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Differential responses of human and rat cartilage to degrading stimuli in-vitro

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Abstract—Human cartilage biopsies incubated for 2 days in-vitro with 15% synovial fluid from rheumatoid arthritis patients contained less glycosaminoglycans (GAG) than control biopsies. Recombinant human (rHu-) interleukin-1α (IL-1α) and IL-1β at 10 or 100 ng mL^{-1} had no effect on human cartilage GAG levels. Similarly, GAG loss from human cartilage biopsies into medium over 5 days was significantly increased by synovial fluid but unaffected by 100 ng mL^{-1} IL-1α or IL-1β compared with controls. However, when rat femoral head cartilage samples were incubated with 100 ng mL^{-1} rHu-IL-1α or IL-1β for 5 days there was a significant increase in GAG loss from the cartilage into medium, whilst human synovial fluid significantly decreased the loss of GAG from rat cartilage into medium, compared with controls. The results demonstrate that human and rat cartilage differ from each other in their responses to degrading stimuli and suggest that animal cartilage may have limited application for the screening of drugs intended for the treatment of human arthritides.

Potential antirheumatic drugs are often screened for cartilage-protecting activity, or cartilage-damaging side-effects, using in-vitro cultures of cartilage of rat (Desa et al 1988; Clay et al 1989), bovine (Rainsford 1985, 1986), rabbit (Comper et al 1981), mouse (De Vries et al 1988) or dog (Palmoski & Brandt 1983, 1984; Muir et al 1988). However, recent evidence suggests that cartilage from various species differ from each other in their responses to degrading stimuli such as interleukin-1 (IL-1). For example, it has been reported that IL-1β degrades rat cartilage after 5 days of culture (Clay et al 1989) whilst IL-1α is without effect (Desa et al 1988). On the other hand, both IL-1α and IL-1β degrade bovine and porcine cartilage after 2 or 6 days of culture (Dingle et al 1979; Saklatvala et al 1983, 1985). In contrast, we have recently shown that human cartilage is not degraded in-vitro by IL-1α or IL-1β (0·1–100 ng mL^{-1}) after 2 or 6 days of culture, even though synovial fluid from arthritis patients did degrade the same cartilage (Hollander et al 1991).

In view of these differences, the use of animal tissue to screen for drugs which protect human cartilage must be considered questionable. However, it is not yet clear whether the differences are truly a reflection of species variation or merely due to the use of different methodology from one laboratory to another. To distinguish between these possibilities, we have examined the degradation of human cartilage by IL-1 using two different types of culture system, one measuring changes in cartilage glycosaminoglycans (GAG) concentration, the other measuring GAG loss from cartilage into medium. In addition, we have compared the degradation of rat and human cartilage in one of these systems.

Materials and methods

Collection of synovial fluid. Samples of knee synovial fluid were obtained from patients attending the Rheumatology Clinic at Bristol Royal Infirmary. All patients fulfilled the American Rheumatism Association criteria for classical or definite rheumatoid arthritis. The synovial fluid samples were collected in sodium citrate (3% w/v) and centrifuged at 800 *g* for 10 min to remove cells and debris. After treatment with hyaluronidase (30 units mL^{-1}, Sigma) for 30 min at 37°C, cells were stored at −70°C until use.

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Human cartilage samples. Slices of normal human articular cartilage were taken from the femoral heads of patients who were undergoing hemi-arthroplasty following osteoporitic subcapital fracture of the femoral neck. Only macroscopically normal cartilage was used. Biopsies of 3 × 3 mm were obtained from the cartilage slices using a biopsy punch.

Rat cartilage samples. The femoral head cartilage was removed intact from both hips of female Wistar rats aged 30–32 days. The samples were washed thoroughly with phosphate buffered saline, pH 7·2, before use in culture experiments.

Changes in the GAG concentration of human cartilage. Each biopsy of human cartilage was cut in half and the two pieces were placed in separate wells of a 24-well plate. One half (control biopsy) was cultured in 2 mL of supplemented medium consisting of RPMI-1640 containing 2 mM l-glutamine, 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (all from Gibco), 25 mM Hepes (Flow Labs, UK) and 5% normal human AB-serum (Blood Transfusion Centre, Bristol) which was heat-inactivated (56°C for 30 min) before use. The other half (treated biopsy) was cultured in 2 mL supplemented medium (as above) containing one of the following stimuli: 15% synovial fluid, recombinant human (rHu-) IL-1α (a gift from Hoffman La-Roche, New Jersey) or rHu-IL-1β (a gift from Roussel, UCLAF). Each synovial fluid or cytokine was tested on at least 5 biopsies alongside at least 5 paired control biopsies. The cultures were incubated for 48 h at 37°C in an atmosphere of humidified 5% CO_2/air. At the end of the culture period the plates were stored at −20°C until the GAG concentration in each biopsy could be determined. The change in GAG concentration induced by each stimulus was estimated using the calculation:

$$\% \text{ Reduction in cartilage GAG} = 100 - \left(\frac{\text{Mean [GAG] in treated biopsies}}{\text{Mean [GAG] in control biopsies}} \times 100\right)$$

GAG loss from cartilage into medium. In order to compare GAG loss from human and rat cartilage it was necessary to culture samples in a medium which was suitable for use with tissue from both species. Accordingly, the following medium was used (Clay et al 1989): Dulbecco's modified Eagle's medium (DMEM) containing 25 mM Hepes, 5 g L^{-1} glucose, 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 1 mM sodium pyruvate (all from Gibco) and 5% foetal calf serum (Gibco) which was heat inactivated (56°C for 30 min) before use. Human cartilage biopsies were cut in half and placed in separate wells of a 24-well plate. One half was the control biopsy and the other the treated biopsy. Similarly for rats, cartilage from the left hip was used as the control sample whilst cartilage from the right hip was the treated sample. The paired samples were all incubated in medium alone for 48 h at 37°C in an atmosphere of humidified 5% CO_2/air. rHu-IL-1α, Hu-IL-1β or 15% synovial fluid (see above) was then added to the treatment wells, but not the control wells, and the plates were incubated for a further 5 days. At the end of the culture period the plates were stored at −20°C until the GAG concentration in the cartilage samples and in the medium could be determined. The GAG loss from each sample into medium was estimated for control and treatment groups using the calculation:

$$\% \text{ GAG loss into medium} = \frac{[\text{GAG}] \text{ in the medium}}{[\text{GAG}] \text{ in the medium} + [\text{GAG}] \text{ remaining in the sample}} \times 100$$

Processing of samples. Each cartilage sample was blotted dry, weighed and then placed in an Eppendorf tube containing 0·5 mL of papain (16·3 units mL^{-1}, Sigma) in digestion buffer consisting of 20 mM disodium hydrogen orthophosphate dihydrate, 1 mM EDTA (both from BDH) and 2 mM dithiothreitol (Sigma). The capped Eppendorf tubes were incubated in a water bath at 65°C. Since it took a longer time to digest rat cartilage than human cartilage, samples from experiments with human cartilage only were incubated for 2 h whilst samples from experiments in which human and rat cartilage were compared were incubated for 3 h. Portions of the digested samples were then diluted as necessary with digestion buffer before being assayed for GAG. Portions of medium from the culture wells were mixed in a 1:1 ratio with papain (16·3 mL^{-1}) in digestion buffer and incubated for 3 h at 65°C. They were then assayed, without dilution, for GAG.

GAG assay. Whale chondroitin sulphate standards (Sigma) were prepared in digestion buffer. Duplicate 10 μL amounts of the diluted cartilage digests and standards, or 20 μL amounts of the digested medium samples and standards, were added to the wells of a round-bottom microtitre plate. Estimation of the sulphated GAG in samples and standards was then carried out using a modified version of the colorimetric method of Farndale et al (1982, 1986), as recently described by Hollander et al (1991).

Statistical analysis. The significance of differences between groups of data was estimated using the 2-tailed Mann-Whitney U-test. $P < 0.05$ was taken as significant.

Results

Changes in the GAG concentration of human cartilage. Biopsies of human cartilage incubated for 2 days with 15% synovial fluid contained less GAG than control biopsies (mean % reduction in cartilage GAG ± s.d. for 18 synovial fluid samples was 17·2 ± 10·2%; Fig, 1). In equivalent experiments using 10 or 100 ng mL^{-1} of IL-1α or IL-1β, no such change in cartilage GAG could be detected (Fig. 1).

GAG loss from human cartilage into medium. In order to confirm that human cartilage is degraded by synovial fluid but not IL-1, the alternative method of measuring GAG loss into medium was used. The results (Fig. 2) show that synovial fluid significantly increased GAG loss from human cartilage into medium over 5 days (mean % GAG losses ± s.d. for 6 synovial fluid samples and 6 controls were 12·4 ± 3·8% and 3·8 ± 1·4%, respectively; $P < 0.002$), whilst IL-1α and IL-1β once again had no significant effect.

GAG loss from rat cartilage into medium. In contrast to the results with human cartilage, loss of GAG from rat cartilage into medium was significantly reduced by synovial fluid over 5 days of culture, despite using the same technique (Fig. 3). The mean % GAG losses ± s.d. for 11 synovial fluid samples and 11 controls were 11·3 ± 4·8% and 23·4 ± 5·7%, respectively ($P < 0.02$).

Conversely, both IL-1α and IL-1β significantly increased the GAG loss from rat cartilage into medium (Fig. 3). The mean % GAG losses ± s.d. for 100 ng mL^{-1} IL-1α (n = 10) and 10 controls were 32·7 ± 6·3% and 19·8 ± 1·7%, respectively ($P < 0.002$). The equivalent values for 100 ng mL^{-1} IL-1β

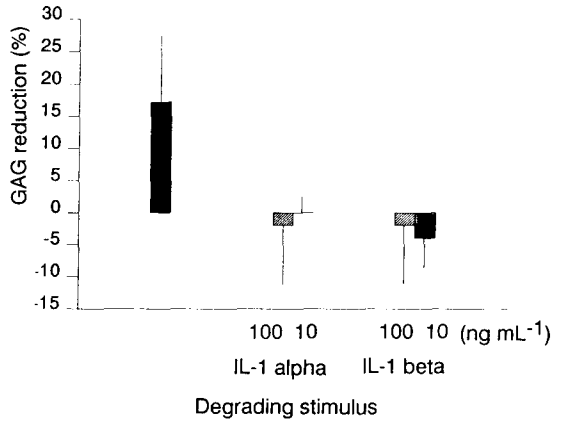

FIG. 1. Degradation of human cartilage over 2 days by 15% synovial fluid (SF; n = 18) or IL-1α and IL-1β at 10 ng mL^{-1} (n = 6 for each cytokine) or 100 ng mL^{-1} (n = 3 for each cytokine). Results are shown as the reduction of GAG in treated biopsies, expressed as a percentage of the paired control biopsies.

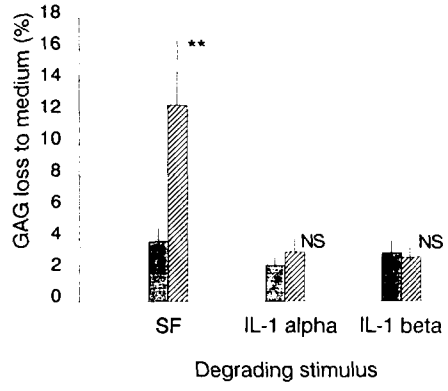

FIG. 2. GAG loss from human cartilage into medium over 5 days, induced by 15% synovial fluid (SF; n = 6), 100 ng mL^{-1} IL-1α (n = 9) or 100 ng mL^{-1} IL-1β (n = 10). Results are shown for treated biopsies (striped bars) and the paired control biopsies (dotted bars). NS = not significant; ** $P < 0.002$ vs paired control.

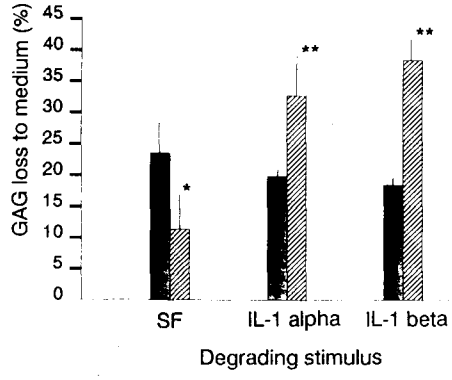

FIG. 3. GAG loss from rat cartilage into medium over 5 days, induced by 15% synovial fluid (SF; n = 11), 100 ng mL^{-1} IL-1α (n = 10) or 100 ng mL^{-1} IL-1β (n = 10). Results are shown for treated biopsies (striped bars) and the paired control biopsies (dotted bars). * $P < 0.02$ vs paired control; ** $P < 0.002$ vs paired control.

($n=10$) and 10 controls were $38{\cdot}4\pm4{\cdot}0\%$ and $18{\cdot}4\pm1{\cdot}2\%$, respectively.

Discussion

The results of this paper show clearly, for the first time, that human and rat cartilage differ in their responses to degrading stimuli, even when identical culture systems are used. Human cartilage was degraded by arthritic synovial fluid, irrespective of which method (change in cartilage GAG or loss of GAG from cartilage into medium) was used to measure the effect. However, rHu-IL-1α and IL-1β failed to degrade the human cartilage in either culture system. In contrast, rat cartilage was degraded by both IL-1α and IL-1β, as judged by loss of GAG into the medium over 5 days. The results with IL-1β are in agreement with previously published data (Clay et al 1989) whilst the results with IL-1α contradict the findings of Desa et al (1988), who were unable to degrade rat cartilage with IL-1α. We are unable to account for these differences at present, although it may be a result of variations in the activity of IL-1α, depending on the source of the cytokine. Surprisingly, arthritic synovial fluid failed to degrade rat cartilage, but significantly reduced the loss of GAG into medium compared with controls. It may be that the effects of cartilage-degrading cytokines in the synovial fluid are off-set by cartilage-protecting molecules. However, any such protective molecule is clearly without effect on human cartilage (see above) and is probably equally ineffective on bovine cartilage, which can be degraded in-vitro by human arthritic synovial fluid (Silverman et al 1984; Saxne et al 1988).

These results strongly suggest that the use of animal cartilage to test new drugs for human cartilage protective, or damaging, activity could be very misleading and such screening would best be carried out using human cartilage. Further studies are required to examine the factors which control normal cartilage matrix turnover in humans, compared with other species.

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