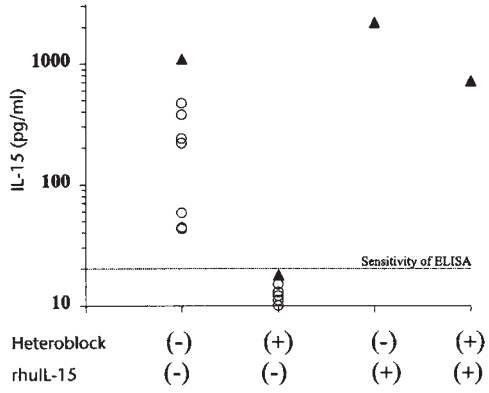
A role for IL-15 in rheumatoid arthritis?

To the editor—McInnes *et al.* have reported a potential role for interleukin-15 (IL-15) in the pathogenesis of rheumatoid arthritis (RA)[1,2]. The *in vitro* functional data in support of their hypothesis rest, in part, on the very high concentrations of IL-15 detected in synovial fluid of patients with RA. Fourteen of the seventeen RA patients reported with detectable IL-15 appear to have levels ranging between 10 and 1200 ng/ml. Several groups have documented IL-15 protein production by activated human monocytes using immunoblot analysis, immunohistochemical staining and ELISA, but the levels of soluble IL-15, when detected, have never been in this range.

We assayed synovial fluid from nineteen RA and seven osteoarthritis (OA) patients in a blinded manner using a sandwich ELISA for human IL-15 prepared in a similar fashion to that reported by McInnes *et al.* None of the OA patients had detectable IL-15. Eight of the nineteen RA patients had detectable IL-15 in synovial fluid, but in the range of 0.04–1 ng/ml. However, when additional aliquots from seven of these positive patient samples were first mixed with a high concentration of Heteroblock (1 mg/ml, Bozeman, MT), an active blocking reagent that eliminates interference caused by heterophilic antibodies in sandwich ELISAs, levels of IL-15 were all below 0.02 ng/ml (level of sensitivity).When an aliquot from one of these patient samples was spiked with recombinant human (rhu) IL-15, the ELISA detected the cytokine in the presence of Heteroblock (see Fig.).

These data indicate that the detection of relatively high levels of IL-15 soluble protein in the synovial fluid of RA patients by sandwich ELISA may reflect non-specific binding of rheumatoid factor to primary and secondary antibodies used as reagents in the ELISA. This is not without precedent[3]. If our findings are confirmed, this may call into question the role of IL-15 as a T cell stimulus and chemoattractant in RA given that the *in vitro* data in support of this required in excess of 10 ng/ml IL-15 for activation[1,2,4]. This does not necessarily exclude a role for IL-15 in RA, as localized production of lesser amounts by activated mononuclear cells and/or synovial cells could be important.



Effect of Heteroblock on the detection of IL-15 in RA synovial fluid by ELISA.

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McInnes et al. reply—Shah *et al.* suggest that measurement of interleukin-15 (IL-15) levels in synovial fluid may be complicated by the presence of rheumatoid factor (RF) (although we note that the level of IL-15 recovered from 'spiked' SF in their assay was reduced in the presence of Heteroblock). We also considered this possibility after our initial observations[1] and subsequently reported a revised concentration range for IL-15 in RA based on data obtained using a commercially available ELISA (R&D Systems)[2]. We have now detected IL-15 in 30 of 53 synovial fluid samples obtained from patients with both seropositive (RA) and seronegative inflammatory arthropathies, in the range 10–1128 pg/ml (median of positive samples, 198 pg/ml). In contrast to Shah *et al.*, we were unable to detect significant reductions in IL-15 measured when rheumatoid factor was removed from the synovial fluid using γ-globulin coated polystyrene beads (Rapitex RF, Behring). These data were confirmed in a novel IL-15 receptor-based capture assay. This employed soluble IL-15Rα generated in our laboratory, coated onto ELISA plates into which diluted SF and then biotinylated anti-IL-15 antibody were sequentially added, before colorimetric estimation. IL-15 concentrations in RA SF measured in this assay closely correlated with the figures determined by ELISA ($r = 0.937$, $P<0.001$), indicating that RF interference could not account for the IL-15 levels detected by ELISA.

Others have provided evidence for relevant IL-15 expression within RA synovial membrane, including the presence of high levels of IL-15 mRNA in treated as compared with non-treated RA patients[5] and immunohistochemical localization of IL-15 using specific monoclonal antibodies, the binding of which can be effectively neutralized by prior addition of recombinant IL-15 (refs. 1,6). Moreover, the presence of bioactive IL-15 is suggested by the ability of anti-IL-15 antibodies to neutralize chemotactic activity contained in RA synovial fluids[4].

That the absolute concentrations of IL-15 found in these studies may be lower than those used in our *in vitro* experiments does not, in our opinion, obviate the hypothesis that IL-15 can play an important role in RA pathogenesis. The local 'peri-cellular' and therefore relevant cytokine concentration may exceed that detected in adjacent inflammatory effusions[7]. Several authors have now documented T cell activation by a combination of cytokines including IL-15, TNFα and IL-6, indicating that such a pathway can operate in RA[2,8]. Moreover, we have recently observed that administration of soluble IL-15Rα abrogated collagen-induced arthritis in DBA/1 mice[9].

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