beyond

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STRATEGIES TO TARGET SYNOVITIS
A broad range of strategies to target inflammatory synovitis is currently being explored. Most approaches require the identification of a single molecule/pathway that is tractable to modulation in the clinic. In general, molecular activities with relevant biological effects are sought within the appropriate target lesion—namely, the synovium. Thereafter, such an activity may be targeted in rodent models of arthritis and the effects on inflammation and articular destruction measured. A moiety with plausible biology and bioactivity in model systems may then move to phase I “proof of concept” studies and thereafter to clinical development (fig 1). The successful targeting of tumour necrosis factor α (TNFα) represents an example of such an approach whereby an effective therapeutic agent has been derived.1,2 However, even after TNF blockade, unmet clinical need clearly persists in inflammatory arthritis. Major challenges remain, in particular the identification of therapeutic responders a priori, and the rational choice of additional targets, either as independent primary therapeutic targets or as synergistic biological targets for future combination applications. This short review will test interleukin 15 (IL15) in this model of new biological development to evaluate its therapeutic potential. Thereafter, an alternative approach will be considered for comparative purposes, whereby a model in which multiple pathways within the synovium are targeted simultaneously using a strategy borrowed from the host-parasite relationship arising from evolutionary pressures over millennia (fig 1).

INTERLEUKIN 15 AS A THERAPEUTIC TARGET
Our group has recently sought mechanisms whereby innate and acquired immune responses interact during chronic inflammation, with particular emphasis on cytokine biology. Many cytokines present within the synovium derive from macrophages and synovial fibroblasts.3 IL15, a cytokine with structural similarities to IL2,4 is produced primarily by macrophages and as such attracted our attention at an early stage as a potential regulator of synovial inflammation. It is now clear that IL15 exhibits plausible biological effectors function, is detectable in inflamed synovial tissues and, finally, when suppressed in rodent models of articular inflammation offers therapeutic utility.

Plausible biology
IL15 mRNA is broadly expressed throughout numerous normal human tissues and cell types, including activated monocytes, dendritic cells, and fibroblasts.5,6 IL15 mRNA expression, however, is not synonymous with protein detection in tissues, reflecting tight regulatory control of translation and secretion. IL15 is subject to significant post-transcriptional regulation via 5′-UTR AUG triplets,3 regulatory elements, and a further putative C-terminus region regulatory site. Two isoforms of IL15 subject to altered glycosylation are thus generated—secreted IL15 (48-aa) from a long signalling peptide and an intracellular IL15 form localised to non-endoplasmic regions in both cytoplasmic and nuclear compartments from a short signalling peptide (21-aa).7 Cell membrane expression may be crucial in mediating extracellular function rather than secretion and, in part, explains the difficulty in detecting soluble IL15 in biological systems.

Factors that drive endogenous IL15 release are as yet poorly understood. Exogenous agents include human herpesvirus 6 and 7, Mycobacterium leprae, Mycobacterium tuberculosis, Staphylococcus aureus, lipopolysaccharide, and ultraviolet irradiation. IL15 binds a widely distributed heterotrimeric receptor (IL15R) which consists of a β-chain (shared with IL2) and common γ-chain, together with a unique α-chain (IL15α), that in turn exists in eight isoforms.5 Subsequent signalling is through at least JAK1/3 and STAT3/5. Additional signalling through src related tyrosine kinases and Ras/Raf/MEK to fos/jun activation is also proposed. High affinity (10^5 M^-1) with slow off-rate make IL15RA in soluble form a useful and specific inhibitor in biological systems. IL15 exhibits broad proinflammatory effects that are summarised in fig 2. The target described are of relevance to current models of synovitis pathogenesis. Of particular importance may be its ability to initiate and sustain T cell chemokinesis, functional polarisation, cognate interactions with macrophages and memory compartment maintenance, NK cell activation, and neutrophil effector function. Details of these and broader effects have recently been reviewed.8

Plausible expression profile
IL15 mRNA (taqman PCR, nested reverse transcriptase-polymerase chain reaction (RT-PCR)) and protein (immunohistochemistry, enzyme linked imunosorbent assay (ELISA), receptor capture assay) are detected in rheumatoid arthritis (RA) synovial membrane at higher levels than in reactive arthritis or psoriatic synovial biopsy specimens.3 Low levels of IL15 are also present in sera of up to 40% of patients with RA, although variable levels have been reported in distinct populations.1 Serum IL15 expression does not correlate with disease subsets thus far recognised. Whereas RA serum TNFα levels correlate with the presence of germinal centres in parallel synovial biopsies, IL15 levels were raised, with either germinal centres or diffuse lymphocytic infiltrative patterns.9 IL15 expression has also recently been detected in synovial membrane derived from patients with juvenile rheumatoid arthritis,6 associated with IL18, IL12, and interferon γ (IFNγ) expression. Spontaneous production of IL15 by primary RA synovial membrane cultures and by isolated synovial fibroblasts is reported.10 In long term cultures of synovial tissues, outgrowth was found to be dependent upon the presence of T

Abbreviations: ACR, American College of Rheumatology; CIA, collagen induced arthritis; IFN, interferon; IL, interleukin; IL15Ra, interleukin 15 receptor antagonist; RA, rheumatoid arthritis; TNFα, tumour necrosis factor α
cells, which in turn lead to local release of IL15, IL17, and fibroblast growth factor-1. Factors that in turn drive synovial IL15 expression are unclear. Activated T cells can induce IL15 expression in macrophages by cognate interactions. Exposure of synovial fibroblasts to TNFα or IL1β also induces high levels of IL15 expression, although rarely in secreted form. In dermal fibroblasts TNFα but not IFNγ induces membrane expression of IL15, which in turn can sustain T cell growth. A further pathway promoting IL15 production has been suggested in studies of synovial embryonic growth factor expression through the wingless 5 and frizzled 5 ligand pair. Thus IL15 is detected in synovial tissues, as are factors that can initiate and sustain its expression.

Effector function of IL15 in synovium is largely predicted from its basic biology. Its effects in synovial tissues have been recently reviewed. In particular, IL15 sustains T cell/macrophage interactions to promote activation and cytokine release by the latter. Similar interactions between T cells and fibroblast-like synoviocytes with endogenous positive feedback loops have also been demonstrated. Of interest, secreted IL15 was not detected, indicating that functionally active membrane IL15 may be involved. IL15 can also promote T cell migration and survival, NK cell activation, synovial neutrophil activation and survival. Complex networks involving secreted and cognate pathways are therefore predicted in which cytokine secretion and adhesion molecule expression interact to sustain chronic leucocyte activation. IL15 apparently operates as a critical factor in these pathways, probably in synergy with other locally release cytokines including TNFα, IL18, IL12, and IL6.

Successful targeting in vivo in models
Three approaches have been considered to block IL15—namely, neutralising antibodies, soluble interleukin 1 receptor
IL15—proof of concept?

The foregoing data suggest that IL15 has many features of an attractive therapeutic target in inflammatory synovitis. A fully human monoclonal anti-IL15 monoclonal antibody has recently been generated that neutralises human IL15 (HuMax-IL15). Preliminary analysis of data arising from a phase I dose ascending safety and tolerability study performed in Europe suggests that IL15 can be neutralised safely with no immediate effects on leucocyte subsets, and with no significant short term clinical toxicity. Moreover, American College of Rheumatology (ACR) 20 (ACR20), ACR50, and ACR70 responses were of the same order as those achieved in studies using TNF blocking agents as monotherapy. These data must, however, be interpreted with caution because no placebo control was included throughout the experimental design; phase II data are awaited. Nevertheless, the study does provide early proof of concept that the above model can be useful in detecting therapeutic targets, with promise for subsequent intervention studies.

CONCLUSIONS

In this short summary I have illustrated two distinct strategies for targeting chronic inflammation in inflammatory arthritis. The genome and proteomic revolution are already yielding numerous additional “targets” for consideration in the preclinical development. “Biological” versus “small molecule” inhibitory approaches are currently debated in the main. The data shown above would argue for a broader discussion, in which consideration is given not only to new, single target identification but also to development of new strategies, using elucidation of broad mechanisms of immune subversion drawn from natural examples of successful immune modulation.

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