Cytokines and Focal Loss of Cartilage in Osteoarthritis

Sir—It is well established that IL-1 and TNF-α can degrade animal cartilage both in vitro and in vivo. However, it remains uncertain whether these cytokines contribute to the process which leads to focal loss of cartilage in osteoarthritis (OA). Credence is lent to this theory by the demonstration that the overall activity of cartilage-degrading enzymes, known to be produced by chondrocytes in response to IL-1 and TNF-α, is increased in OA [1]. In addition, cells from OA synovium contain a higher proportion of IL-1-secreting cells than normal synovium [2]. TNF-α is secreted by OA synovium [3] and IL-1, at least at concentrations in the 10 ng/ml range, can degrade human articular cartilage explants in vitro [4]. However, it is unclear whether the amounts of TNF-α produced by OA synovium are greater than those produced by normal synovium or if the concentrations of IL-1 attained in OA joints are likely to reach the levels which can stimulate chondrocytes. Similarly, it is uncertain whether TNF-α can degrade OA cartilage. To address these questions, we compared the concentrations of IL-1β and TNF-α in 24 h culture supernatants from OA and non-artritic (NA) synovial membranes, examined the ability of low concentrations of IL-1β to stimulate glycosaminoglycan (GAG) loss from OA cartilage explants, and also asked whether TNF-α can reproducibly stimulate such GAG loss.

OA and NA synovium was cultured for 24 h, the supernatants collected, and TNF-α and IL-1β concentrations measured by sandwich ELISAs as described previously [5]. To measure the effects of IL-1β and TNF-α on explants, cartilage was obtained from OA patients undergoing surgery for total knee replacement (n = 10, mean age 71 yr for the IL-1β study; n = 8, mean age 66 yr for the TNF study). Cartilage biopsies (6–10 replicates/patient) from the femoral condyles were cut in half and cultured for 14 days in either medium alone or in medium containing either IL-1 or TNF [4]. The culture supernatants were collected and GAG levels measured colorimetrically [6]. The results are expressed as μg GAG released (μg GAG retained/mg wet weight cartilage) in the case of IL-1, and as μg GAG released/mg dry weight cartilage in the case of TNF.

The concentrations of TNF-α and IL-1β found in OA and NA synovium are recorded in Fig. 1. TNF-α concentrations are found to be higher than IL-1β in OA synovium but similar in NA synovium.

![Figure 1](https://example.com/figure1.png)

**FIG. 1.**—Comparison of TNF-α and IL-1β concentrations in supernatants from OA and NA synovium cultured for 24 h.
concentrations were significantly higher ($P = 0.004$, $t$-test with Welch’s correction for non-equal variances on log-transformed values) in supernatants from cultured OA synovium ($0.71 \pm 1.05$ ng/ml, range 0.02–5.0, $n = 35$) than in NA synovium supernatants ($0.15 \pm 0.14$ ng/ml, range 0.04–0.54, $n = 13$). Similarly, IL-1β concentrations were significantly higher ($P < 0.0006$, $t$-test with Welch’s correction for non-equal variances on log-transformed values) in supernatants from cultured OA synovium ($0.25 \pm 0.24$ ng/ml, range 0.006–0.69, $n = 11$) than in NA synovium supernatants ($0.007 \pm 0.006$ ng/ml, range <0.003–0.017, $n = 6$). The concentrations of TNF-α and IL-1β in OA synovium supernatants, but not in NA synovium supernatants, were closely related (OA, $r = 0.92$, $P < 0.0001$, $n = 11$; NA, $r = 0.28$, $P = 0.60$, $n = 6$, Pearson’s product moment correlation).

The cumulative GAG released from groups of OA cartilage explants ($n = 10$) stimulated with IL-1β (0.01 ng/ml) was significantly higher ($P < 0.001$, paired $t$-test) than that of those cultured with medium alone (5.45 $\pm$ 2.23 compared to 3.22 $\pm$ 1.19 µg/mg). The cumulative GAG release from groups of OA cartilage explants ($n = 8$) stimulated with TNF-α (10 ng/ml) was also significantly higher ($P < 0.001$, paired $t$-test) than that of those cultured with medium alone (35.0 $\pm$ 15.4 compared to 22.7 $\pm$ 7.2 µg/mg). Note that the apparent difference in GAG released from the group of OA explants cultured with IL-1 as compared with TNF is due to the difference in the way the results are expressed.

These results support the hypothesis that the cartilage catabolic cytokines IL-1β and TNF-α contribute to cartilage loss in OA. Firstly, the results confirm that OA synovium secretes higher levels of IL-1β than NA synovium and demonstrate, for the first time, that OA synovium secretes higher levels of TNF-α than NA synovium. It is notable that those OA synovia secreting most TNF-α are also those which secrete most IL-1β, since these two cytokines could synergize in their effects on cartilage. It may be asked whether the IL-1 and TNF in the supernatants are biologically active. Work to be published elsewhere [7] shows that incubation of OA synovium supernatants with normal chondrocytes increases the number of TNF receptors they express. The effect is inhibited by antisera to IL-1β, but not normal sera, and the inhibitory effect is abrogated by addition of recombinant IL-1β. Secondly, the results demonstrate that a concentration of IL-1β likely to be attained in OA joints, as judged by comparison with the concentrations found in OA synovium supernatants, significantly stimulates GAG loss from OA cartilage explants. Finally, TNF-α reproducibly stimulated GAG release from OA cartilage explants, albeit at a concentration slightly above the range found in OA synovium supernatants. But how can cytokines produced by OA synovium cause focal loss of cartilage? Recently [8] we showed that cartilage explants from different regions of femoral condyles from both OA and NA joints vary in their susceptibility to TNF-α, and that chondrocytes from these regions also vary in the numbers of p55-TNF receptors they express. Strikingly, both for OA and NA cartilage, a direct relationship exists between p55-TNF chondrocyte receptor expression and the susceptibility of cartilage explants from the different regions to TNF-α. Thus, at times when the OA synovium produces high concentrations of cartilage catabolic cytokines, focal loss of cartilage will occur where the chondrocytes are susceptible to their effects. It remains to determine how catabolic cytokine receptor numbers on chondrocytes become upregulated during the development of OA.

Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD
Accepted 2 June 1997


Prolonged Healing of Malleolar Ulcers after Fibrinolytic Treatment in a Woman with Systemic Sclerosis

Sir—Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterized by endothelial cell injury and progressive fibrosis of skin and internal organs [1]. As a consequence of micro- and macrovascular damage, skin ulcerations and autoamputation of fingers and toes may ensue [2, 3]. We wish to report on a SSc patient whose skin ulcers, recalcitrant to conventional immunosuppressive agents, healed after protracted fibrinolytic treatment. A 28-yr-old Indian woman was admitted in January 1996 because of painful and progressive ulcerations of her medial malleoli. These had developed 4 months after limited cutaneous SSc had been diagnosed in January 1994. Her medication then consisted of penicillamine 250 mg daily. During her admission, a skin biopsy...