**EXTENDED REPORT**

Interleukin 7 stimulates tumour necrosis factor α and Th1 cytokine production in joints of patients with rheumatoid arthritis

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**Background:** A large number of activated T cells are found in the joints of patients with rheumatoid arthritis (RA). Interleukin 7 (IL7), a T cell growth factor and a regulator of Th1 and Th2 cytokine production, is produced by synoviocytes from patients with RA.

**Objective:** To investigate the effect on proinflammatory cytokine production of synovial fluid mononuclear cells (SFMC) and the mechanism by which IL7 influences CD4+ T cell activity in patients with RA.

**Methods:** In a cross sectional group of patients with RA, IL7 levels were compared with those of healthy controls and related to disease activity. The effect of IL7 on cytokine production was tested by RA SFMC and on SF CD4+ T cells in the presence of mononuclear cells (MC). Production of tumour necrosis factor α (TNFα), IL1β, interferon γ (IFNγ), and IL4 was measured by enzyme linked immunosorbent assay (ELISA) and by single cell FACS analysis. Expression of the IL7 receptor α chain on CD4+ T cells (essential for IL7 signalling) was assessed. Direct effects of IL7 on isolated synovial fluid (SF) CD4+ T cells were studied by cytokine analysis. By neutralisation of IL12 in MC cultures, indirect effects of IL7 on T cells through accessory cells were studied.

**Results:** IL7 serum levels were higher in patients with RA than in healthy controls and related positively with C reactive protein levels. IL7 stimulated TNFα production by SFMC and very potently stimulated IFNγ and TNFα production by SF CD4+ T cells. These effects were probably mediated through the IL7 receptor α chain, which was abundantly expressed on SF CD4+ T cells. Besides the direct stimulation of T cell cytokine production by IL7, its action was partly dependent on IL12, indicating that IL7 also stimulates accessory cell function, leading to T cell activation.

**Conclusion:** IL7 stimulates proinflammatory cytokine production of intra-articular CD4+ T cells and accessory cells from patients with RA. The correlation with measures of disease activity indicates that IL7 might substantially contribute to the perpetuation of Th1 and TNFα mediated proinflammatory responses in patients with RA.

In humans, IL7, produced by stromal cells at lymphopoietic sites (bone marrow, thymus, spleen, and gut), is a factor known for its role in T and B lymphocyte development. IL7 is a critical growth factor in early human T cell development and has been shown to prime human naive CD4+ T cells, in the absence of accessory cells, for proinflammatory (IFNγ, IL2) and anti-inflammatory cytokines (IL4). IL7 can also stimulate proliferation and, under certain conditions, cytokine secretion of memory T cells. These data suggest that IL7, present in the joints of patients with RA, may be an important immunoregulatory cytokine.

The purpose of this study was to investigate whether IL7 may play a part in proinflammatory or anti-inflammatory intra-articular T (helper) cell regulation. For this reason, IL7 levels in patients with RA were compared with those of healthy controls and related to C reactive protein (CRP) levels, as a marker of disease. Furthermore, synovial fluid mononuclear cell (MC) and T cell activation by IL7 was studied. Because of the linkage of major histocompatibility complex class II molecules (HLA-DR1

**Abbreviations:** CRP, C reactive protein; DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme linked immunosorbent assay; IFNγ, interferon γ; IL, interleukin; MC, mononuclear cells; RA, rheumatoid arthritis; SF, synovial fluid; SFMC, synovial fluid mononuclear cells; TNFα, tumour necrosis factor α
and DR4) with RA, \(^{22}\) molecules which participate in the stimulation of CD4+ T cells, in particular the role of IL7 in CD4+ T cell activation, were studied.

PATIENTS AND METHODS

Patients
For IL7 assessment, blood samples from 34 consecutive patients with RA visiting our outpatient clinic were taken. Patients fulfilled the 1987 revised American College of Rheumatology criteria\(^{22}\) and ranged in age from 30 to 77 years, with a mean (SD) age of 50 (13) years. Duration of the patients’ disease ranged from 1 to 20 years, with a mean disease duration of 5 (4) years. Twenty three patients were rheumatoid factor positive. Eleven patients received methotrexate, seven took sulfasalazine, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving azathioprine. In addition, eleven patients received methotrexate, seven took sulfasalazine, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate, three were receiving hydroxychloroquine, three were receiving methotrexate.

Cytokine analysis

For TNF\(\alpha\), IFN\(\gamma\), and IL4 analysis, media were harvested and freed of cellular material by centrifugation (five minutes, 900 \(g\)), frozen in liquid nitrogen, and stored at \(-20^\circ\text{C}\). TNF\(\alpha\), IFN\(\gamma\), and IL4 protein in the culture supernatant were determined by ELISA (see below).

Figure 1  
(A) Serum IL7 levels of patients with RA (n=34) compared with an age and sex matched healthy control group (n=32, HC). Mean (SEM) values are given. Patients with RA showed a significantly higher serum IL7 level than HC (p<0.05). (B) The serum IL7 levels in patients with RA (n=32) correlated with CRP.
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Serum levels of IL7 are increased in patients with RA compared with healthy controls

Serum levels of IL7 in patients with RA (n=34) were on average almost fivefold higher than in an age and sex matched healthy control group (n=32, p<0.05, fig 1A). IL7 serum levels did not differ between rheumatoid factor positive or negative patients.

CRP levels, available in 23 patients with RA, correlated positively with serum IL7 levels ($r_s=0.406$, p<0.05, fig 1B).

IL7 stimulates TNFα production by SFMC and TNFα and IFNγ production by SF T cells

Because IL7 is produced by fibroblasts from RA joints, the effect of IL7 on synovial fluid MC and T cell activation was investigated. IL7, at concentrations of 10 ng/ml, significantly stimulated TNFα production by SFMC (fig 2, on average 157%). IL1β production was undetectable and was not stimulated by IL7. IFNγ production by SFMC from only one donor was stimulated by IL7 (from <10 to 138 pg/ml), whereas IL4 in each case remained undetectable. To analyse if T cells were modulated in their responsiveness, culture of SFMC for five days with IL7 was followed by T cell stimulation for 24 hours. Upon this T cell stimulation, increased TNFα and IFNγ production was observed.

Figure 2  IL7 stimulates TNFα production by RA SFMC. SFMC ($1\times10^6$/ml) were incubated without or with IL7 [10 ng/ml] for five days. Mean (SEM) values are given (n=4). Asterisks indicate significant difference compared with control culture (*p<0.05).

Figure 3  Effect of IL7 on cytokine production by RA synovial T cells. (A) SFMC ($1\times10^6$/ml) were incubated with or without IL7 [10 ng/ml] for five days, followed by T cell-specific stimulation with CD3/CD28 for 24 hours. Secretion of TNFα, IFNγ, and IL4 during this 24 hour period was assessed by ELISA. Mean (SEM) values are given (n=8). Asterisks indicate significant difference compared with control culture (*p<0.05, **p<0.01). (B) A dose range of IL7 is shown. Mean (SD) values of two separate experiments are given. Note the difference in scale of the y axis for IFNγ and TNFα compared with IL4.
production by IL7 were seen (fig 3A). IL4 production was not significantly influenced by IL7 pretreatment. Similar effects were seen with peripheral blood mononuclear cells from patients with RA (data not shown). The effect on TNF\(\alpha\) and IFN\(\gamma\) production was already evident at very low levels of IL7 and was dose dependent (fig 3B).

Previously we have shown that cytokine secretion induced through CD3/CD28 stimulation of MC is more than 90% T cell-specific.\(^{24}\) Furthermore, analysis of individual T cells showed that the increase in TNF\(\alpha\) and IFN\(\gamma\) production was associated with an increase in the number of TNF\(\alpha\) and IFN\(\gamma\) expressing CD4\(^+\) T cells (fig 4A, representative donor; fig 4B, means). Less than 5% of CD3\(^-\) cells produced IFN\(\gamma\) (on average 4.9%) or TNF\(\alpha\) (on average 2.4%). No significant change in the IL4-producing CD4\(^+\) T cells was found. To a smaller extent the increase in TNF\(\alpha\), IFN\(\gamma\), and IL4 production was due to IL7 stimulated cytokine secretion by CD3\(^+\)CD4\(^-\) T cells (on average from 3.9 to 10.6%, 4.5 to 10.3%, and 0.43 to 0.93% of total T cells, respectively).

**Synovial fluid CD4\(^+\) T cells express the IL7\(\alpha\) receptor and respond directly to IL7**

Down regulation of the IL7\(\alpha\) receptor (CD127) has been shown upon maturation of naive neonatal CD4\(^+\) T cells to cytokine secreting memory cells.\(^{18}\) Because most SF T cells express the memory phenotype (CD45RO\(^+\)) we analysed CD127 expression levels on these cells. CD127 was abundantly expressed on synovial CD4\(^+\) T cells from patients with RA (fig 5A). The
expression was comparable with peripheral blood memory CD4+ T cells and higher than that of naive peripheral blood T cells (data not shown). The functionality of the IL7α receptor was shown by incubating isolated CD4+ T cells with IL7. IL7 not only significantly increased TNFα and IFNγ but also IL4 production, although IL4 levels were much lower (fig 5B).

**IL7 also stimulates T cell responses through accessory cells**

In addition to the direct effect of IL7 on T cells, other pathways of proinflammatory cytokine induction might exist as well.

IL7 has been shown to activate cytokine secretion by monocytes which express CD127 (this study, data not shown). To analyse the involvement of IL12, which is mainly produced by synovial macrophages and a potent stimulator of Th1 cell activity, anti-IL12 neutralising antibodies (5 µg/ml) were added to SFMC during stimulation with IL7. This resulted in an inhibition of IL7-stimulated IFNγ production by more than 50% (p<0.01), in unchanged TNFα production, and in enhanced IL4 production (97%, p<0.05, fig 6). In an identical control experiment (RA SFMC, n=3) it was shown that these effects were specific, because IL7-induced IFNγ

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**Figure 5**  
(A) Expression of the IL7 receptor α chain (CD127) on isolated synovial CD4+ T cells (black histogram) compared with control staining (open histogram). (B) Isolated CD4+ T cells (5×10^5/ml) were stimulated with CD3/CD28 in the absence or presence of IL7 (10 ng/ml) for 10 days. T cells were subsequently stimulated for 24 hours with CD3/CD28 alone, and cytokine secretion was assessed. Mean (SEM) values of three patients are given. Asterisks indicate significant difference compared with control culture (p<0.05).

**Figure 6**  
Effect of IL12 neutralising antibodies (aIL12) on IL7-stimulated cytokine production by RA synovial T cells. SFMC (1×10^6/ml) were incubated without (open bars) or with IL7 (10 ng/ml, black bars) and IL12 neutralising antibodies (5 µg/ml, grey bars) for five days, followed by a T cell-specific stimulation with CD3/CD28 for 24 hours. Secretion of TNFα, IFNγ, and IL4 was assessed by ELISA. Mean (SEM) values of eight patients are given. Asterisks indicate significant differences compared with control cultures (p<0.05). † Indicates significant differences between IL7 and IL7/anti-IL12 cultures (p<0.01).
production (from 8.7 (4.4) to 19.0 (2.5) ng/ml) was inhibited by anti-IL12 (to 12.0 (4.7) ng/ml), but not by irrelevant isotype control antibodies (5 µg/ml, to 22.7 (0.6) ng/ml). The isotype control antibodies also did not change IL7-induced TNFα production.

**DISCUSSION**

In humans, IL7 has been recognised as a factor regulating B and T cell development, myeloopoiesis, and activating monocytes/macrophages, natural killer cells, and (cytotoxic) T cells. Little is known about the role of IL7 in patients with RA. Nurse-like cell clones from the bone marrow of patients with RA have been shown to secrete IL7 and to promote survival and enhance the function of B cells. Our study shows that IL7 may have a proinflammatory role in patients with RA. IL7 was increased in serum from patients with RA compared with healthy controls and correlated with a marker of inflammation (CRP). The source of IL7 in RA serum is not known, but may include stromal cells in lymphoepithelial tissue and cells at the inflammatory sites. For the latter it has been shown that synovocytes of patients with RA can produce IL7. The relation between IL7 and markers of inflammation has also been found in patients with juvenile RA, in whom levels of IL7 in plasma were significantly higher than in healthy children and correlated with markers of inflammation (plasma IL6 levels, CRP) and disease (systemic features). In support of the role of IL7 in increasing the acute phase responses is the stimulatory effect of IL7 on TNFα production by RA SFMC (this study) and the previously described effects on IL6 and IL1β production by monocytes/macrophages. TNFα, IL6, and IL1 are all cytokines which can increase acute phase responses.

Because of the rather low production of TNFα, we could not identify the source of TNFα production by single cell analysis. This TNFα production may result from direct stimulation of T cells (this study) or direct stimulation of macrophages. Furthermore, because IL7 can enhance expression levels of costimulatory molecules (for example, CD69), it might mediate T cell dependent regulation of TNFα by macrophages, similar to IL11. Hence, IL7 might contribute to increased T cell and macrophage TNFα production in patients with RA, and promote joint inflammation and destruction.

Although IL7 has been shown to have a role in the IL4 production of naive CD4+ T cells, the present data indicate that IL7 has a minor effect on IL4 production of RA SF memory CD4+ T cells and merely stimulates proinflammatory T cell activity. In the presence of accessory cells IL7 stimulates Th1 cytokine secretion (IFNγ) without having a significant influence on Th2 cell (IL4) activity, which is in agreement with T cell cytokine profiles found in the RA joints.

The effect of IL7 on RA CD4+ SF T cells present in SFMC was, at least partly, directly on the T cells. SF CD4+ T cells expressed the IL7 receptor α chain and were, when isolated, strongly stimulated in their TNFα and IFNγ production by IL7. In addition to the direct effect of IL7 on CD4+ T cells, part of the effect of IL7 on Th1/Th2 cytokine production was mediated by the production of IL12 because blockade of IL12 resulted in a significant decrease in Th1 cell activity (IFNγ production) and increase in Th2 activity (IL4 production). This IL12 is probably produced by antigen presenting cells, in particular monocytes/macrophages which are major producers.

The present data also suggest that in patients with RA, IL7 may give rise to IL4 production, in the absence of stimulatory accessory cells, or when the activity of synovial accessory cells is modulated, for example by IL12 blockade.

T cell TNFα production (after pretreatment with IL7 and T cell stimulation) seems to be regulated differently from IFNγ production because, in contrast with IFNγ production, this TNFα is not blocked by anti-IL12. In agreement with this we found that IL10 similarly blocked IL7-induced IFNγ production, but not TNFα production (data not shown). Because IL10 inhibits many cytokines produced by macrophages, including IL12, this indicates that the dichotomy between TNFα and IFNγ may not be dependent on factors produced by accessory cells. T cell TNFα production may be more dependent on direct T cell stimulation by IL7, or IL7-induced T cell-accessory cell membrane interactions.

In addition to the effect of IL7 on CD4+ T cells, cytokine production by RA CD4+ T cells was stimulated by IL7 as well. This is in agreement with the stimulation of cytokoticy activity of CD8+ T cell clones by IL7 which has been shown previously, and it indicates that IL7 might stimulate a generalised arthriticogenic T cell response in RA.

As far as we know, this study is the first to show an association between IL7 and a marker of RA disease activity and demonstrates that IL7 stimulates SFMC and synovial (CD4+) T cells, producing proinflammatory responses associated with secretion of TNFα and IFNγ. This effect is achieved by direct binding to the IL7α receptor on T cells and indirectly through accessory synovial mononuclear cells. The immunostimulatory ability of IL7 and the presence of IL7 in RA joints indicate that IL7 can substantially contribute to the continuing inflammatory response in RA and that patients with RA might benefit from treatment strategies in which inhibition of IL7 is considered.

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