Effects of murine recombinant interleukin 1 on intact homologous articular cartilage: a quantitative and autoradiographic study

WIM B VAN DEN BERG,[1] FONS A J VAN DE LOO,[1] WIL A ZWARTS,[1] AND IVAN G OTTERNESS[2]

From the [1]Department of Rheumatology, University Hospital St Radboud, Nijmegen, The Netherlands; and the [2]Department of Immunology, Pfizer Central Research, Groton CT

SUMMARY   Murine recombinant interleukin 1 (IL1) was tested for its ability to affect intact murine articular cartilage. IL1 caused enhanced proteoglycan degradation and severe inhibition of chondrocyte synthetic function at a concentration of 3 U/ml (100 pg/ml). Inhibition of proteoglycan synthesis appeared to be delayed in onset but occurred consistently after 24 hours. Pulse chase experiments made it clear that proteoglycan degradation and inhibition of proteoglycan synthesis are two distinct actions of IL1. No indications were obtained for selective degradation of either newly synthesised or processed proteoglycan. Moreover, chondrocyte synthetic activity appeared to be inhibited uniformly throughout the cartilage matrix, i.e., no evidence was found for selective suppression of cells in certain regions. IL1 uptake measurement in the cartilage, using [^{125}I]IL1, yielded a partition coefficient far below 1, and autoradiography demonstrated a faint but even distribution within the cartilage matrix. The coordinated induction of enhanced breakdown of proteoglycan and inhibition of proteoglycan synthesis, with such low concentrations of IL1 reaching the chondrocytes, underlines the impressive destructive potential of IL1.

Key words: proteoglycan synthesis, cytokines, murine cartilage.

The term interleukin 1 (IL1) was originally introduced to describe a 17 kilodalton protein, secreted by activated cells of the macrophage/monocyte lineage, that augmented T cell proliferation to mitogens and antigens.[1] This property was formerly known as lymphocyte activating factor (LAF) activity. It has now become evident that IL1 can affect multiple cell types and functions. For instance, IL1 may mediate fever, hepatic secretion of acute phase proteins, lymphocyte chemotaxis, fibroblast proliferation, and alterations in bone metabolism.[2] Recent work using recombinant IL1 has confirmed that these several biological activities reside within one molecule.[3] Earlier studies had suggested that IL1 is also involved in cartilage destruction in arthritic joints. Coculture of cartilage and synovial tissue resulted in severe chondrocyte mediated

breakdown of cartilage proteoglycans. The substance responsible for this effect was termed catabolin.[4] Similar effects were described for mononuclear cell factor,[5] a product of stimulated human blood monocytes. When purified to homogeneity, catabolin and mononuclear cell factor were shown to have properties identical to those of IL1.[6 7] It is now known that IL1 stimulates the release of metalloproteinases from chondrocytes.[8–10] Interestingly, IL1-like activity has been identified in synovial fluid from patients with various types of joint disease.[11 12] Moreover, raised levels of cartilage derived proteases have been detected in cartilage specimens from patients with osteoarthritis.[13 14]

In the present study we describe the effects of murine recombinant IL1 on chondrocyte metabolism, using anatomically intact homologous articular cartilage—the murine patella. Apart from an enhancing effect on proteoglycan breakdown, IL1 was shown to inhibit severely chondrocyte proteoglycan synthesis as measured by ^{35}S incorporation.

Autoradiography was performed to detect potential variability in IL1 sensitivity of chondrocytes in various regions. Finally, IL1 was radiolabelled to determine its partition in intact cartilage.

Materials and methods

PREPARATION OF IL1

The published amino acid sequence of murine IL1α was used to synthesise a cDNA fragment and this was expressed in *Escherichia coli*. The protein consisted of the carboxy terminal amino acids 115 to 270 of the IL1 propeptide. The molecule was purified from inclusion bodies and refolded into the biologically active form (G Daumy, to be published). Cyanogen bromide fragmentation was used for structural confirmation. Biological activity was verified in multiple assays (Otterness *et al*, in preparation). In the LAF assay the IL1 was found to give 1 unit activity consistently in the 10–40 pg/ml range. Screening for endotoxin activity (Limulus assay) was negative up to a concentration of 100 µg/ml IL1. The recombinant IL1 preparation was stored at −20°C and showed consistent LAF activity over the period studied. Experiments were performed with two batches of IL1 prepared on different occasions. Effects on chondrocyte synthesis and cartilage breakdown were completely similar.

LAF ACTIVITY

Murine thymocytes from mice aged 6–7 weeks were cultured for four days in the presence of 1 µg/ml phytohaemagglutinin and various IL1 concentrations. Thymocytes were cultured at a concentration of 10^7/ml in 200 µl wells, and [^{3}H]thymidine was added (37 kBq/well) during the last day. One unit of LAF activity was defined as the IL1 concentration giving half the plateau of IL1 induced comitogenic thymocyte proliferation.

CARTILAGE ASSAY

Whole murine patellae were isolated from C57Bl mice leaving the patella embedded in a minimal amount of surrounding tissue and used to measure chondrocyte metabolism as described previously.[15][16] In brief, patella specimens were cultured in RPMI-5% fetal calf serum (Flow) under 5% CO_2 with or without IL1. Culture medium was refreshed every day. To determine chondrocyte proteoglycan synthesis the specimens were subsequently pulsed with 740 kBq [^{35}S]sulphate/ml for three hours. After washing, fixation in formalin (10%), and decalcification in formic acid (5%) the patella could easily be punched out of the adjacent tissue. The ^{35}S content of each patella, which is a reliable measure of the

[^{35}S]glycosaminoglycan content,[17][18] was measured by liquid scintillation counting.

To measure the potential release of labelled proteoglycan into the medium 100 µl aliquots of the three hour [^{35}S]sulphate pulse medium of control and IL1 incubations were treated with 1% cetylpyridinium chloride. Non-labelled proteoglycan was added as a carrier. After two hours at 37°C the precipitate was spun down at 10 000 g for 30 minutes and the pellet was washed five times with 0·1% cetylpyridinium chloride to remove adherent [^{35}S]sulphate.[17] To correct for loss of [^{35}S]proteoglycan during this procedure the following standard was included in the experiment: [^{35}S]proteoglycan (2000 cpm) from bovine cartilage supplemented with 74 kBq [^{35}S]sulphate.

To determine IL1 mediated degradation the patellae were prelabelled with [^{35}S]sulphate (6–24 h) before IL1 exposure. After the culture period with IL1 the amount of ^{35}S retained in the patellar cartilage was measured and expressed as a percentage of the ^{35}S content of control cartilage cultured without IL1.

To enable comparison of IL1 effects on newly synthesised and more extensively processed proteoglycans mice were injected intraperitoneally with 74 kBq/g body weight, and labelled patellae were isolated at days 1, 2, and 4. The right patella was cultured in the presence of IL1 for 24 hours and the left patella was cultured without IL1.

The IL1 effect was always expressed in comparison with the value for the contralateral joint of the same animal. This approach minimises variation due to interindividual difference in ^{35}S incorporation.[15]

AUTORADIOGRAPHY

Patella specimens were fixed and decalcified as described above. After histological processing 6 µm sections were prepared and mounted on gelatin coated slides. These were dipped in K_s emulsion (Ilford, Basildon, Essex, England) and exposed for three to five weeks. After this period the slides were developed and stained with haematoxylin and eosin.

CARTILAGE HISTOLOGY

Patella sections were stained with alcian blue for 24 hours. The dye was solved in an acetate buffer (0·025 M, pH 5·4) at a concentration of 0·06%, in the presence of 0·3 M $MgCl_2$. Decreased staining of the cartilage layer reflects loss of proteoglycan. Similar results were obtained with Safranin O or Giemsa staining.

UPTAKE OF RADIOLABELLED IL1

^{125}I labelling of IL1 was performed by the method of

Bolton-Hunter. Iodinated IL1 was separated from free ^{125}I by Sephadex G-25 fractionation. The labelled preparation retained full biological activity, and the specific activity was approximately 37 kBq/µg.

Patellae were incubated with [125I]IL1 in phosphate buffered saline (pH 7·4) for various time periods at 37°C. At the end of the incubation the specimens were rinsed once, blotted dry, and fixed in 10 ml of 2·5% glutaraldehyde or formalin (10%)/ethanol for 30 minutes. This was followed by decalcification in 5% formic acid overnight. After a short fixation period the whole cartilage layer can easily be stripped from the underlying bone,[17] and the amount of [125I]IL1 present in the cartilage can be counted. The procedure of fixation of cartilage associated protein allows the release of free ^{125}I from the tissue: slight contamination of labelled protein solutions with free ^{125}I would otherwise introduce significant errors owing to the relatively high uptake of free ^{125}I compared with that of poorly penetrating proteins. The uptake of [125I]IL1 is expressed as a partition coefficient, which is the concentration in the cartilage divided by the concentration in the incubation medium. To obtain the partition volume of the patellar cartilage strip the cumulative wet and dry weights of 10 specimens were determined. The fluid phase of one patella strip was approximately 0·05 µl.

Results

INHIBITION OF CHONDROCYTE PROTEOGLYCAN SYNTHESIS

Initially, whole patellae were incubated with various concentrations of murine recombinant IL1 for 48 hours, followed by a three hour pulse with [35S]sulphate, to define the effective concentration range of IL1. IL1 caused a dose dependent inhibition of [35S]proteoglycan synthesis (Table 1). Concen-

trations of IL1 of 0·1 U/ml or below were essentially without effect. A plateau of maximal inhibition was reached at IL1 concentrations of 3 U/ml and higher. In multiple experiments inhibition ranged between 50 and 65% with an IL1 concentration of 3 U/ml. Control incubations with recombinant IL2 (Amersham, England), tested in doses up to 30 U/ml, failed to inhibit proteoglycan synthesis. To verify that we were really measuring IL1 mediated inhibition of synthesis and not enhanced breakdown of new proteoglycan synthesised at the same rate we checked the culture supernatant for labelled proteoglycan fragments. The amount released during the three hour ^{35}S pulse period was less than 10% of the amount incorporated in the cartilage for both the control incubation and after 48 hours of IL1 exposure, and was therefore negligible.

Next, the effect on sulphate incorporation of time of exposure to IL1 was explored. After 24 hours IL1 exposure the suppression of chondrocyte synthesis (^{35}S incorporation) was variable (5–20%), only rarely reaching statistical significance. Exposure of patellar cartilage to IL1 for 48 hours consistently resulted in extensive inhibition of proteoglycan synthesis (Table 2). This effect seems not to depend upon exposure of the cartilage to IL1 for 48 hours as incubation with IL1 for 24 hours followed by a

Table 2   *Time dependence of IL1 effect on synthesis*

Time (h)	IL1 (U/ml)	35S incorporation† (cpm/patella)	Inhibition (%)
24	—	737 (163)	—
24	3	575 (86)	22
48	—	626 (95)	—
48	3	288 (46)	54*

Patellae were exposed to IL1 for 24 or 48 hours, followed by a three hour pulse with [35S]sulphate.
*p<0·001, compared with 24 hour value (Mann-Whitney).
†Values are the mean (SD) of six cartilage specimens.

Table 1   *Dose dependent effect of IL1 on 35S incorporation*

IL1 concentration* (U/ml)	35S incorporation† (cpm/patella)	Inhibition (%)
—	357 (33)	—
0·03	345 (19)	3
0·1	309 (25)	13
0·3	288 (34)	19
1·0	227 (32)	36
3·0	161 (39)	55
10·0	125 (37)	65
30·0	139 (31)	61

Patellae were incubated for 48 hours.
*Values are U/ml, based on thymocyte proliferation.
†Values are the mean (SD) of six cartilage specimens.

Table 3   *Time lag in IL1 effect on synthesis*

Incubation with IL1 (h)	Length of chase (h)	35S incorporation* (cpm/patella)	Inhibition (%)	p Value†
—	48	540 (67)	—	
3	45	452 (35)	16	0·001
24	24	294 (52)	46	NS
48	—	278 (35)	49	

Patellae were incubated with IL1 (3 U/ml) for various periods, followed by a chase without IL1. At the end of the incubation patellae were pulsed with [35S]sulphate for three hours.
*Values are the mean (SD) of six cartilage specimens.
†p Values compared with 48 hour IL1 exposure (Mann-Whitney).

24 hour incubation with medium only led to the same total effect (Table 3). These data indicate that IL1 mediated suppression of chondrocyte proteoglycan synthesis is delayed in onset. We never observed a suppression exceeding 25% at 24 hours, even with IL1 concentrations up to 100 U/ml. This indicates that slow diffusion of IL1 into the cartilage, reaching a sufficient level of IL1 near the chondrocyte in a retarded fashion, cannot be the reason for this time lag. We also looked for selective effects of IL1 on chondrocyte incorporation of [35S]sulphate in different regions of the patella by performing autoradiography on semiserial patellar sections of cultured specimens. Uniform labelling of the cartilage layer was obtained after 48 hours' culture and three hours' [35S]sulphate exposure (Fig. 1a). When the patellae were cultured in the presence of IL1, clear cut suppression of labelling intensity was observed (Fig. 1b). No evidence was obtained for a differential sensitivity to IL1 when the central or marginal regions, or the chondrocytes in superficial and deeper layers, were compared.

DEGRADATION OF [35S]PROTEOGLYCANS

Patellae prelabelled with [35S]sulphate for six hours were cultured with or without IL1, and the amount of 35S retained in the patellar cartilage was determined. Significantly less 35S was retained in cartilage cultured in the presence of IL1 (Table 4), indicating that proteoglycan degradation was enhanced. The action of IL1 was not influenced by the presence of fetal calf serum in the medium (Table 4). When larger concentrations of IL1 (up to 100 U/ml) were used, only slightly higher effects on breakdown were seen; not always reaching statistical significance compared with effects obtained with 3 U/ml.

To investigate whether there might be a preferential effect of IL1 on newly synthesised proteoglycan compared with older, more fully processed proteoglycan we carried out the following experiment. Effects of IL1 were compared on cartilage with variably processed proteoglycans, and for optimal physiological processing of labelled proteoglycans labelling was performed in vivo. From earlier studies it was known that the amount of [35S]proteoglycan in patellar cartilage, after a single injection of [35S]sulphate, reaches a plateau between two and six hours and gradually decreases thereafter.[15] Therefore the effect of IL1 on patellar cartilage isolated on days 1, 2, or 4 after [35S]sulphate injection was determined (Table 5). Patellae taken at day 1 or 2 and cultured for 24 hours in the presence of IL1 both contained significantly less 35S than those cultured in the absence of IL1. The enhancement of IL1 mediated breakdown was of the same order of magnitude for days 1 and 2. A similar result was found when the comparison was made between days 1 and 4. We repeated this experiment

Table 4   *IL1 mediated breakdown*

35S Labelling* (h)	IL1 (U/ml)	Fetal calf serum (%)	35S content† cpm/patella	%‡
6	—	—	1047 (179)	—
6	3	—	775 (151)	74
6	—	5	1060 (244)	—
6	3	5	748 (110)	71
24	—	5	1897 (183)	—
24	3	5	1367 (189)	72

*Patellae were prelabelled with [35S]sulphate for either six or 24 hours, followed by incubation for 24 hours with or without IL1.
†Values are the mean (SD) of six cartilage specimens.
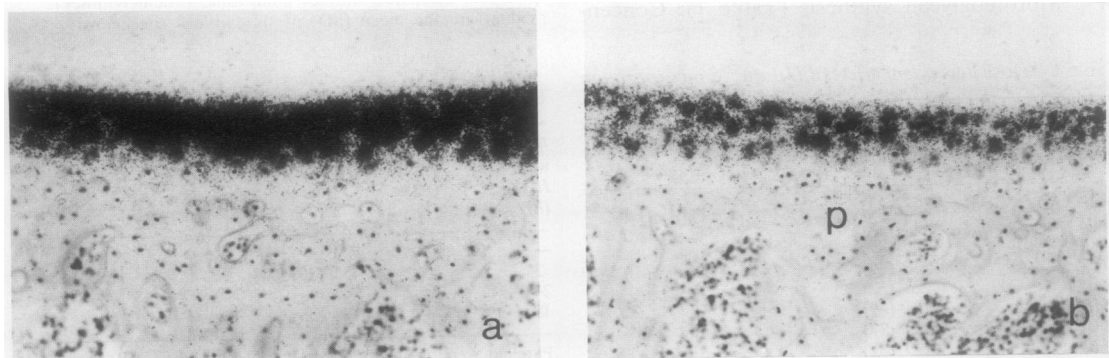‡Compared with its proper control.

Fig. 1   *Autoradiography of patellar cartilage after 48 hours' exposure to (a) medium or to (b) 3 U IL1/ml, followed by a three hour pulse with [35S]sulphate. Note the distinct labelling in (a) and the uniformly diminished labelling above all cells after IL1 exposure in (b). p=patella.*

Table 5 *Comparison of the effect of IL1 on newly labelled and more processed proteoglycan*

Experiment*	Time (h)	IL1 (U/ml)	^{35}S content† cpm/patella	%‡
A	24	—	248 (63)	—
A	24	3	185 (39)	75
A	48	—	228 (63)	—
A	48	3	180 (42)	79
B	24	—	380 (108)	—
B	24	3	275 (59)	72
B	96	—	302 (76)	—
B	96	3	221 (47)	73

Patellae were isolated and cultured for 24 hours with or without IL1, one patella without and the contralateral patella of the same animal with IL1.
*Mice were given an injection with 1·85 (exp A) or 2·78 (exp B) MBq [^{35}S]sulphate.
†Values are the mean (SD) of cartilage specimens of seven mice.
‡Compared with its proper control.

three times, with identical results. Finally, to verify that these results were not an artefact of radio-labelling, patellae were cultured for two days and then stained histologically. IL1 induced clear depletion of proteoglycan in the metabolically active superficial layer of the patella (Fig. 2).

IL1 UPTAKE IN CARTILAGE
IL1 was radiolabelled to permit measurement of its effective concentration in cartilage. The labelled preparation retained full biological activity as tested in the cartilage and in the LAF assay. Patellae were incubated with [^{125}I]IL1 for various time periods. The uptake reached equilibrium within 30 minutes, but the partition coefficient of the anionic IL1 (pI 5) remained far below 1 (Table 6).

A partition coefficient of 0·2 indicates that the local concentration is about five times less than the

IL1 concentration in the incubation medium. In contrast, the cationic protein amidated bovine serum albumin (aBSA; pI 8·5–9)[19 20] accumulated to a high extent in the cartilage and equilibrium was not reached even after four hours (Table 6).

Figure 3 depicts the autoradiography of IL1 and aBSA partition. In contrast with the high affinity of aBSA for the patellar cartilage (Fig. 3b), IL1 was predominantly taken up by the surrounding tissue (Fig. 3a). Higher magnification of the cartilage (Figs 3c and d) showed that most of the IL1 radiolabel was present as a superficial lining layer, and that the quantitative measurement of the partition (Table 6) strongly overestimated the actual concentration within the matrix. If, after [^{125}I]IL1 exposure, the patella specimens were washed with saline and then prepared for autoradiography, labelling was no longer detectable, either at the cartilage surface or within the matrix. The label present within the matrix showed a diffuse pattern over the whole cartilage area.

OTHER EFFECTS OF MURINE RECOMBINANT IL1
Murine IL1 (pI 5) was examined in a number of

Table 6 *IL1 uptake in patellar cartilage*

Incubation time (min)	Partition coefficient* IL1	aBSA†
10	0·08	3·8
30	0·15	ND†
60	0·16	10·4
120	0·14	20·2
240	0·16	33·7

*The partition coefficient is defined as the concentration in the cartilage divided by the concentration in the surrounding fluid.
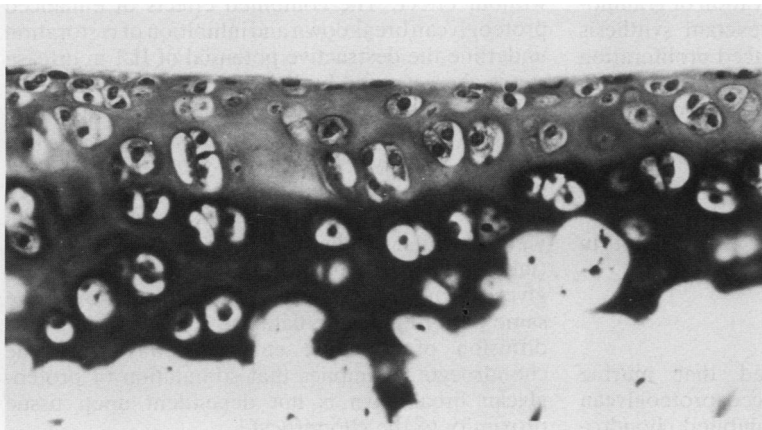†aBSA=amidated bovine serum albumin; ND=not done.

Fig. 2 *Staining of the patellar cartilage after 48 hours' IL1 exposure. Note the distinct loss of staining in the superficial layer, which is the metabolically active part of the cartilage (see Fig. 1). Halo formation around chondrocytes was not evident.*
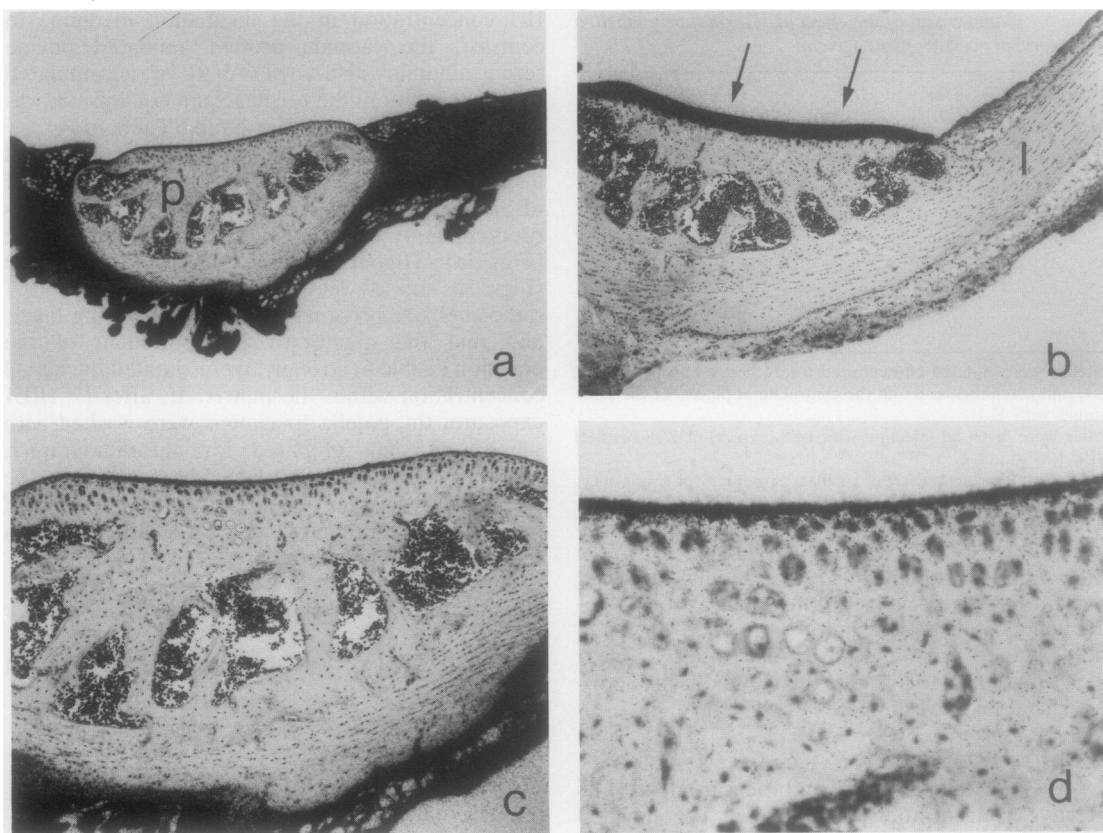
Fig. 3 *Autoradiography, four hours after incubation of patellae with (a, c, d) [^{125}I]IL1 or with (b) amidated bovine serum albumin ([^{125}I]aBSA). (a) IL1 is predominantly present in the surrounding connective tissue and ligament structures. (b) The cationic protein aBSA shows a high affinity for the patellar cartilage (arrows) and deeply penetrates the matrix. (c) and (d) are at higher magnification than (a) to show that IL1 is hardly present within the cartilage matrix. p=patella; l=ligament.*

other assays to compare its activity with that of native IL1. In vivo, in the mouse, we found it to induce fever, leucocytosis, i.e., elevation of granulocyte numbers, and acute phase reactant synthesis (serum amyloid A). In vitro it induced proliferation of thymocytes in the LAF assay. In the latter assay the IL1 was found to give 1 unit of activity consistently in the 10–40 pg/ml range. Preliminary experiments further indicated that the IL1 preparation also caused cartilage breakdown and proteoglycan synthesis inhibition in vivo upon intra-articular injection in knee joints (manuscript in preparation).

Discussion

The present study demonstrated that murine recombinant IL1 not only enhanced proteoglycan breakdown but also extensively inhibited chondro-cyte proteoglycan synthesis in *intact* murine articular cartilage. As a control the lymphokine IL2 was without effect. The combined effects of enhanced proteoglycan breakdown and inhibition of restoration underline the destructive potential of IL1 in disease states characterised by IL1 overproduction.

IL1 mediated loss of proteoglycans from the cartilage matrix does not seem to be a selective process. Newly synthesised proteoglycan and proteoglycan processed for four days are affected equally (Table 5). Earlier studies with catabolin yielded similar results. Release of newly synthesised (labelled) proteoglycans and unlabelled proteoglycans from catabolin treated cartilage followed the same pattern.[21] These data are consistent with free diffusion of catabolic enzymes away from the chondrocyte. It implies that stimulation of proteoglycan breakdown is not dependent upon tissue proximity to the chondrocyte.

Upon stimulation with IL1 chondrocytes have been shown to release metalloproteinases capable of degrading both collagen and proteoglycan.[8] [22] Enzymes found in the medium of IL1 treated cartilage cultures are in the latent form and must be activated before they can be enzymatically assayed.[22] This suggests that IL1 stimulated the release of latent metalloproteinases from chondrocytes and that a proportion of the enzyme is activated in situ in the matrix. Suggestive evidence for this mechanism stems from detailed analysis of cartilage proteoglycans after stimulation with IL1.[23]

In addition to proteoglycan degradation, IL1 induced extensive inhibition of chondrocyte proteoglycan synthesis. This inhibition appears to be retarded in onset. Effects were variable and not impressive at 24 hours but very significant at 48 hours. A similar delay in onset of action was described for catabolin.[24] So far, the second messenger involved in the effect of IL1 on synthesis has not been elucidated. A recent report, however, clearly demonstrated the presence of specific IL1 receptors on chondrocytes.[25] One could argue that the time lag in IL1 action on proteoglycan synthesis is compatible with the suggestion that IL1 may stimulate new synthesis of degradative enzymes and that the apparent inhibition seen at 48 hours is therefore due to a high level of proteoglycan degrading enzymes and more severe breakdown of new proteoglycans synthesised at a normal, i.e., unchanged, rate. No evidence has been found, however, for a faster, more extensive degradation of newly synthesised proteoglycan during the short ^{35}S pulse after long term IL1 exposure. Tyler did not observe enhanced amounts of ^{35}S labelled proteoglycan fragments in the culture medium of such IL1 treated and briefly ^{35}S pulsed cartilage,[24] and in this study we obtained similar results. Moreover, our pulse chase experiment made it clear that 24 hour IL1 exposure, followed by a chase without IL1, resulted in the same extent of inhibition of synthesis (Table 3). This makes it highly unlikely that the inhibition of synthesis is due to enhanced breakdown as it is known that proteoglycan breakdown ceases within 24 hours of removal of IL1.[21] Taken together, our data clearly indicate that IL1 has two effects on cartilage: firstly, enhanced degradation of proteoglycan; secondly, extensive inhibition of proteoglycan synthesis via an as yet unknown mechanism.

IL1 has to penetrate the cartilage before it is active. Uptake of a protein in hyaline cartilage depends on both its size and charge. Earlier studies from our group showed that proteins with an isoelectric point (pI) above 8·5 have a high affinity for the negatively charged cartilage and easily penetrate and accumulate in the dense matrix.[19] [20] [26–28] The data obtained for IL1 uptake in the present study indicate a partition coefficient of 0·2, pointing to concentration of mediator inside the cartilage five times lower than in the surrounding fluid. This value is consistent with published results for cartilage distribution of other anionic proteins of similar molecular weight.[29] Our autoradiography made it clear, however, that most of the radiolabel was located at the surface and that the actual IL1 concentration within the matrix was far less. These observations stress the need for autoradiographic control of radiolabel studies. The high amount of label at the surface is just a consequence of the technique used and does not reflect IL1 binding at that site. In uptake studies patella specimens are not washed, and surface binding of attached label does occur after fixation. When washed with saline before fixation, this label is not detectable anymore. Another relevant issue in uptake studies with labelled proteins is the potential change in behaviour due to labelling. We chose the mild iodination according to Bolton-Hunter and checked that the [^{125}I]IL1 retained its full biological activity in our cartilage assay. Moreover, at most one ^{125}I molecule is introduced per IL1 molecule, making a behavioural change highly unlikely. Loss of activity in our cartilage assay was clear cut after choramine-T iodination of IL1 (data not shown). Earlier observations from our laboratory have indicated that penetration of anionic proteins increases when the cartilage matrix is depleted of proteoglycans.[20] [26] This indicates that IL1 penetration and interaction with chondrocytes could be expected to increase in already damaged cartilage of an inflamed joint. Thus IL1 effects may be slightly higher in fibrous cartilage than in dense hyaline cartilage owing to penetration differences. Although the two forms of this mediator, IL1α (pI 5) and IL1β (pI 7–8), differ considerably in isoelectric point, neither form has a pI above 8·5, and even IL1β would not be expected to accumulate in hyaline cartilage.[28] Slight differences in potency between IL1α and IL1β, as noted in cartilage assays,[7] are not likely to be caused by disparity in uptake, and probably relate more to differences in IL1 chondrocyte (receptor) interaction.

In experimental models of murine joint inflammation, such as zymosan or antigen induced arthritis, chondrocyte proteoglycan synthesis was already significantly suppressed at day 1.[16] [30] From the present kinetic data it seems unlikely that IL1 is directly responsible for this early suppression. Diffusion of mediators of acute inflammation such as hydrogen peroxide[31] or neutrophil enzymes such as elastase[32] [33] may play a part in this early phase. After the first day IL1 seems a likely candidate for

the continuing suppression of proteoglycan synthesis. The suppression found in the models of experimental arthritis was of the same order of magnitude (50–60%) as that obtained with IL1 in vitro. In vivo it was observed that there was a higher vulnerability of chondrocytes in certain regions of the articular cartilage.[30] For instance, suppression of sulphate incorporation was more pronounced in chondrocytes of the central area of the patellar cartilage than in the margins.[30] Evidence for an enhanced susceptibility of particular chondrocytes to IL1 effects was not observed in vitro in the present study. This could indicate that IL1 is not the mediator of the cartilage damage seen in these models. In vivo, however, load bearing may give rise to regional differences. Moreover, diffusion of proteolytic inhibitors or other protective factors from the margins, supplied in vivo, may lead to more pronounced suppression in the central area.

The importance of IL1 as a mediator of cartilage destruction in chronic destructive arthritis is yet to be determined. IL1 activity has been detected in synovial fluid of patients with rheumatoid arthritis.[11][12] Inhibitors are also present in high amounts, however, and measurement of consistent IL1 activity depends upon separation of IL1 and its inhibitors by gel chromatography.[34][35] Some inhibitors appear only able to block the IL1 comitogenic assay,[36][37] but recently an inhibitor has been described which blocked fibroblast collagenase production,[38] and this may be an important regulator of tissue destruction. IL1 must penetrate cartilage and escape from (local) inhibitors before it is active. The recent observation that IL1 could induce joint inflammation and cartilage degradation[39–41] provides further evidence for its role as a key mediator. Experiments with selective (synthetic) inhibitors of IL1 effects, or IL1 synthesis and release, are needed to elucidate this issue fully.

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