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Polymorphonuclear leukocytes induce damage to the articular cartilage in acute immunologic arthritis in rabbits

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A major feature of inflammatory joint disease such as rheumatoid arthritis is the extensive degradation of the articular cartilage which may eventually lead to irreversible loss of joint function. During acute inflammatory flares, which occur sporadically in rheumatoid arthritis, vast numbers of polymorphonuclear leukocytes (PMNL) accumulate in synovial exudates. In acute antigen-induced arthritis in rabbits [1], which exhibits many features of the early phase of rheumatoid arthritis, large numbers of PMNL also accumulate in synovial exudates and this is accompanied by up to 40% loss of the articular cartilage proteoglycan [2]. Studies carried out *in vitro* [3–6] have demonstrated that proteases secreted by activated PMNL may contribute to the loss of proteoglycan from the articular cartilage matrix. However, definitive evidence *in vivo* that PMNL contribute directly to the damage to the articular cartilage observed in rheumatoid arthritis and experimental models of arthritis is lacking. A reversed passive Arthus reaction (RPAR) in the rabbit knee joint also has several features in common with an acute flare reaction of rheumatoid arthritis in that it involves a transient oedema and PMNL infiltration into the joint cavity [7]. Using a RPAR in the rabbit knee joint we have investigated the relationship between immune complex mediated PMNL infiltration into the joint cavity and damage to the articular cartilage.

Materials and methods

Antibodies to bovine serum albumin (BSA) were raised in New Zealand White (NZW) rabbits and partially purified by ammonium sulphate precipitation and DEAE-cellulose chromatography as previously described [7]. A RPAR was produced in the right knee joint of female NZW rabbits (2.5–3.5 kg, Ranch Rabbits, Crawley Downs, Sussex) by intra-articular injection of 600 μg of specific anti-BSA protein in 1.0 ml of pyrogen-free saline followed immediately by an i.v. injection of 20 mg BSA antigen (Sigma). The proteoglycan content of articular cartilage was assessed by measuring the glycosaminoglycans (GAG) which are the heteropolysaccharide side chains of proteoglycan. Briefly, articular cartilage slices from the ends of the femurs and tibia were washed in saline, blotted dry then heated at 100° for 15 min in 0.5 ml of 0.4% sodium dodecyl sulphate. After cooling the cartilage was digested by the addition of 1 mg of protease K (Sigma) in 10 mM Tris–HCl pH 7.6 containing 1mM calcium chloride and incubating at 55–60° for 24 hr. Following centrifugation the supernatants were stored at −20° and subsequently assayed for GAG, hydroxyproline and DNA content as described by Whiteman [8], Huszar [9] and Hinegardner [10] respectively.

Results and discussion

Shortly after (1–2 hr) initiation of a RPAR in the rabbit knee joint there was a rapid infiltration of leukocytes (>98% PMNL) into the joint cavity. Peak PMNL levels were observed at 10 hr ($5.35 \pm 0.2 \times 10^7$ per ml exudate) then declined to approximately 20% and <2% of peak levels by 24 and 48 hr respectively. The PMNL infiltration was associated with a parallel extravasation of plasma into the joint cavity measured by leakage of ^{125}I rabbit serum albumin [7]. The severity of both parameters was directly proportional to the concentration of antibody injected into the joint. No inflammation was detected in joints injected with sterile pyrogen-free saline.

Following initiation of a RPAR in the knee joint there was a progressive loss of GAG from the articular cartilage for at least 72 hr when compared with cartilage from the contralateral saline injected joint as shown in Fig. 1. This is indicative of proteoglycan loss from the cartilage matrix. Identical profiles were obtained when the GAG content was standardised against DNA (as shown in Fig. 1) or hydroxyproline (data not shown). No change in the hydroxyproline content of the articular cartilage was detected during the 72 hr following initiation of the RPAR suggesting that collagen is not lost from the cartilage matrix during this reaction.

The possibility that the infiltrating PMNL may contribute to the loss of proteoglycan from the articular cartilage was investigated in control and neutropenic animals. As shown in Fig. 2 the GAG content of articular cartilage taken from control animals 3 days after production of a RPAR in the knee joint showed a statistically significant loss of GAG content when compared to the saline injected contralateral joint. In neutropenic animals PMNL did not infiltrate the joint cavity and no loss in GAG content of the articular cartilage was detected following production of a RPAR in the knee joint (Fig. 2). These results suggest that the PMNL

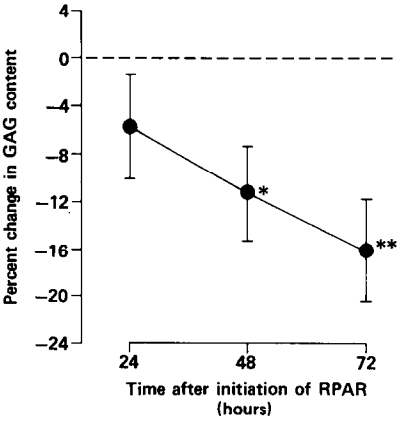

Fig. 1. Time course of the proteoglycan loss from the articular cartilage following initiation of a RPAR in the rabbit knee joint. RPAR was produced in the right knee joint, as described under Materials and Methods, and 1 ml of pyrogen-free saline ("Steriflex", The Boots Company PLC) was injected into the left knee joint. At the times indicated the animals were killed with intravenous pentobarbitone sodium ("Euthatal", May & Baker), the cartilage from the ends of the femur and tibia were dissected, digested and analysed for GAG and DNA as described under Materials and Methods. The proteoglycan content of the articular cartilage from the RPAR affected and saline injected joints was calculated as μg GAG per μg DNA and the difference between the joints expressed as a percentage of the saline control values in the same animals. Each point is the mean ± SEM of 8 animals per time point and * indicates $P < 0.05$ and ** $P < 0.01$ compared to controls assessed by a one sample Student's *t*-test.

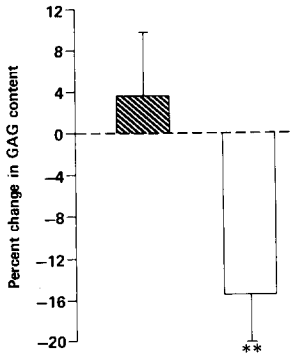

Fig. 2. Effect of neutropenia on proteoglycan loss from the articular cartilage following initiation of a RPAR in the rabbit knee joint. Animals were made neutropenic by i.v. injection of 1.75 mg/kg nitrogen mustard (mustine hydrochloride, The Boots Company PLC) and three days later a further i.v. injection of 0.88 mg/kg was given. A second injection is necessary to maintain neutropenia throughout duration of the experiment. The leukocyte content of blood was measured with a Coulter counter and the percentage of PMNL determined on blood smears stained with Geimsa's stain. On days 3 through to 6 after treatment with nitrogen mustard the PMNL content of blood was <3% of normal. Shortly after the second nitrogen mustard treatment a RPAR was produced in the right knee joint, as described under Materials and Methods, and 1 ml of pyrogen-free saline was injected into the left knee joint. Three days later the animals were killed with i.v. pentobarbitone sodium and the proteoglycan content of the cartilage from the ends of the femur and tibia were dissected, digested and analysed for GAG and DNA as described under Materials and Methods. The change in proteoglycan content between RPAR affected and saline injected joints was calculated as described in the legend to Fig. 1. The open histogram bars are control and cross-hatched bars the neutropenic animals. Histogram shows the mean ± SEM of 10 animals per group and ** indicates $P < 0.01$ compared to controls assessed by a one sample Student's *t*-test.

which infiltrate the joint during a RPAR contribute to the destruction of the articular cartilage matrix. Although N-mustard also depletes circulating monocytes these cells were not detected in exudate taken from knee joints affected with a RPAR. Therefore, it is unlikely that monocytes contribute direct damage to the articular cartilage in this model. It is possible that N-mustard treatment could affect another cell type, for example the chondrocyte or synoviocyte, which may contribute to the destruction of the cartilage matrix. However, the loss of GAG from the cartilage matrix is most likely caused by the release of degradative enzymes released from leukocytes during phagocytosis of immune complexes formed during the RPAR [11]. It is interesting that GAG continued to be lost from the articular cartilage after the number of PMNL in the joint cavity had markedly declined. This is consistent with the observation of Sandy *et al.* [6] that a PMNL-derived elastase-like enzyme, which degrades proteoglycan, could be extracted from articular cartilage taken from the affected knee joints of rabbits with antigen-induced arthritis. It is possible therefore that during a RPAR, enzymes released from PMNL due to phagocytosis of immune complexes bind to or penetrate the articular cartilage where they exert a destructive effect on the cartilage matrix long after PMNL disappear from the joint cavity. An alternative explanation is that tissues resident within the joint could be responsible for damaging the articular cartilage, perhaps triggered by a PMNL-derived factor.

It was surprising that collagen also was not lost from the articular cartilage matrix since PMNL-elastase can degrade collagen [12]. Several explanations for this are possible. For example, (1) collagen is less sensitive to degradation by elastase [12], (2) collagen fibres could be cleaved but not lost from the matrix, (3) the enzyme may fail to gain access to collagen within the matrix, or (4) proteoglycan specific proteinases may be responsible for destruction of the proteoglycan.

In a recent publication, Pettipher *et al.* [13] demonstrated that Interleukin 1 (IL-1), a polypeptide released by activated macrophages and thought to be a key mediator in host responses to infection and inflammation, when injected into the rabbit knee joint induced leukocyte infiltration into the joint cavity and proteoglycan loss from the articular cartilage. The presence of leukocytes in the joint cavity may not by itself account for all the proteoglycan loss since leukocyte infiltration into the joint cavity, stimulated by intra-articular injection of endotoxin, did not cause proteoglycan loss. In fact the IL-1 induced proteoglycan loss is not dependent on leukocytes (Pettipher, Higgs and Henderson, personal communication) but may be due to its direct action on chondrocytes causing them to secrete degradative enzymes which destroy the cartilage matrix [13]. Thus the mere presence of leukocytes within the joint space is not necessarily sufficient to cause damage to the articular cartilage. It appears that in addition to a chemotactic substance which is necessary to induce their recruitment, leukocytes also require activation by phagocytic stimuli, for example immune complexes, in order to damage the articular cartilage. During acute flares which occur in rheumatoid arthritis, PMNL within the joint cavity may become activated by immune complexes associated with the articular cartilage [14–16] thereby inducing damage to the cartilage by a process analogous to that reported here.

A similar study to that reported here has been conducted using the Dumonde Glynn model of antigen-induced monoarticular arthritis [17]. In contrast to our observations using a RPAR, leukocyte depletion by N-mustard failed to prevent GAG loss from cartilage following intraarticular antigen challenge. These different observations probably have a mechanistic basis. The RPAR described here is an acute PMNL-dependent inflammation which occurs when intravenous antigen and intra-articular antibody combine to form immune complexes within the joint tissues [7]. In the Dumonde Glynn model antibodies are raised by actively sensitising animals with antigen. On subsequent intra-articular challenge with appropriate antigen an immunological reaction is induced within the joint tissues which includes the formation of immune complexes and the generation of cytokines such as IL-1 which may stimulate cartilage breakdown [18, 19]. Since IL-1 induced proteoglycan loss is not dependent on leukocytes (see above) such a mechanism could account for the failure of PMNL depletion to prevent GAG loss in the Dumonde Glynn model as reported by Henderson *et al.* [17].

In summary, following initiation of a RPAR in the rabbit knee joint a transient PMNL-enriched inflammatory exudate occurred within the joint cavity. This was accompanied by a time-dependent loss of GAG from the articular cartilage. In rabbits made neutropenic by treatment with N-mustard a RPAR failed to induce an exudate within the joint space and no loss of GAG occurred from the articular cartilage. These observations support the hypothesis that the infiltration of PMNL into the joint cavity during a rheumatoid flare may contribute to destruction of the articular cartilage.

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Imperial Chemical Industries PLC
Pharmaceuticals Division
Bioscience I Department
Mereside, Alderley Park
Macclesfield, Cheshire
SK10 4TG, U.K.

STEPHEN J. FOSTER*
C. JANE CUNLIFFE
MARGARET E. MCCORMICK

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* Author for correspondence.