Increased proteolytic cleavage of $\alpha_1$-antitrypsin ($\alpha_1$-proteinase inhibitor) in knee-joint synovial fluid from patients with rheumatoid arthritis

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The neutral serine proteinase, elastase, is released in large amounts by neutrophils at sites of inflammation, where it can act on a broad range of substrates, including elastin, proteoglycans and structural collagen [1]. Thus elastase has been implicated in proteolytic tissue damage within the rheumatoid joint [2, 3].

$\alpha_1$-Antitrypsin (AAT), also known as $\alpha_1$-proteinase inhibitor, is the major inhibitor of elastase. However, in samples of knee-joint synovial fluid taken from patients with rheumatoid arthritis (RA), much of the AAT is inactive as an inhibitor [4, 5]. Wong & Travis [6] found that AAT isolated from a sample of stored RA synovial fluid was oxidized at a critical active-site methionine residue, suggesting that the synovial fluid AAT is inactivated by reactive oxygen species in a manner analogous to that which occurs in the bronchoalveolar lavage fluid of patients with pulmonary emphysema [7].

AAT (54 kDa) inhibits elastase by forming a stable 1:1 AAT-elastase complex (83 kDa). The complex slowly dissociates, leaving inactive 50 kDa and 4 kDa fragments of AAT. A newly described neutrophil metalloproteinase [8, 9] also cleaves AAT to give 50 kDa and 4 kDa fragments which have lost inhibitory capacity, although the presence of this metalloproteinase in RA synovial fluid has not been demonstrated. We have therefore screened synovial fluid samples for the presence of proteolytic fragments of AAT, with a view to assessing whether, in addition to oxidation, proteolysis might play a role in the functional deficiency of AAT in RA synovial fluid.

Synovial fluid was aspirated from 17 patients with RA and 10 patients with osteoarthritis (OA). Serum was obtained from 11 normal control subjects. Most samples were analysed within 24 h of collection. A few samples were stored for a maximum period of 3 days at either 4°C or -60°C. Over this time period there were no significant changes in the results obtained. The molecular form of AAT in samples was determined by SDS/PAGE followed by Western blotting and immunostaining. The details are described in the legend to Fig. 1. The blots were analysed using a digital imaging system (Seescan) to quantitate the intensities of bands corresponding to the various molecular forms of AAT.

Several molecular forms of AAT were visible on Western blots of human synovial fluid (Fig. 1). Both native (54 kDa) and proteolytically cleaved (50 kDa) forms of AAT were present in all the samples. A 4 kDa band was not detected, presumably due to a lack of immunochromenically reactivity. A weak band corresponding to the AAT-elastase complex (83 kDa) was sometimes detectable in the RA synovial fluid samples (five out of 17), in the OA synovial fluid (two out of 10) and in the normal serum samples (two out of 11). In agreement with these results, others [10] have shown by an enzyme-linked immunosorbent assay that only a very small proportion of the AAT in RA/OA synovial fluid exists as a complex with elastase.

Fig. 2 shows the results obtained by digital-imaging analysis of the blots, where for each sample the intensity of the 50 kDa band has been expressed as a fraction of the 54 kDa band intensity. In the RA synovial fluids this ratio was 0.30±0.16 (mean ± s.d.). In OA synovial fluids the 50 kDa band was significantly less intense, giving a ratio of 0.15±0.09 ($P<0.01$). Similarly, normal serum showed a significantly lower proportion of cleaved AAT (ratio 0.16±0.08; $P<0.01$). In the five RA synovial fluid samples in which an 83 kDa band could be detected, this band accounted for 2–25% of the total protein staining.

We have shown that RA synovial fluid contains, on average, about twice as much cleaved AAT as OA synovial fluid or normal serum. The proteinase(s) responsible for producing the 50 kDa fragment of AAT is unknown. The 50 kDa band might be generated by slow dissociation from the AAT–elastase complex, but this seems unlikely in view of the apparently low level of complex in the synovial fluids. It is possible that one or more of the three latent metalloproteinases known to be present in synovial fluid [11] are involved, since a neutrophil metalloproteinase capable of cleaving a 4 kDa fragment from AAT in vitro has recently

Abbreviations used: AAT, $\alpha_1$-antitrypsin ($\alpha_1$-proteinase inhibitor); RA, rheumatoid arthritis; OA, osteoarthritis.

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The aim of the present study was to measure the effects of growth factors on collagen biosynthesis and collagenase activity by dermal fibroblasts cultured in dermal equivalents.

A dermal equivalent is fabricated by the incorporation of a dermal fibroblast-extracellular matrix-growth factor interactions. Such dermal equivalents may remain attached to the culture well or may be detached after polymerization and allowed to float. Collagen production over 4 days was measured in attached dermal equivalents seeded with 5 x 10^4 cells in Costar 24-well culture plates by the incorporation of anhydride-labelled collagen and 1^4C proline into collagenase-sensitive, trichloroacetic acid-precipitable protein. Collagenase action over 15 days was measured in detached collagen gels containing [3H]acetic acid labelling and 1 x 10^4 fibroblasts by the measurement of total active and latent (i.e. non-cleaved) collagen. The addition of TGF-β over the range 0.5-10 ng/ml stimulated collagen production in the culture medium allowed to float. Collagen production over 4 days was measured in attached dermal equivalents seeded with 5 x 10^4 cells in Costar 24-well culture plates by the incorporation of [3H]proline into collagenase-sensitive, trichloroacetic acid-precipitable protein. Collagenase activity in the supernatant was measured in collagen gels containing [3H]acetic acid and [3H]proline by the measurement of total active and latent collagen. The addition of TGF-β over the range 0.5-10 ng/ml stimulated collagen production in the culture medium allowed to float. Collagen production over 4 days was measured in attached dermal equivalents seeded with 5 x 10^4 cells in Costar 24-well culture plates by the incorporation of [3H]proline into collagenase-sensitive, trichloroacetic acid-precipitable protein. Collagenase action over 15 days was measured in detached collagen gels containing [3H]acetic acid labelled collagen and 1 x 10^4 fibroblasts by measuring the release of soluble, radioactive peptides. The effects of transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF) were studied and serum was either eliminated from the culture medium or reduced to 2% at 24 h before the assay to minimize any effects of growth factors and mitogens present in serum.

Growth factors modulate collagen production and collagenase action by skin fibroblasts in a dermal equivalent model system

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A dermal equivalent is fabricated by the incorporation of fibroblasts into a hydrated collagen gel [1]. The collagen lattice is contracted and reorganized by the fibroblasts in a process similar to that occurring during wound contraction. Recent reports have suggested that some growth factors found at the wound site have a role in extracellular matrix reorganization (‘remodelling’) during wound repair [2] and that the dermal equivalent system may be used to study fibroblast-extracellular matrix-growth factor interactions.

The aim of the present study was to measure the effects of growth factors on collagen biosynthesis and collagenase activity by dermal fibroblasts cultured in dermal equivalents. Human dermal fibroblast monolayer cultures were established from foreskins obtained at circumcision. They were grown and maintained in Dulbecco's modified Eagle's medium and used at passages 4-12. Hydrated collagen gels seeded with fibroblasts were prepared from rat-tail collagen [3]. Such dermal equivalents may remain attached to the culture well or may be detached after polymerization and allowed to float. Collagen production over 4 days was measured in attached dermal equivalents seeded with 5 x 10^4 cells in Costar 24-well culture plates by the incorporation of [3H]proline into collagenase-sensitive, trichloroacetic acid-precipitable protein [4]. Collagenase activity in the supernatant was measured in collagen gels containing [3H]acetic acid labelling and 1 x 10^4 fibroblasts by measuring the release of soluble, radioactive peptides. The effects of transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF) were studied and serum was either eliminated from the culture medium or reduced to 2% at 24 h before the assay to minimize any effects of growth factors and mitogens present in serum.

The addition of TGF-β over the range 0.5-10 ng/ml stimulated collagen production in the culture medium between 3.8- and 6.1-fold (compared with controls) with the maximum increase being observed at 4 ng of TGF-β/ml but falling off at higher concentrations. A similar distribution was observed in the gel matrix (2.7-3.2-fold increase). The stimulatory effect of TGF-β on collagen production was shown not to be due to cell proliferation. The addition of TGF-β (0.1-10 ng/ml) inhibited collagenase activity after 24 h, but inhibition was overcome by day 10 (Fig. 1a). In contrast, PDGF stimulated collagenase activity by up to 5.8-fold by day 15 (Fig. 1b). The levels of total active and latent (i.e. trypsin-treated) collagenase were determined. In the presence of TGF-β, a decrease in the levels of the active form

[Fig. 2. Proportion of cleaved (50 kDa) AAT relative to native (54 kDa) AAT, in healthy controls and patients with either OA or RA]

Western blots were scanned using a digital imaging system and the intensity of the 50 kDa band was expressed as a fraction of the 54 kDa band intensity. The horizontal bar (±) indicates the mean value within each group. Data were parametrically distributed and statistical significance was tested by Student's t-test (see the text).

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