Monocytes/macrophages rather than PMN are involved in early cartilage degradation in cationic immune complex arthritis in mice

P. L. E. M. Van Lent, A. Blom, A. E. M. Holthuysen, C. W. M. Jacobs,*
L. B. A. Van De Putte, and W. B. Van Den Berg

Department of Rheumatology and *Department of Nephrology, University Hospital, St. Radboud, Geert Grooteplein, The Netherlands

Abstract: We investigated the involvement of polymorphonuclear granulocytes (PMN) and monocytes in cartilage degradation in immune complex-mediated arthritis (ICA). ICA induced with lysozyme-anti-lysozyme in the murine knee joint is characterized by a major influx of PMNs followed by monocytes and marked cartilage proteoglycan (PG) depletion developments within 2 days. Around 60% of 35S-prelabeled PG is lost at day 2. Influx of cells was manipulated using interleukin-1 (IL-1) receptor antagonist (IL-1ra) or antibodies to adhesion molecules. Cellular infiltrate was analyzed on hematoxylin-stained joint sections. Early systemic treatment with IL-1ra highly reduced PMN influx, whereas monocyte influx was hardly diminished. PG loss was not significantly reduced, declining from 62% in controls to 47% in IL-1ra-treated mice. Total blockade of cell influx was found after intravenous treatment with monoclonal antibodies 5C6 (anti-CD11b/CD18:anti-CR3) or NIMP.R14 (25—30 kDa protein mainly present on PMN) and PG loss was reduced to 5—10%. A similar reduction was observed after prior depletion of circulating PMNs with total body irradiation. Because amounts of IL-1 produced in leukopenic and control arthritic joints are comparable, this suggests that IL-1 is only marginally involved in PG loss in the first phase of ICA. This study indicates that monocytes rather than PMN might be involved in PG loss in this form of arthritis, either directly or by local activation of synovial layer cells of the joint.

Key Words: experimental arthritis • interleukin-1 • proteoglycan depletion

INTRODUCTION

One of the main pathological features of acute human and experimental arthritis is cartilage damage [1—3]. Proteoglycans (PG), important constituents of the cartilage matrix, are degraded and set free in the joint cavity. Besides degradation, the formation of new PGs by the chondrocyte is strongly inhibited thus preventing recovery of the matrix [1]. The exact mechanism(s) involved in PG degradation is still unknown but recent studies suggest that PG are degraded between the G1-G2 domain by an unknown enzyme called aggrecanase [4]. The presence of neutrophils and monocytes/macrophages in joint inflammation is well documented. Some studies suggested that the neutrophil is involved in the initial stages of cartilage degradation [5—7], whereas others gave more emphasis to the monocyte/macrophage [8—10]. The capability of these cell types to induce cartilage destruction has been extensively studied in vitro. The neutrophil releases many substances with the potency to degrade PG like serine [11] or metalloproteinases [12]. Earlier studies done by us revealed that, under in vitro conditions, elastase was the dominant factor involved in PG degradation [13]. In addition, the monocyte/macrophage also causes release of proteoglycans if co-cultured with a cartilage specimen [14, 15]. Metalloproteinases have been shown to be important mediators of PG degradation in such experiments [15]. However, although a great deal of in vitro evidence has been obtained that indicates that neutrophils and monocytes/macrophages are involved in cartilage destruction, their active role in vivo is still a matter of debate. During the first phase of arthritis induced by immune complexes, severe cartilage damage is observed within a few days after induction both in mice [16] and rabbits [17]. In the period that cartilage degradation develops, neutrophils form the major component of the cell infiltrate, whereas monocyte number is low. Because complete blockade of cell influx prevented the larger part of cartilage degradation it seemed likely that the neutrophil is the dominant cell involved in cartilage degradation. However, elastase,

Abbreviations: PMN, polymorphonuclear granulocytes; ICA, immune complex-mediated arthritis; PG, proteoglycan; IL-1ra, interleukin-1 receptor antagonist; MIP-2, macrophage inflammatory protein-2; PLL, poly-L-lysine; EDC, 1-ethyl-3(3-dimethyl-aminopropyl)carbodiimide; DMPA, N,N-dimethyl-1,3-propanediamine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; FTTC, fluorescein isothiocyanate.

Correspondence: Dr. P. L. E. M. Van Lent, Department of Rheumatology, Geert Grooteplein Zuid 8, 6525 GA Nijmegen, The Netherlands.

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the dominant cartilage-degrading factor under in vitro conditions seemed unimportant in vivo because arthritis induction caused similar cartilage degradation in elastase-deficient Beige mice compared with controls [18]. The latter observation casts some doubt on the role of the neutrophil as the dominant cartilage-degrading cell.

The objective of the present in vivo study was to further investigate the relative role of neutrophils and monocytes/macrophages in cartilage degradation in the early phase of experimental arthritis. As a model, immune complex-induced arthritis (ICA), which is induced with lysozyme-antilysozyme in the murine knee joint, was studied. ICA is characterized by early IL-1 production, influx of inflammatory cells, and severe cartilage degradation of proteoglycans from the cartilage matrix [16]. In the first part of the study, kinetic studies on histological sections were done to correlate influx of neutrophils and monocytes to the onset of cartilage degradation. Subsequently the involvement of these cell types in cartilage degradation was studied by complete or selective blocking of the influx of inflammatory cells into the arthritic joint. Complete inhibition of cell influx was achieved with anti-adhesion antibodies or total body irradiation. Selective inhibition of PMN influx was obtained by IL-1ra treatment, in line with the recent observation that IL-1ra inhibits chemokine macrophage inhibiting factor (MIP-2), responsible for PMN attraction in the kidney [19]. The effect on cartilage degradation was measured either semi-quantitatively in total knee joint sections stained with safranin-o or quantitatively by measuring the release of [35S]sulphate-labeled proteoglycans from the patellar cartilage matrix. Our study suggests that monocytes rather than PMN are involved in early cartilage damage during acute arthritis.

**MATERIALS AND METHODS**

**Animals**

Normal male C57 BL/6 mice were obtained from Jackson. They were fed a standard diet and tap water ad libitum. They were used between 8 and 12 weeks of age.

**Chemicals**

Poly-L-lysine (PLL), lysozyme, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide were obtained from Sigma Chemical Company, St. Louis, MO. N,N-dimethyl-1, 3-propanediamine was obtained from BDH Chemicals Ltd., Poole, U.K.

**Lysozyme coupling to PLL**

Lysozyme was coupled to PLL according to the method of Danon [20] using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as an activator and PLL as a nucleophil as described previously [16]. Free carboxyl groups of the protein were then coupled to amino groups of PLL. The molecular mass was raised, whereas the isoelectric point remained high, as was determined in a 5% polyacrylamide slab gel with 0.8% ampholines (pH gradient from 3.5 to 9.5). After coupling lysozyme to PLL, the products were purified by gel chromatography (Sephadex G75). The first peak coming from the column was used for eliciting ICA. The molecular mass appeared to be 74 kDa, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

**Induction of ICA**

Three micrograms of PLL-coupled lysozyme were injected into the right knee joint of mice that were previously (4 h before PLL-lysozyme injection) given specific antisera (0.2 mL) directed against lysozyme intravenously. These antisera were raised in rabbits and the titer for lysozyme appeared to be 15 (serum dilution) as measured with an enzyme-linked immunosorbent assay [16]. The antisera was decomplemented by heating at 56 °C for 30 min. The left knee joint was injected with saline and served as control. Additional controls were mice that got normal rabbit serum instead of specific anti-lysozyme. Intra-articular injection of 3 μg of PLL:lys into the knee joint in the absence of specific anti-lysozyme antibodies did not cause inflammation or cartilage damage.

**Histology**

Total knee joints of mice were isolated 1, 3, 6, 9, 12, 18, 24, or 48 h after the induction of arthritis. For standard histology, tissue was fixed in 4% formaldehyde in phosphate-buffered saline during 3 days at room temperature, decalcified in 5% formic acid in phosphate-buffered saline during 7 days, and subsequently dehydrated and embedded in paraffin. Paraffin sections were cut at 7 μm and mounted on gelatin-coated slides. Hematoxylin/eosin (H & E) staining was performed to study the inflammatory cells and safranin-o staining was performed for studying proteoglycan depletion of the cartilage matrix [21].

**Measurement of inflammation and cell influx**

H & E-stained sections were used to measure the severity of the inflammation. Infiltrate and exudate were scored separately. Infiltrate is defined as influx of leukocytes in the synovial layer, whereas exudate represents the influx of leukocytes in the joint cavity. The severity was determined using an arbitrary score (0–3), performed blindly by two observers. Mild cellularity throughout the joint was scored 1, higher cellularity 2, and very high cellularity was scored 3.

Furthermore, H & E-stained sections were also used to make a distinction between infiltrating PMN and mononuclear cells. This distinction could be made on the basis of cellular morphology using a magnification of 400×. The amount and type of cell were determined in a well-defined area in synovium and synovial cavity by use of an ocular graticule. The infiltrated cells were determined in the synovial layer ventrally at the lateral and medial side of the patella. The exudate cells were determined in the joint cavity adjacent to areas in which infiltrate was measured. Five serial sections spacing 140 μm of each animal were counted.

**In vivo manipulation of cell influx**

Manipulation of cell influx was accomplished by various approaches. In the first approach, mice were depleted of neutrophils by total body irradiation. A single dose of 7.5 Gy was applied at a dose rate of 2 Gy/min, with a 13-MeV electron beam from a linear accelerator (CGR Saturne, Buc, France). Treatment was started 4 days before induction of arthritis.

In the second approach, monoclonal antibodies directed against CR3 (5C6: rat anti mouse: IgG2b, kindly provided by S. Gordon, University of Oxford) or a 25- to 30-kDa epitope mainly present on neutrophils (NIMP.R14: rat anti-mouse: IgG2b, kindly provided by M. Strath, National Institute for Medical Research, London) were used. The IgG fraction present in the ascitis fluid was affinity purified by protein G. Both antibodies have been shown to inhibit migration of cells into inflammatory fields [22, 23]. Binding of these monoclonal antibodies to activated PMNs and peritoneal monocytes/macrophages was tested by FACS analysis.

Monoclonal antibodies (either 5C6 or NIMP.R14, 0.5 mg) were given 18 h before arthritis induction. In control groups, irrelevant IgG2b monoclonal antibodies were given.

In the third approach, selective blockade of neutrophil influx was accomplished with the use of human IL-1ra (kindly provided by Synergen,
Fig. 1. Total knee joint sections of mice were screened for infiltrate and exudate cells 1, 3, 6, 9, 12, 18, 24, and 48 h after induction of ICA. Synovial infiltration and exudate cells were scored on five semi-serial sections of each specimen, spaced 140 μm apart (A). In each section, infiltrate and exudate was graded in medial and lateral areas extending from the patella to the tibia. Scoring was done in a blinded fashion by two observers. Synovial infiltrate and joint cavity exudate were graded on a scale from 0 to 3, according to the amount of inflammatory cells in synovium and cavity, respectively. Maximal cellular influx is defined as the most that was observed in a given set of experiments. 0, no influx; 1, minimal influx; 2, marked influx; 3, maximal influx. Seven mice were measured at each time point. Mean values ± sd were determined. Note that inflammatory cells infiltrate the joint cavity at the synovial/cartilage junction at 3 (B) and 6 h (C). More cells scattered throughout the synovial layer were found at 24 h (D). In control knee joints in which phosphate-buffered saline was injected no cell influx was observed (data not shown; H & E; original magnification × 100). F, femur; P, patella; JS, joint space; S, synovial layer.


**RESULTS**

**Kinetics and topography of cell influx and cartilage degradation in early ICA**

To relate kinetics and location of cartilage degradation to influx of PMNs and monocytes at defined sites of the joint, total knee joints were dissected at various time points (1, 3, 6, 9, 12, 18, 24, and 48 h) after arthritis induction. Sections were stained with H & E. Figure 1A shows that cell influx started at 3 h. Infiltrate shows a continuing increase, which reached its maximum at 24 h, whereas exudate reached maximal values at 18 h. Cells mainly entered through blood vessels lying in the synovial layer adjacent to the cartilage layers between 3 (Fig. 1B) and 6 h (Fig. 1C) and were found throughout the synovium at 24 h (Fig. 1D).

Characterizing the inflammatory cells by light microscopic examination at high magnification it was found that PMNs first entered the synovial layer at 3 h followed by mononuclear cells at 6 h after ICA onset (Fig. 2). At 6 h, the contribution of monocytes to the total amount of inflammatory cells was only 10%, but increased thereafter to 30 and 50% at 24 and 48 h, respectively (Fig. 2).

In addition, cartilage degradation was measured in safranin-o-stained sections of the same joints. PG degradation was scored in an arbitrary way by determining loss of red staining from the cartilage matrix. Figure 3A shows that PG degradation started between 12 and 18 h after arthritis induction, thus at a time-point that both PMN and mononuclear cells were present in the joint. PG loss started at the edge of the cartilage layers of patella and femur just adjacent to the blood vessels through which the inflammatory cells entered the joint (Fig. 3, B and C). PG loss was

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**Measurement of PG degradation**

**Semi-quantitative**

Total knee joint sections were stained with safranin-o (0.1% safranin-o in aqua dest during 10 min at room temperature). The intensity of red staining of the cartilage matrix is a measure for PG content in the cartilage layer. PG loss was expressed as the percentage of total red staining of control noninflamed intact patellar or femoral layers. An arbitrary scale of 0–3 was used with the following range: 0, zero; 1, low; 2, medium; 3, high PG loss.

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**Quantitative**

To study the effect of ICA on in vivo degradation of GAGs, patellas were prelabeled by intraperitoneal injection of carrier-free sodium sulfate in RPMI ($^{35}$SO$_4$, Du Pont, Boston, MA; 50 μCi/mouse), 4 h before ICA induction. $^{35}$S was incorporated in newly formed GAGs. More than 90% of the incorporated radioactivity was confined to the patellar cartilage layer compared with the whole patella [26]. After $^{35}$S incorporation, patellas were isolated and patellar content was determined according to the method of Van den Berg et al. [27]. In brief, patellas were fixed in 2% formaldehyde and decalcified in 4% formic acid. After this treatment the patella can easily be separated from the surrounding tissue and the labeled PG content can be determined. Cartilage degradation is defined as loss of $^{35}$S from the inflamed patella. The loss of $^{35}$S is measured by comparison of $^{35}$S content of the arthritic patella and the contralateral non-arthritic patella.
Fig. 3. Loss of proteoglycans from the cartilage was determined 1, 3, 6, 9, 12, 18, 24, and 48 h after ICA induction. Proteoglycan degradation was measured by loss of red staining in total knee joint sections stained by safranin-o. Red staining was measured using an arbitrary scale from 0 to 3 (A), according to the amount of PG loss in patella and femur, respectively. Maximal PG loss is defined as the most that was observed in a given set of experiments. PG loss: 0, zero; 1, low; 2, medium; 3, high. Values represent the mean ± sd of seven mice per time point. No PG loss was seen 1 h after ICA induction (B). PG loss started at the synovial/cartilage junction between 12 and 18 h (C). Maximal PG loss was observed at 48 h after ICA induction (D). Injection of phosphate-buffered saline alone has no effect on PG loss (data not shown; safranin-o, original magnification × 100). P, patella; S, synovium; F, femur; JS, joint space; C, cartilage.
The amount of infiltrate and exudate cells was determined in total knee joint sections of mice 24 and 48 h after ICA induction in control and IL-1ra-treated mice. Control mice had empty osmotic pumps implanted in the peritoneal cavity. Seven mice were measured at each time point. Synovial infiltrate and joint cavity exudate were graded on a scale from 0 to 3 according to the amount of inflammatory cells present in synovium and joint cavity, respectively: 0, no influx; 1, minimal influx; 2, marked influx; 3, maximal influx. Values were statistically evaluated using the Mann-Whitney U test (*P < 0.05). Note the significant lower amounts of inflammatory cells in infiltrate at day 1 and both exudate and infiltrate at day 2 after IL-1ra treatment.

maximal at 48 h after arthritis induction (Fig. 3D) and recovered thereafter. Full replenishment of PG in the cartilage matrix was found at day 7 after induction (data not shown).

Selective blockade of neutrophil but not monocyte influx by IL-1ra treatment

Because both PMN and mononuclear cells are present during the onset of early cartilage degradation, blockade of one of the cell types can give information about the contribution of the other cell type in cartilage degradation. The approach we chose was blocking IL-1 effects by IL-1ra, which recently has been shown to selectively block PMN influx in inflamed areas of the kidney [19]. IL-1ra was given systemically, using mini-osmotic pumps that continuously release 1.2 mg IL-1ra per day. Implantation of mini-osmotic pumps was done 2 days before arthritis induction to reduce stress. To check the efficacy of IL-1ra treatment, washouts of synovial specimen, taken 6 h after the onset of arthritis, were tested for IL-1, using a sensitive IL-1-bioassay. In contrast to IL-1 levels in arthritic controls (20–40 pg/mL), IL-1 could not be detected in the IL-1ra group (below 1 pg/mL). Studying hematoxylin-stained knee joint sections we found in the IL-1ra-treated group a significant decrease in influx of inflammatory cells both at day 1 and day 2 after arthritis induction: synovial infiltrate and exudate was significantly inhibited at day 1 and 2, respectively (Fig. 4). In IL-1ra-treated animals, inflammatory cells were mainly detected within the synovial layer and only a few cells resided within the joint space, in contrast to control arthritic joints in which numerous inflammatory cells were detected both in synovium and joint space.

Selective effects on influx of particular cell types was further analyzed by H & E staining. In the IL-1ra-treated group, neutrophils were significantly decreased at days 1 and 2 after arthritis onset (Table 1 and Fig. 5, A and B). It was of interest that the absolute number of mononuclear cells was not changed by the IL-1ra treatment (Table 1). This was found both in infiltrate and exudate. High-power micrographs show abundant PMN and monocytes/macrophages in day 2 arthritic joints (Fig. 5C), whereas in IL-1ra-treated arthritic joints predominantly monocytes/macrophages were found (Fig. 5D).

Monocyte influx during ICA is sufficient for the larger part of cartilage degradation

PG loss of patellar and femoral surfaces was scored semiquantitatively in safranin-o-stained sections. Surprisingly, although IL-1ra treatment almost completely inhibited influx of neutrophils, PG loss (Fig. 6) was still high. In the IL-1ra-treated mice, PG loss as detected by loss of red staining was similar at the lateral but less at the medial site of the cartilage layer if compared with control arthritis. IL-1ra treatment inhibited PG loss from patella and femur not significantly at day 1 and day 2 compared with arthritic controls. Furthermore, we determined PG loss quantitatively by measuring loss of prelabeled 35S proteoglycans from patellas. At day 2 after ICA induction 62% degradation of proteoglycans was found. In the IL-1ra-treated group, there was still 47% PG release from the cartilage layer, which was not significantly different from control arthritic joints.

Complete blockade of PMN and mononuclear cell influx by total body irradiation or anti-adhesion antibodies

Whether inflammatory cells are necessary for cartilage degradation seen within this model was further studied by total blockade of cell influx at the onset of arthritis using either total body irradiation or antibodies directed against membrane epitopes (5C6;NIMPR14). The latter antibodies

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<th>Treatment</th>
<th>Neutrophils</th>
<th>Monocytes</th>
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<tr>
<td></td>
<td>Infiltrate</td>
<td>Exudate</td>
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<tr>
<td>ICA day 1</td>
<td>119 ± 36</td>
<td>84 ± 12</td>
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<tr>
<td>ICA day 2</td>
<td>18 ± 3*</td>
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<td>ICA day 2</td>
<td>145 ± 14</td>
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<td>ICA day 2</td>
<td>25 ± 6*</td>
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Absolute numbers of PMN and monocytes/macrophages were determined in infiltrate and exudate cells 1 and 2 days after ICA induction in control and IL-1ra-treated mice. Cell numbers were counted at a well-defined area in synovium and joint cavity between patella and femur using an ocular graticule (magnification × 400). Note that IL-1ra treatment significantly diminished PG numbers but not monocyte numbers. Five serial sections per animal were studied. Values represent the mean ± so of six animals. *P < 0.05 by the Mann-Whitney U test.
were tested by FACS analysis using activated murine PMNs or mononuclear cells. 5C6 (anti-CD11b CD18) preferentially binds to mononuclear cells and less to PMNs. In contrast NIMPRI4 predominantly binds to PMNs and only weakly to mononuclear cells (Fig. 7). 5C6 treatment had no effect on numbers of peripheral PMNs or mononuclear
Fig. 6. The effect of IL-1ra treatment on cartilage degradation was determined 24 and 48 h after ICA induction either semi-quantitatively by loss of red staining in patella and femur in total knee joint sections stained by safranin-o or quantitatively by release of 35S-labeled proteoglycans from patellar cartilage. Red staining was measured using an arbitrary scale from 0 to 3. PG loss: 0, zero; 1, low; 2, medium; 3, high. In IL-1ra-treated animals, cartilage degradation was still high (not significant from controls) both 24 (A, D) and 48 h (B, E) after ICA induction. Loss of 35S prelabeled PG was measured at 48 h after ICA induction. PG content in the patella derived from the arthritic joint (arthritic) is compared with PG content in the patella from the noninflamed contralateral knee joint. Again high PG loss was observed in the IL-1ra-treated group (47%) not significantly different from that observed in control arthritis (62%; C). Values represent the mean ± sd of two experiments. In each experiment at least six mice per time point were tested. Values were statistically evaluated using the Mann-Whitney U test (* P < 0.05).
concentration (ng/ml)

Fig. 7. Binding of monoclonal antibodies to activated PMNs and monocytes/macrophages were tested by FACS analysis. PMNs or monocyte/macrophages were incubated with monoclonal antibody (NIMPR14;5C6) or no monoclonal antibody (irrelevant IgG2b) for control, additionally stained with FITC-conjugated goat anti-rat and analyzed by FACS. NIMP.R14 preferentially bound activated PMN (A), whereas 5C6 preferentially bound normal or activated monocytes/macrophages (B). No significant binding of control monoclonal antibody was found (data not shown). Peritoneal exudate cells were isolated from normal mice or from mice 2 days after intraperitoneal injection of 40 pg of LPS. The plastic adherent cells appeared to be monocytes/macrophages. PEC, peritoneal exudate cells; LPS PEC, lipopolysaccharide-stimulated peritoneal exudate cells; PMN, polymorphonuclear cells; GFL, mean green fluorescence per cell.

Fig. 8A: Binding of monoclonal antibodies to activated PMNs and monocytes/macrophages were tested by ELISA. PMNs or monocyte/macrophages were incubated with monoclonal antibody (NIMPR14;5C6) or no monoclonal antibody (irrelevant IgG2b) for control, additionally stained with FITC-conjugated goat anti-rat and analyzed by FACS. NIMP.R14 preferentially bound activated PMN, whereas 5C6 preferentially bound normal or activated monocytes/macrophages. No significant binding of control monoclonal antibody was found (data not shown).

DISCUSSION

In this study we obtained evidence that in an arthritic knee joint in the absence of infiltrating inflammatory cells PG loss was almost completely blocked. Moreover, in the presence of merely monocytes/macrophages, severe PG loss is found not significantly different from PG loss found in control arthritic joints in the presence of both neutrophils and monocytes/macrophages.

Migration of both neutrophils and monocytes to the inflamed knee joint in our non-T cell-mediated ICA model was fully blocked by the monoclonal antibody 5C6 (anti-CD11b) or NIMPR14. Although 5C6 binds preferentially to monocytes/macrophages, whereas NIMPR14 binds preferentially to PMN, some binding was found to the other cell type. Blockade of 5C6 or NIMPR14 epitopes on both cell types might explain the full blockade of cell influx after ICA induction. Moreover these antibodies may have their effect after binding of inflammatory cells to endothelial cells, which may result in reduction of adhesion molecules necessary for penetration.

CD11b seems to be a crucial adhesion molecule involved in cell migration to non-T cell-mediated inflammatory areas because full blockade was also found in the pulmonary response to aerosolized lipopolysaccharide [28] or the cutaneous response to tumor necrosis factor (Shwartzmann reaction) [28]. In T cell-mediated inflammatory processes, however, such as adjuvant arthritis, CD11a and CD11c appeared to be more important [29], NIMPR14, which is directed against an unknown 20- to 25-kDa surface protein and preferentially binds to neutrophils also completely prevented influx of both cell types. Similar results were again found in the Shwartzmann reaction [23].

It was of interest that neither NIMPR14 nor 5C6 treatment was able to inhibit cell influx completely in the T cell-mediated antigen-induced arthritis [unpublished results], suggesting that, in this model, different adhesion molecules are important for cell influx in the joint.

Full blockade of inflammatory cells to the arthritic joint prevented cartilage degradation from 60% in controls to 5—10% in leukopenic joints. Similar results were found in knee joints of neutropenic rabbits in which arthritis was induced by either immune complexes [17] or LPS [30]. Our findings indicate that sole activation of resident cells of the synovial tissue by immune complexes, in the absence of cell influx, is not causing major cartilage degradation. However, we did find marked production of inflammatory mediators like IL-1 in murine leukopenic arthritic joints, which appeared to be even higher than in control arthritic joints [18]. Local production of IL-1 by resident cells may well be responsible for the 5—10% PG loss, as measured in neutropenic arthritic joints, by activating chondrocytes cells as detected by hemalog. NIMPR14 treatment, however, showed 30—40% reduction of peripheral PMNs. Both irradiation or pretreatment of mice with 0.5 mg of either intravenous NIMPR14 or 5C6 completely prevented cell influx as seen 48 h after arthritis induction (Fig. 8A).

Cell influx is necessary for cartilage degradation

PG loss as measured in safranin-o-stained sections was almost absent in irradiated or antibody-treated arthritic joints (Fig. 8B). If cartilage degradation was measured by loss of 35S-prelabeled proteoglycans we found between 50 and 60% degradation in control arthritic joints. Prevention of cell influx almost completely protected cartilage destruction and proteoglycan loss was only between 5 and 10% (Fig. 8C). Nevertheless there was still production of inflammatory mediators in such joints. IL-1 production was similar or even elevated in arthritic joints devoid of leukocytes (data not shown).
or synovial cells to release PG-degrading enzymes. Although the amount of IL-1 formed in the leucopenic arthritic joint still causes high inhibition of PG synthesis [18], it may be too low to cause severe PG loss. Higher concentrations of IL-1 are needed to cause PG degradation compared with PG synthesis. Moreover, direct effects of IL-1 on PG loss are only marginal in mouse cartilage if compared with, e.g., bovine cartilage [31, 32].

In addition, we selectively blocked the neutrophil from entering the arthritic joint. Recent studies done by Tam et al. have shown that IL-1ra treatment during acute glomerulonephritis preferentially inhibited neutrophil influx,
whereas monocyte influx was hardly affected [19]. IL-1ra treatment inhibited mRNA levels of the neutrophil chemoattractant factor MIP-2 by 72%, whereas the monocyte chemoattractant factor (MCP-1) was only inhibited by 27%. In our study the number of monocytes was not altered significantly by the IL-1ra treatment, whereas PMN influx decreased considerably. These results suggest that IL-1 is not responsible for monocyte influx during ICA. Other cytokines like interferon-γ [33] or transforming growth factor-β [34] or direct activation of resident cells by cationic IC may be responsible for production of chemotactic factors directing monocyte influx, like MCP-1.

We further studied cartilage degradation quantitatively by 35S-PG release and found that PG degradation continued for the larger part in the IL-1ra treated group (from 62% in controls to 47% in IL-1ra-treated animals). In combination with the finding that the minor loss of PG (5–10%) in neutropenic arthritic joints is probably mediated by IL-1, this strongly suggests that cartilage destruction in this IC model is caused by an IL-1-independent monocyte/macrophage activation. In other models like antigen-induced arthritis, we also found that, using IL-1ra treatment, IL-1 is not a key mediator directly involved in the PG degradation observed in the acute phase of this arthritis [24], but has a role in synovial cell activation and propagation of late PG degradation. Moreover, IL-1 has been shown to be the crucial cytokine involved in inhibition of PG synthesis [35]. So in the recovery phase starting shortly after the initial PG degradation, the absence of IL-1 allows for restoration of the PG content in the cartilage [24]. Also in ICA, a more rapid recovery of PG content was observed in IL-1ra-treated animals at day 7 (data not shown) but such an effect was not seen as early as day 2 after ICA.

In control ICA, cartilage degradation started between 12 and 18 h, a time-point at which monocytes comprise only 10–30% of the total inflammatory cell population. In control arthritic joints, PG loss started at the synovial cartilage junction. A similar pattern of cartilage degradation was found in IL-1ra-treated animals. At these sites, the majority of monocytes/macrophages but also expression of pro-inflammatory cytokines are detected [36]. This might suggest that monocytes residing in the synovial layer near the cartilage might be more important in cartilage degradation than exudeate cells entering the joint space. If substances released by free-floating cells like enzymes or oxygen radicals were important for cartilage degradation, one would expect a more homogenous release of PG from patellar, tibial, and femural surfaces because joint movement will equally disperse enzymes in the joint space. The released substances are probably neutralized in the synovial fluid, which has been shown to contain a wide array of inhibitors [37].

The monocyte/macrophage produces more than one hundred substances ranging from 30 (nitric oxide) to 440,000 daltons (fibronectin) [38]. Proteinases like metalloproteinases are synthesized after stimulation and production takes some time. PMN release their contents directly after stimulation. The time gap of 9 h between the first appearance of neutrophils and cartilage degradation does not favor this cell type as important for degradation, although it could be a cumulative effect. Furthermore, the start of cartilage degradation at the synovial/cartilage junction at the moment that monocytes are present might suggest that released proteinases might escape from enzyme inhibitors present in the synovial fluid by the close contact.

In addition, monocytes/macrophages may act indirectly by activating fibroblasts. Studies done by Janusz and Hare showed that the interaction between these two cell types elevated cartilage degradation [15]. During activation of synovial cells many enzymes are released, most of them in their inactive form. Monocytes have been shown to produce urokinase-type plasminogen activator (u-PA) and tissue-type plasminogen activator (t-PA) [39]. These substances might increase proteolytic activity by transforming the enzyme proform into its active counterparts. Furthermore monocytes/macrophages are important producers of cytokines like IL-1 or tumor necrosis factor, which have been shown to activate fibroblasts or resident lining macrophages that have been shown to direct the larger part of the inflammatory response [40, 41]. Under chronic inflammatory conditions the contribution of infiltrating inflammatory cells might be less important [42]. Under such circumstances, resident fibroblasts and macrophages might become more sensitive and develop higher potency to degrade cartilage components after contact with cell-activating triggers.

This study indicates that in vivo the monocyte rather than the neutrophil seems to be involved in cartilage degradation during arthritis induced by immune complexes. Recent clinical studies have claimed that, during rheumatoid arthritis, macrophages rather than other cell types like T or B cells show strong correlations to cartilage degradation [8, 43]. As immune complexes are generally found in the synovial and cartilagenous layers of rheumatoid arthritis patients [44], this mechanism may well play a role in cartilage damage that develops during early rheumatoid arthritis. Because activated monocytes/macrophages do release a spectrum of substances that may damage cartilage either directly or indirectly, a therapy that leads to suppression of the activated monocyte/macrophage might be promising.

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