

REPORT

Role of interleukin 15 and interleukin 18 in inflammatory response

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Interleukin 15 (IL15) and interleukin 18 (IL18) are cytokines produced principally by macrophages during innate immune response and subsequently profoundly influence adaptive immunity. Recent studies have shown that IL15 and IL18 play an influential part in inflammatory response. Here we present recent data mainly from our own laboratories illustrating the importance of IL15 and IL18 in the induction and perpetuation of chronic inflammation during experimental and clinical rheumatoid synovitis.

The interactive role of innate and adaptive immunity has attracted considerable current interest. A large number of mediators have been implicated, particularly those derived from the innate response that can drive the adaptive immunity. In this short review, we will summarise the work carried out in our laboratory on the roles of IL15 and IL18 in adaptive immune response and in perpetuating chronic organ specific inflammatory reactions. We will focus primarily on rheumatoid arthritis (RA).

The processes that initiate and perpetuate RA are currently unclear. Successful clinical targeting of tumour necrosis factor α (TNF α) therefore represents an exciting and important advance, not only in therapeutics, but also in the understanding of disease pathogenesis. However, non-responder or partial responder patients are not infrequent and inflammatory disease usually flares up on discontinuation of treatment.^{1,2} This carries significant pathogenetic implications for existing disease models. Moreover, it exemplifies the clinical necessity for generation of further novel, pathogenesis led interventions. One approach to detecting novel synovial inflammatory pathways is to establish events that regulate synovial TNF α production. Recently, our group has explored the expression of novel cytokine activities within RA synovial membrane that could perpetuate inflammatory synovitis, in particular through modulation of T lymphocyte function and TNF α expression by IL15 and IL18.

IL15 IS PROINFLAMMATORY IN RA

IL15 is a 14 kDa–15 kDa cytokine expressed at the mRNA level in numerous normal human tissues in a broad range of cell types, including activated monocytes, dendritic cells, osteoclasts, and fibroblasts.^{3,4} The heterotrimeric IL15 receptor (IL15R) includes the IL2R β chain, and γ chain, together with a unique α chain (IL15 α), which is alternately spliced to yield three active forms, each capable of high affinity binding to IL15.^{4,5} Whereas IL2R α is primarily expressed on activated T cells, IL15R α mRNA has been identified in numerous human tissues and cells, including activated T cells. IL15R α deficient mice exhibit lymphopenia because of reduced proliferation and homing of mature lymphocytes, particularly of the CD8⁺ subset.⁶ Like IL2, the IL15R $\alpha\beta\gamma$ complex signals through JAK1/3 and STAT3/5.^{4,5}

Our initial studies focused upon IL15 mRNA and protein expression in RA synovial membrane.⁷ Lining layer macrophages represent the predominant cellular source in situ, although expression in fibroblast-like synoviocytes and in endothelial cells is also evident.^{7–9} IL15 can be detected by ELISA in around 60% of RA (but not osteoarthritis) synovial fluids (SF), at median concentrations similar to those of TNF α detected in parallel assays.^{10,11} In a series of studies, Dayer and colleagues have shown that after stimulation with non-physiological mitogens, paraformaldehyde fixed T cells and T cell clones induce proinflammatory cytokine production by macrophages and fibroblasts through cell contact.¹² We found that freshly isolated synovial T cells similarly induce TNF α synthesis by blood or synovial derived macrophages through cell membrane contact, with no requirement for secretory factor synthesis.¹³ This activity is maintained in vitro by addition of IL15. These data showed for the first time that a fundamental property of synovial T cells is the capacity to drive TNF α production through cell contact, and that IL15 represents one factor capable of sustaining this activity.

IL15 confers similar properties upon CD45RO⁺ PB T cells, such that IL15 activated peripheral blood cells from RA patients induce TNF α synthesis in synovial macrophage/synoviocyte co-cultures. Our neutralisation studies implicated at least CD69, LFA-1 and ICAM-1 in these interactions,¹³ although other ligand pairs are probably involved including at least CD11b and CD40L/40.^{14,15}

Considerable information about the implications of cytokine expression in synovitis has been obtained using inflammatory arthritis models. Administration of recombinant IL15 during priming with type II collagen (CII) in incomplete Freund's adjuvant induces development of an erosive inflammatory arthritis in DBA/1 mice, which closely resembles that obtained in positive control mice receiving collagen in complete Freund's adjuvant. Soluble murine IL15 receptor α (smIL15R α) administration provides a mechanism to manipulate IL15 bioactivities in vivo. smIL15R α administration after antigen challenge suppresses development of collagen induced arthritis in DBA/1 mice, associated with delayed development of anti-collagen specific antibodies (IgG2a) and with reduced collagen specific interferon γ (IFN γ) and TNF α production in vitro.¹⁶ Finally, in preliminary studies, we have shown that shIL15R α suppresses the development of collagen induced arthritis (CIA) in a primate model (unpublished). Feedback loops such as these could therefore perpetuate synovial inflammation through T cell/macrophage interactions. Together these data clearly show that IL15/IL15R interactions are important in the development of arthritogenic immune responses in vivo.

Abbreviations: IFN γ , interferon γ ; IL15, interleukin 15; IL18; interleukin 18; RA, rheumatoid arthritis; TNF α , tumour necrosis factor α ; SF, synovial fluid; CIA, collagen induced arthritis

It has been shown that IL15 is essential for the proliferation and maintenance of CD8⁺ memory cells but has little or no effect on naive CD8⁺ cells or CD4⁺ T cells.¹⁷⁻¹⁹ However, we have recently shown that IL15, at high concentrations, induced proliferation of both naive and memory CD4⁺ and CD8⁺ cells.²⁰ IL15 also increased the differentiation of Type 1 (IFN γ producing) and Type 2 (IL5 producing) CD4⁺ and CD8⁺ T cells under IL12 and IL4 driving conditions, respectively. However, IL15 alone was not efficient in stimulating cytokine production of these cells in the absence of T cell subset driving cytokines (IL12 or IL4) and/or simultaneous TCR activation. Together, these results show that IL15, at high dose, is a pan-T cell growth factor. The apparent requirement of IL15 for the maintenance of memory CD8⁺ cell in vivo may reflect the exceptionally restricted nature of this subpopulation of cells for IL15. The inability of IL15 alone to stimulate cytokine synthesis also suggests that IL15 on its own does not drive antigen specific T cells to exhaustion. The levels of these cells are maintained by IL15 and they are only mobilised to carry out effector functions when subsequently confronted with specific antigens.

IL18 AND RA PATHOGENESIS

IL18 is an 18 kDa glycoprotein derived by enzymatic cleavage of a 23 kDa precursor, pro-IL18, by at least caspase 1.²¹ Pro-IL18 expression is widespread, including monocyte/macrophages, dendritic cells, Kupffer cells, keratinocytes, articular chondrocytes, synovial fibroblasts and osteoblasts, and within adrenal cortex and pituitary gland.²² IL18 mediates bioactivities through a heterodimeric receptor consisting of α and β chains that are widely expressed on naive T lymphocyte subsets, NK cells, macrophages, neutrophils, and chondrocytes. IL18R α , characterised earlier as IL1R related protein (IL1Rrp) binds IL18 at relatively low affinity (in the range of 10⁻⁸ M). Generation of IL18R α deficient mice confirmed that this receptor is nevertheless essential for signalling.²³ IL18R β chain, initially termed IL1 receptor accessory protein-like (AcPL) is related and similar to IL1RacP in that it does not bind ligand directly, but rather binds to the complex formed by IL18/IL18R α chain²⁴ generating the likely high affinity complex. Thus far the signal transduction pathway known for IL18R is identical to that of IL1R. We recently detected IL18 in the synovial compartment of patients with RA.²⁵ Whereas IL18 mRNA was found in both RA and osteoarthritis synovial membranes, IL18 protein was reproducibly detected by histological examination and ELISA only in RA derived tissues. IL18 expression was localised in RA synovial membranes in cells of dendritic morphology within lymphocytic aggregates and in lining layer areas. Subsequent double staining confirmed expression in both CD68⁺ macrophages and fibroblast-like synoviocytes.

Addition of IL18 consistently induced TNF α , GM-CSF, and IFN γ production by RA synovial membrane or SF mononuclear cultures in vitro. IL18 induced cytokine production was significantly increased by coincident addition of IL12 and/or IL15, and suppressed by IL10 and TGF β . That IL18 was acting not only through lymphocyte activation, but also through direct effects on macrophages was confirmed using intracellular cytokine staining.²⁵ These data show that a primary function of IL18 could be directly promoting TNF α production through binding to macrophage IL18R. Importantly, dose response studies show that only very low concentrations (down to 1 pg/ml) of IL15, IL18, and IL12 respectively are required to induce TNF α production in vitro. In contrast with IL15, we found that IL18 activation of synovial T cells does not change subsequent T cell/macrophage interactions directly. However, addition of recombinant IL18 to cytokine activated, formalin fixed T cell/monocyte co-cultures synergistically upregulates TNF α production mediated through direct effects of IL18 upon the target monocyte population (unpub-

lished observations). Thus IL18 synergistically and potently increases the proinflammatory potential of T cell/monocyte interactions that are in turn induced by IL15.

Factors regulating IL18 in synovial membrane are as yet unclear. IL18 expression in monocytes is complicated by constitutive expression of mRNA. IL18 mRNA and protein expression are however upregulated in vitro in fibroblast-like synoviocytes by IL1 β and TNF α , suggesting the existence of positive feedback loops linking the well recognised monokine predominance with cytokine production and Th/c 1 cell activation in synovial immune responses. Finally, IL18 also induced nitric oxide (NO) release by RA synovial membranes in vitro. As NO inhibits caspase 1 activity, this provides a potential feedback loop, whereby IL18 may regulate its own cleavage.²²⁻²⁶

To directly investigate the effect of endogenous IL18 expression, we generated IL18 deficient mice on a DBA/1 background. These mice develop significantly delayed onset and milder severity of CIA, which is characterised by reduced TNF α concentrations in serum and in spleen cultures in vitro, and by suppressed CII specific Th1 responses in vitro.²⁷ Compatible with this, antibody mediated IL18 neutralisation suppresses streptococcal cell wall induced arthritis through an IFN γ independent mechanism²⁸ and IL18BP-Fc fusion retards established CIA comparable with etanercept.²⁹ Finally, we have shown that anti-IL18 antibody suppresses development of carrageenan induced paw inflammation by directly suppressing TNF α expression,³⁰ suggesting that IL18 can operate upstream of TNF α production in vivo. Together, these data strongly suggest that the net effect of IL18 expression is proinflammatory, at least in the context of antigen driven articular inflammation.

Recently, we have found a naturally spliced variant of human IL18R α , generating a soluble form of IL18R α , which is secreted from monocytic cells and neutralises IL18 activity (Wei *et al*, unpublished). There are at least five more such variants of different sizes induced in human blood mononuclear cells and murine spleen cells under different conditions. Thus, there could be a family of spliced variants of naturally produced soluble IL18R α , possibly playing distinct functions in the regulation of IL18 activity for the maintenance of immunological homeostasis. Such molecules may be exploited for therapeutic intervention in inflammatory diseases associated with the over expression of IL18.

CONCLUSION

Evidence from in vitro studies in RA derived tissues and in vivo using the collagen induced arthritis model show that whereas a clear relation with TNF α regulation has been demonstrated, evidence has also been provided for proinflammatory activity independent of TNF α . Moreover, our studies show the potential for striking cytokine synergy in promoting synovial inflammation. Our choice of IL18, an IL1-like cytokine (NF- κ B dependent) and IL15, an IL2-like cytokine (STAT3/STAT5 dependent) may partially explain this synergism although this remains to be explored. Increased expression of IL15 and IL18 extends beyond RA to include several human inflammatory diseases. Thus, the biological activities elucidated here for IL15 and IL18 will probably be of general importance. The ultimate significance of IL15 and IL18 expression in autoimmune disease in vivo however requires confirmation in human intervention in development to perform such studies.

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