Polymorphonuclear granulocytes in rheumatic tissue destruction. III. An electron microscopic study of PMNs at the pannus-cartilage junction in rheumatoid arthritis

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SUMMARY Metatarsophalangeal and metacarpophalangeal joints from 3 patients with rheumatoid arthritis were investigated electron microscopically with regard to the occurrence of polymorphonuclear granulocytes (PMNs) at the pannus-cartilage junction. In all 3 cases PMNs could be detected at the junction and within the cartilaginous matrix. PMN cytoplasmic processes surrounded collagenous islands in the cartilage. From the morphological findings it is deduced that PMNs are cells capable of destroying cartilage in inflammatory joint diseases, in particular in rheumatoid arthritis.

Using enzyme histochemical and immune histological investigations we recently demonstrated the appearance of polymorphonuclear granulocytes (PMNs) at the pannus-cartilage junction in rheumatoid joints.1-4 As some doubt remains about the specificity of the naphthol-AS-D-chloroacetate esterase reaction5 for the demonstration of PMNs with regard to the content of this enzyme in monocytes,6 we tried to characterise PMNs at the pannus-cartilage junction by electron microscopy.

Material and methods

Surgically removed metatarsophalangeal and metacarpophalangeal joints were fixed in buffered formalin. After decalcification in Versen (Titriplex III) the specimens were bisected. One half of the tissue was processed by the routine paraffin method, stained with haematoxylin-eosin and the naphthol-AS-D-chloroacetate esterase reaction.5 The second half was stored in formalin. Joints from 3 patients (Table 1) with accumulations of PMNs at the pannus-cartilage junction, proved by light microscopy, were chosen for electron microscopy. From areas corresponding to the histological PMN-rich foci small pieces of the articular cartilage with adherent pannus tissue were dissected and rinsed in water. Thereafter they were postfixed in osmic acid, dehydrated, and embedded in Araldite. Semithin sections were prepared with a Reichert Ultramicrotome OMU 3 and stained with toluidine blue. The ultrathin sections were stained with uranyl acetate and lead citrate. The sections were examined with a Zeiss EM 10 electron microscope.

Table 1 Summary of the clinical data of the patients

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Clinical diagnosis</th>
<th>Duration of disease (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 16951/78</td>
<td>f</td>
<td>61</td>
<td>RA</td>
<td>16</td>
</tr>
<tr>
<td>E 8528/79</td>
<td>m</td>
<td>60</td>
<td>RA</td>
<td>36</td>
</tr>
<tr>
<td>E 20266/79</td>
<td>f</td>
<td>60</td>
<td>RA</td>
<td>8</td>
</tr>
</tbody>
</table>

Results

Even in the formalin prefixed tissue ultrastructural details of the cartilaginous matrix and the cells were remarkably well preserved. Vacuoles in pannus tissue cells may be due to this fixation process. PMNs identified by their condensed nuclear chromatin, electron-dense cytoplasmic granules, intracytoplasmic lipid droplets, and the absence of rough endoplasmic reticulum were found in each of the 3 patients. These inflammatory cells were rarely present in the pannus tissue outside the cartilage. However, they often appeared in the immediate vicinity of the cartilage in the pannus-cartilage
junction (Fig. 1). In some areas PMNs had invaded deep into the cartilage matrix, surrounded by the collagenous fibres of the cartilage. Some inflammatory cells were even observed in chondrocytic lacunae with remnants of chondrocytes (Fig. 2). In most instances the PMNs were surrounded by a distinctive cellular membrane. Cytoplasmic processes of these inflammatory cells extended deep into the cartilaginous matrix. Islands of collagenous fibres were separated by the cytoplasmic processes from the adjacent cartilage (Fig. 3). The collagenous fibres usually showed a preserved periodicity. However, in some instances small fibres without obvious periodicity were present in the neighbourhood of the PMNs.

Discussion

In the inflammatory destruction of cartilage proteoglycans and collagenous fibres are destroyed.

Enzymes of mononuclear inflammatory cells and of synoviocytes are usually regarded as the most important destructive agents. However, there is some evidence that chondrocytes themselves may be involved in cartilage degradation.

The participation of PMNs in rheumatic tissue destruction is usually neglected. According to Hadler et al., there is no positive correlation between the activity of lysosomal enzymes in the synovial fluid and the degree of cartilage destruction. Moreover it is said that PMNs are absent at the pannus-cartilage junction.

In contrast to the results of the morphological investigations quoted above Mohr and Wessinghage demonstrated the presence of PMNs at the pannus-cartilage border by a histochemical study. Moreover by the combination of histochemical and immunohistological methods PMNs were detected at the pannus-cartilage junction.

The present ultrastructural investigation confirms these previous results. By electron microscopy PMNs were observed as isolated cells attached to the cartilage or invading the cartilaginous matrix and even chondrocytic lacunae. In some areas PMNs were seen accumulating at the pannus-cartilage border as described by Ugai et al. in their in-vitro experiments using the incubation of cartilage (from BSA-injected joints of immunised rabbits) with PMNs.
The infiltration of the cartilage by PMNs is by no means unknown. It has been reported in human septic arthritis\textsuperscript{24–27} and is also found in experimental adjuvant arthritis in the rat.\textsuperscript{28, 29}

However, the morphological relationship between these inflammatory cells and cartilage does not necessarily mean the participation of these cells in cartilage destruction. Nevertheless, this localisation of the inflammatory cells may be regarded as a possible way by which enzymes of PMNs, capable of degrading proteoglycans\textsuperscript{30–34} and collagenous fibres\textsuperscript{34, 35} may attack cartilage in a microenvironment in the absence of serum inhibitors.

The stimulus responsible for the localisation of PMNs at the pannus-cartilage junction remains obscure. Ugai \textit{et al.}\textsuperscript{38} observed the invasion by PMNs of cartilage pretreated with immune complexes under in-vitro conditions. Whether such a chemotactic mechanism is at work in rheumatoid arthritis is unknown. Moreover, it is possible that cartilage merely acts as a mechanical barrier, thus inhibiting the random migration of PMNs in pannus, resulting in the accumulation of these inflammatory cells at the borderline of the cartilage barrier.\textsuperscript{36} Bacterial remnants were not observed in the present study. However, further investigations should be made to ensure that the described phenomenon is not due to an infectious agent. A surgically induced infiltration of the pannus tissue may be excluded: vascular margination of PMNs was usually absent in the area of accumulation of the inflammatory cells at the pannus-cartilage junction. Intra-articular injections were not performed in these patients during the preoperative period. Finally, it should be mentioned that the presence of PMNs in fibrinoid necrosis of rheumatoid nodules\textsuperscript{37, 38} also favours the idea that these cells are involved in rheumatic tissue destruction.

It is not the aim of this paper to explain inflammatory destruction of cartilage purely on the basis of granulocytic enzymes. From investigations in recent years it has been learned that other cells (monocytes,\textsuperscript{7} the dendritic cell,\textsuperscript{39–40} and synoviocytes,\textsuperscript{7, 8, 10, 11}) are also involved. It therefore appears that inflammatory destruction of cartilage in the rheumatoid joint is probably an enzymatic process due to several kind of cells: it is not a process attributable solely to mononuclear macrophages.

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References


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