

## REPORT

# New strategies to control inflammatory synovitis: interleukin 15 and beyond

I B McInnes, J A Gracie, M Harnett, W Harnett, F Y Liew

*Ann Rheum Dis* 2003;**62**[Suppl II]:ii51–ii54

## 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  $\alpha$  (TNF $\alpha$ ) represents an example of such an approach whereby an effective therapeutic agent has been derived.<sup>1,2</sup> 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.<sup>3</sup> IL15, a cytokine with structural similarities to IL2,<sup>4</sup> 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 effector 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.<sup>4,5</sup> 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 local-

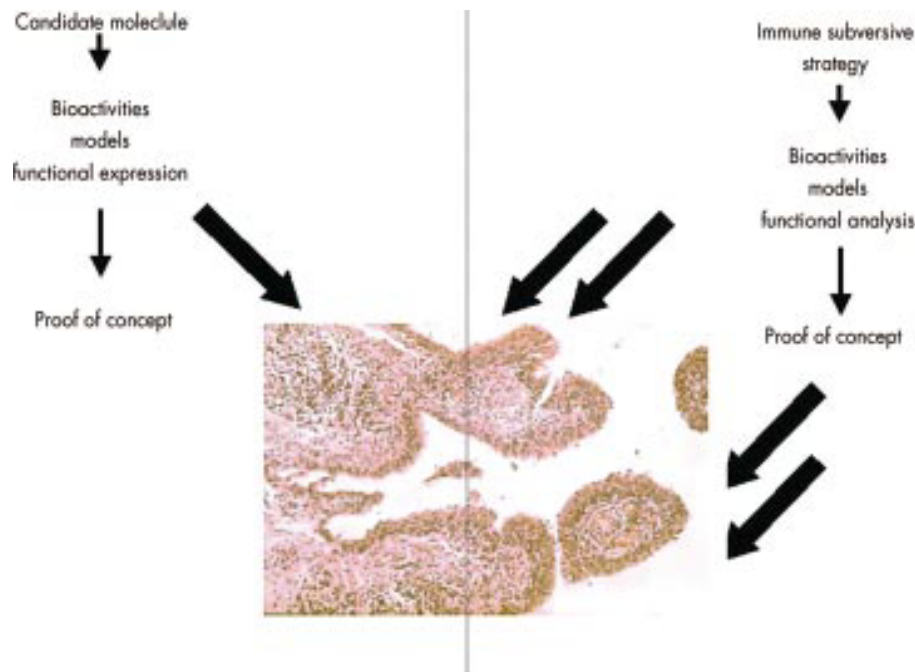
ised to non-endoplasmic regions in both cytoplasmic and nuclear compartments from a short signalling peptide (21-aa).<sup>5</sup> Cell membrane expression may be crucial in mediating extracellular function rather than secretion and, in part, explains the difficulty in detecting soluble IL15 in biological 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  $\beta$ -chain (shared with IL2) and common  $\gamma$ -chain, together with a unique  $\alpha$ -chain (IL15 $\alpha$ ), that in turn exists in eight isoforms.<sup>5</sup> Subsequent signalling is through at least JAK1/3 and STAT3/5. Additional signalling through src related tyrosine kinases and Ras/Raf/MAPK to fos/jun activation is also proposed. High affinity ( $10^{11}$  M<sup>-1</sup>) 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 cells 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.<sup>6</sup>

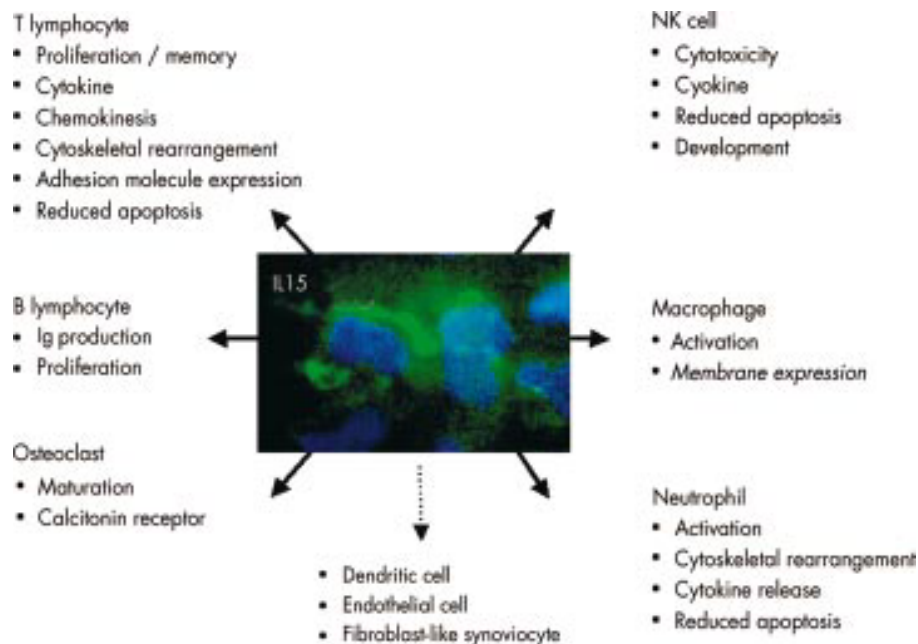
### Plausible expression profile

IL15 mRNA (Taqman PCR, nested reverse transcriptase-polymerase chain reaction (RT-PCR)) and protein (immunohistochemistry, enzyme linked immunosorbent 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.<sup>7</sup> 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.<sup>8</sup> Serum IL15 expression does not correlate with disease subsets thus far recognised. Whereas RA serum TNF $\alpha$  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.<sup>9</sup> IL15 expression has also recently been detected in synovial membrane derived from patients with juvenile rheumatoid arthritis,<sup>10</sup> associated with IL18, IL12, and interferon  $\gamma$  (IFN $\gamma$ ) expression. Spontaneous production of IL15 by primary RA synovial membrane cultures and by isolated synovial fibroblasts is reported.<sup>11</sup> 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 $\gamma$ , interferon  $\gamma$ ; IL, interleukin; IL15Ra, interleukin 15 receptor antagonist; RA, rheumatoid arthritis; TNF $\alpha$ , tumour necrosis factor  $\alpha$ .



**Figure 1** Alternative model approaches, resulting in "proof of concept" clinical studies to target synovitis.



**Figure 2** IL15 exhibits pleiotropic effects on numerous target cell types involved in current models of synovitis pathogenesis.

cells, which in turn lead to local release of IL15, IL17, and fibroblast growth factor-1.<sup>12</sup> 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 TNF $\alpha$  or IL1 $\beta$  also induces high levels of IL15 expression, although rarely in secreted form. In dermal fibroblasts TNF $\alpha$  but not IFN $\gamma$  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 wntless 5 and frizzled 5 ligand pair.<sup>13</sup> 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.<sup>8</sup> 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 TNF $\alpha$ , IL18, IL12, and IL6.<sup>14</sup>

#### Successful targeting *in vivo* in models

Three approaches have been considered to block IL15—namely, neutralising antibodies, soluble interleukin 1 receptor

antagonist (IL15Ra), and mutated IL15 species, usually generated as fusion proteins. Several studies using these diverse approaches have been attempted, or are in progress. Full length soluble IL15Ra administration can suppress development of collagen induced arthritis (CIA), associated with delayed development of anticollagen-specific antibodies (IgG2a) and with reduced antigen-specific cytokine release.<sup>15</sup> Studies are continuing to determine whether small molecule derivatives of soluble IL15Ra are of therapeutic use in the CIA model. An alternative approach has been to generate mutant IL15 forms that can specifically modify IL15 activities. An IL15/Fcγ2a fusion protein that antagonises the activities of IL15 in vitro, suppresses the onset of delayed-type hypersensitivity responses in vivo associated with reduction in CD4<sup>+</sup> T cell infiltration.<sup>16</sup> This fusion protein has also proved effective in vivo in preventing rejection of murine islet cell allografts in combination with CTLA4/Fc.<sup>17</sup>

### 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 20 (ACR20), ACR50, and ACR70 responses were of the same order as those achieved in studies using TNF blocking agents as monotherapy.<sup>18</sup> 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.

### TARGETING MULTIPLE PATHWAYS—LESSONS FROM HOST-PARASITE SYMBIOSIS

An alternative strategy for the detection of individual molecules as targets is reflected in recent observations, in which the autoimmune therapeutic potential of parasite derived moieties has been explored. Such an approach facilitates targeting multiple points in the inflammatory cascade but relies on host-parasite symbiosis to prevent excess host immune suppression. This idea will be briefly explored using as an example the filarial nematode *Acanthocheilonema viteae*.

Understanding modulation of the host immune system by pathogens offers rich therapeutic potential because evolutionary pressures operate over millennia to promote “safe” immune modulation. Parasitic filarial nematodes are often tolerated in human hosts for decades with little evidence of host disease, and this seems to reflect parasite induced suppression of host proinflammatory immune responses. Harnett *et al* previously described a filarial nematode derived, secreted phosphorylcholine-containing glycoprotein, ES-62, with immunomodulatory activities that are broadly anti-inflammatory in nature.<sup>19</sup> Filarial nematodes, arthropod transmitted parasites of vertebrates including humans, achieve long term infection through suppression or modulation of the host immune system. In infected humans, reduced production of IFNγ and increased production of IL4/IL10 together with raised IgG4 levels suggest a predominant Th2/anti-inflammatory phenotype conducive to both parasite survival and host health.<sup>20, 21</sup> Consistent with this, most humans who harbour viable filarial nematodes exhibit little parasite induced inflammatory response or local tissue destruction/disruption.

In recent studies we showed that a secreted product of *A viteae*, ES-62, significantly reduced initiation of inflammatory

arthritis in the CIA model in DBA/1 mice.<sup>22</sup> Crucially, ES-62 was also found to suppress CIA severity and progression when administration was delayed until after clinically evident disease onset. Ex vivo analyses showed that in both cases, the effects were associated with inhibition of collagen-specific proinflammatory/Th1 cytokine (TNFα, IL6, and IFNγ) release. ES-62 was found also to significantly suppress human macrophage activation through cognate interaction with activated T cells, and to suppress lipopolysaccharide induced rheumatoid arthritis synovial TNFα and IL6 production. Although of a preliminary nature, these data provide intriguing proof of concept that parasite derived molecules, or derivatives thereof might be used therapeutically borne out of the long necessity for “tolerable” immune subversion.

### 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.

### ACKNOWLEDGEMENTS

The support of the Nuffield Foundation (Oliver Bird Fund), the Wellcome Trust, and the Arthritis Research Campaign (UK) is acknowledged. Foo Y Liew has been an equal partner throughout these studies. Bernard P Leung, J. Alastair Gracie, Holger Ruchatz, Xiao Qing Wei, provided invaluable contributions (all University of Glasgow). William Harnett (University of Strathclyde) and Margaret Harnett (University of Glasgow) have been instrumental in the host-parasite studies.

### Authors' affiliations

I B McInnes, J A Gracie, M Harnett, W Harnett, F Y Liew, Centre for Rheumatic Diseases, Division of Immunology, Infection and Inflammation, University of Glasgow, UK

Correspondence to: Dr I B McInnes, Centre for Rheumatic Diseases, University Department of Medicine, Level 3, Queen Elizabeth Building, Glasgow Royal Infirmary, 10, Alexandra Parade, Glasgow G31 2ER, Scotland, UK; [i.b.mcinnis@clinmed.gla.ac.uk](mailto:i.b.mcinnis@clinmed.gla.ac.uk)

### REFERENCES

- 1 Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, *et al*. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. *N Engl J Med* 2000;343:1586.
- 2 Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, *et al*. Infliximab and methotrexate in the treatment of rheumatoid arthritis Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N Engl J Med* 2000;343:1594.
- 3 Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. *Cell* 1996;85:307.
- 4 Grabstein KH, Eisenman J, Shanebeck K, Rauch C, Srinivasan S, Fung V, *et al*. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. *Science* 1994;264:965.
- 5 Waldmann TA, Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annu Rev Immunol* 1999;17:19.
- 6 Fehniger TA, Caligiuri MA. Interleukin 15: biology and relevance to human disease. *Blood* 2001;97:14.
- 7 Kotake S, Schumacher HR Jr, Yarboro CH, Arayssi TK, Pando JA, Kanik KS, *et al*. In vivo gene expression of type 1 and type 2 cytokines in synovial tissues from patients in early stages of rheumatoid, reactive, and undifferentiated arthritis. *Proc Assoc Am Physicians* 1997;109:286.
- 8 Liew FY, McInnes IB. Role of interleukin 15 and interleukin 18 in inflammatory response. *Ann Rheum Dis* 2002;61 (suppl III):ii100.
- 9 Klimiuk PA, Sierakowski S, Latosiewicz R, Cylwik B, Skowronski J, Chwiecko J. Serum cytokines in different histological variants of rheumatoid arthritis. *J Rheumatol* 2001;28:1211.
- 10 Scola MP, S Thompson D, Brunner HI, Tsoras MK, Witte D, Van D, *et al*. Interferon-gamma:interleukin 4 ratios and associated type 1 cytokine expression in juvenile rheumatoid arthritis synovial tissue. *J Rheumatol* 2002;29:369.

- 11 **Harada S**, Yamamura M, Okamoto H, Morita Y, Kawashima M, Aita T, *et al*. Production of interleukin-7 and interleukin-15 by fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Arthritis Rheum* 1999;42:1508.
- 12 **Wakisaka S**, Suzuki N, Nagafuchi H, Takeba Y, Kaneko A, Asai T, *et al*. Characterization of tissue outgrowth developed in vitro in patients with rheumatoid arthritis: involvement of T cells in the development of tissue outgrowth. *Int Arch Allergy Immunol* 2000;121:68.
- 13 **Sen M**, Chamorro M, Reifert J, M Corr, Carson DA. Blockade of Wnt-5A/frizzled 5 signaling inhibits rheumatoid synoviocyte activation. *Arthritis Rheum* 2001;44:772.
- 14 **Brennan FM**, A Hayes L, CJ Ciesielski, Green P, Foxwell BM, Feldmann M. Evidence that rheumatoid arthritis synovial T cells are similar to cytokine-activated T cells: involvement of phosphatidylinositol 3-kinase and nuclear factor kappaB pathways in tumor necrosis factor alpha production in rheumatoid arthritis. *Arthritis Rheum* 2002;46:31.
- 15 **Ruchatz H**, Leung BP, X Wei Q, I McInnes B, Liew FY. Soluble IL-15 receptor alpha-chain administration prevents murine collagen-induced arthritis: a role for IL-15 in development of antigen-induced immunopathology. *J Immunol* 1998;160:5654.
- 16 **Kim YS**, W Maslinski, XX Zheng, AC Stevens, XC Li, GH Tesch, *et al*. Targeting the IL-15 receptor with an antagonist IL-15 mutant/Fc gamma2a protein blocks delayed-type hypersensitivity. *J Immunol* 1998;160:5742.
- 17 **Ferrari-Lacraz S**, Zheng XX, Kim YS, Li Y, Maslinski W, Li XC, *et al*. An antagonist IL-15/Fc protein prevents costimulation blockade-resistant rejection. *J Immunol* 2001;167:3478.
- 18 **GenMab**. 2002. [http://www.genmab.com/html/2002\\_09\\_04.shtml](http://www.genmab.com/html/2002_09_04.shtml)
- 19 **Harnett W**, Deehan MR, Houston KM, Harnett MM. Immunomodulatory properties of a phosphorylcholine-containing secreted filarial glycoprotein. *Parasite Immunol* 1999;21:601.
- 20 **Lawrence RA**, Devaney E. Lymphatic filariasis: parallels between the immunology of infection in humans and mice. *Parasite Immunol* 2001 23:353.
- 21 **Hoerauf A**, and N Brattig 2002 Resistance and susceptibility in human onchocerciasis—beyond Th1 vs Th2 *Trends Parasitol* 18:25
- 22 **McInnes IB**, Leung BP, Harnett MM, J Gracie A, F Liew Y, W Harnett. 2003 A novel therapeutic approach targeting articular inflammation using the filarial nematode derived phosphorylcholine-containing glycoprotein, ES-62. *J Immunol* (in press).