Pannus tissue at the cartilage-synovium junction in rheumatoid arthritis.

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Abstract

The cartilage-synovium junction of knees afflicted with rheumatoid arthritis was observed light microscopically using formalin-fixed, decalcified and immunohistochemically stained tissues. Decalcification had little or no influence on immunoreactivity for lysozyme and S-100 protein. All the specimens had pannus formation, which was classified into four types: A) cellular pannus with homogeneous cell pattern, B) cellular pannus of inflammatory cells, C) fibrous pannus with many fibrous bundles, D) fibrous pannus including round cells with scattered fibrous bundles. Type A pannus may be responsible for extensive cartilage degradation, and may occur at the first stage of pannus formation. Type B pannus may occur afterwards, and may be followed by type C pannus at a later stage. Type D pannus was found in two out of 19 specimens. Round cells in type D were positive for S-100 protein and lysozyme, and were probably chondrocytes. The findings indicated that chondrocytes were responsible for cartilage degradation and pannus formation.

Pannus Tissue at the Cartilage-Synovium Junction in Rheumatoid Arthritis

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The cartilage-synovium junction of knees afflicted with rheumatoid arthritis was observed light microscopically using formalin-fixed, decalcified and immunohistochemically stained tissues. Decalcification had little or no influence on immunoreactivity for lysozyme and S-100 protein. All the specimens had pannus formation, which was classified into four types: A) cellular pannus with homogeneous cell pattern, B) cellular pannus of inflammatory cells, C) fibrous pannus with many fibrous bundles, D) fibrous pannus including round cells with scattered fibrous bundles. Type A pannus may be responsible for extensive cartilage degradation, and may occur at the first stage of pannus formation. Type B pannus may occur afterwards, and may be followed by type C pannus at a later stage. Type D pannus was found in two out of 19 specimens. Round cells in type D were positive for S-100 protein and lysozyme, and were probably chondrocytes. The findings indicated that chondrocytes were responsible for cartilage degradation and pannus formation.

Key words : rheumatoid arthritis, cartilage-synovium junction, pannus, S-100 protein, lysozyme

Progressive erosion of the articular cartilage by granulation tissue is a characteristic feature of joint destruction in rheumatoid arthritis (RA) (1). The granulation tissue at the cartilage-synovium junction is proliferative and invasive, and overlays the cartilage to form a pannus. Morphologically, the cartilage-synovium junction consists mainly of macrophage-like cells, fibroblasts, polymorphonuclear cells and "tumor cell-like" immature mesenchymoid cells (2-5). These cells are considered to be derived from synovium-lining cells or to have infil-trated from subsynovial tissue or from the synovial fluid. Histochemical observations have confirmed the presence of activity of lysosomal enzymes in chondrocytes, suggesting that chondrolysis may be caused by some lysosomal enzymes of the chondrocyte itself. The origin of the rheumatoid pannus is still controversial, and recent histologic observations suggested that the pannus tissue over the cartilage in rheumatoid joints may originate from the cartilage itself (6).

Immunohistochemical techniques are used to investigate the localization of enzymes and immune complexes (7-9). Little or no influence of decalcification on immunohistochemi-

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cal staining of formalin-fixed tissue has been reported (10, 11). In this study, cellular derivation at the site of cartilage erosion of formalin-fixed, paraffin-embedded tissues was examined by the peroxidase-antiperoxidase (PAP) method to elucidate the mechanism of joint destruction in RA.

Materials and Methods

Specimens of the cartilage-synovium junction were obtained during total knee replacement from the edge of the patellofemoral aspect of the femur of 19 patients with definite or classical RA. Areas to be examined were selected with the naked eye among the eroded areas of this site, and the cartilage of weight bearing surfaces was avoided so as to exclude mechanical factors of cartilage degradation. The pannus area, if any, was taken with underlying bone. Clinical data of the 19 patients are shown in Table 1. Specimens of the cartilage-synovium junction from 6 patients with osteoarthritis were added for comparison.

The samples were fixed in 10% phosphate-buffered formalin, sliced 3-5 mm thick, decalcified in 10% formic acid for 24 h, dehydrated, and paraffin-embedded. Tissue blocks were sectioned every

3 μm, and sections were dewaxed with xylol and processed to absolute ethanol. These sections of each block were stained with hematoxylin-eosin (H.E.), Safranin-O (12), and PAP (13, 14) for lysozyme, S-100 protein, IgG, IgA and IgM. Primary antisera, secondary antiserum, normal swine serum and PAP complex were obtained from Dakopatts, Denmark (Japan agent: Kyowa Medix, Tokyo). Anti-lysozyme serum and anti-S-100 protein serum were diluted 1:200. Secondary antiserum and PAP complex of Dako universal PAP kit were used. Anti-immunoglobulin sera of Dako PAP kit were used. Trypsinization was carried out for the sections for IgG, IgA and IgM according to the data of Matthews (10). 3,3'-Diaminobenzidine (DAB) was obtained from Sigma Chemical Company, St. Louis, Mo, USA. For negative controls, one slice of each sample was not treated with primary antiserum. For the positive control for S-100 protein, one each of decalcified and non-decalcified schwannoma specimens was stained by the same method. For the positive control for other staining methods, a non-decalcified RA synovium specimen was used. The immunoreactivity between decalcified and non-decalcified specimens was compared. The cartilage-synovium junction of each specimen was examined under a light microscope (Olympus BH-2, Olympus Optical Co., Ltd., Tokyo).

Table 1 Clinical data of the patients with rheumatoid arthritis (RA)

Case No.	Age	Sex	ESR[a] (1h/2h)	RA[b]	CRP[c] (mg/dl)
1	65	F	52/89	+	3.1
2	62	F	94/130	−	8.2
3	55	F	54/96	++	1.0
4	71	F	35/71	++	7.6
5	59	F	84/119	+	13.3
6	73	F	52/90	−	3.2
7	74	F	27/45	−	1.5
8	69	F	107/131	+	9.8
9	57	F	38/78	+	3.6
10	73	F	87/121	++	10.1
11	74	M	89/128	++	16.2
12	62	F	116/147	++	8.4
13	78	F	68/95	++	8.6
14	54	F	41/84	++	4.9
15	56	F	55/95	+	4.8
16	63	F	69/106	++	5.5
17	45	F	82/117	++	3.9
18	48	F	33/69	+	5.5
19	66	F	152/156	+	12.3
Mean±SD	63.1±9.12		70.3±31.8/103.5±27.8		6.92±4.02

a: Erythrocyte sedimentation rate (mm)
b: Rheumatoid arthiitis test (−, +, ++)
c: C-Reactive protein

Results

Pathologic findings. All the specimens obtained from RA patients had pannus tissue at the cartilage-synovium junction. The pannus tissue consisted of various types of granulation tissue extending on or under the cartilage. Some of the specimens had clusters of macrophage-like cells and other inflammatory cells, but little fibrous tissue, while most of the panni had ample fibrous tissue which was either dense or loose. Cellular panni were divided into types A and B according to the density and shape of the cellular components. Type A consisted of homogeneous cell clusters which were round or polyhedral with large nuclei (Fig. 1), and type B consisted of various inflammatory

cells such as lymphocytes, plasma cells, macrophages, or polymorphonuclear cells (Fig. 2). Fibrous panni were divided into types C and D according to the characteristics of the fibrous substance. In type C, many collagen fibers, which occasionally formed dense fibrous scar tissues, filled the pannus tissue which had few cellular components (Fig. 3). The intercellular substance of type D was more homogeneous than that of the type C pannus tissue, and fibrous bundles were scattered among the substance (Fig. 4). Each type of pannus is listed with the case numbers in Table 2. In some specimens, combinations of two to three types were observed. In case No. 4, the pannus tissue covered the cartilage extensively, with pannus tissue of type A at the tip, type B in the center and type C on the synovial tissue side.

Type A pannus tissue invaded the cartilage and the subchondral bone in places, and the cartilage adjacent to type A panni was destroyed more severely than other parts of the cartilage. Most of the specimens had subchondral granulation tissue, and some of the tissue morphologically resembled type A pannus tissue. Type A panni and granulation

Table 2 The types of pannus tissue

Type	Histopathological findings	Case number
A	Cellular pannus with homogeneous cell pattern	4^a $7^b, 8^b, 16^b$
B	Cellular pannus with inflammatory cells	4^a $7^b, 8^b, 16^b$ 10
C	Fibrous pannus with dense fibrous bundles	4^a 1, 2, 3, 5, 6, 9, 11, 12, 13, 17, 18, 19 $14^c, 15^c$
D	Fibrous pannus with sparse fibrous bundles	$14^c, 15^c$

a : Case No. 4 exhibited types A, B and C
b : Case No. 7, 8 and 16 exhibited types A and B
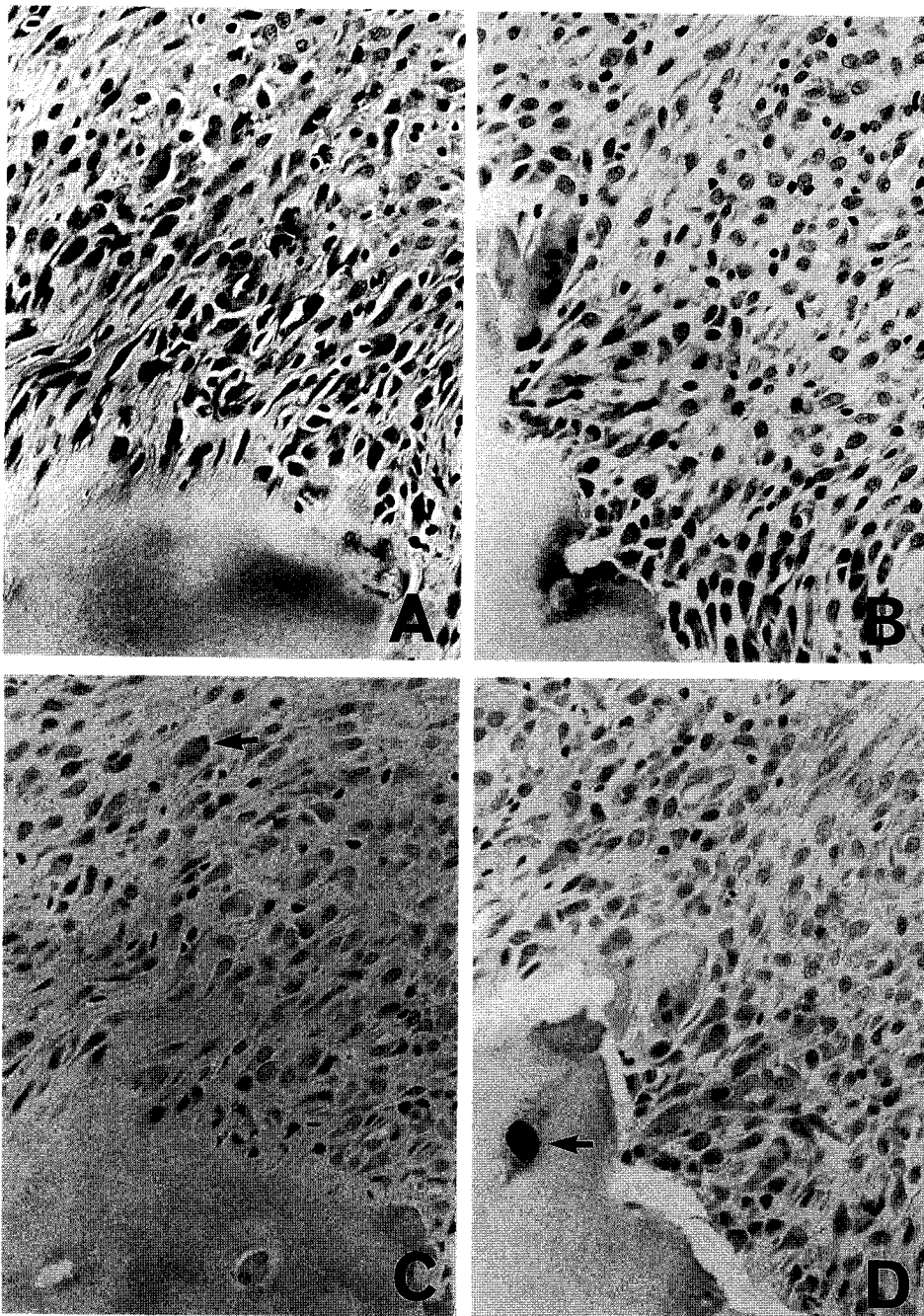c : Case No. 14 and 15 exhibited types C and D

tissue resembling type A panni had little or no vascularization. Occasionally, mild perivascular lymphocyte infiltration was seen in the type A pannus tissue. Type B panni were well vascularized with many lymphocytes and plasma cells infiltrating around blood vessels. Occasionally, polymorphonuclear cells and macrophages were also seen. Most type C pannus tissue had dense collagen fiber bundles with fibroblast-like cells intermixed among them. Some type C pannus tissue consisted of loose fibrous tissue. Type D fibrous panni were distinguished from type C panni on the basis of the homogeneous intercellular substance, in which the fibrous bundles were more sparse and scattered than in type C panni. A part of the homogeneous intercellular substance of type D pannus tissue was stained faintly red with Safranin-O. Blood vessels were occasionally seen in type D pannus tissue.

Immunohistological findings. The cartilage-synovium junction and subchondral bone was examined by the PAP method. The preliminary examination of the influence of decalcification of formalin-fixed tissue on immunoreactivity was performed. Decalcification of a schwannoma specimen had no influence on the immunoreactivity of S-100 protein. Although decalcification slightly influenced the immunoreactivity of synovial lysozyme, the immunoreactivity was judged satisfactory. The time for the DAB reaction was generally prolonged, and the contrast of the color of the DAB reaction was slightly decreased, especially in the synovium-lining cells. Plasma cells positive for IgG, IgA and IgM in decalcified tissues were considerably fewer than in non-decalcified tissues. Therefore, the distribution of lysozyme and S-100 protein-positive cells was examined in this study. In all specimens, most of the chondrocytes were strongly positive for S-100 protein and slightly positive for lysozyme. Some chondrocytes situated in the

Fig. 1   Microphotographs of the junction between the cartilage and type A pannus (Case No. 8). A: Hematoxylin-eosin stain ×330. B: Safranin-O stain ×330. C: Peroxidase-antiperoxidase (PAP) stain (lysozyme) ×330. D: PAP stain (S-100 protein) ×330. Note the uniform cells in the pannus tissue. Few cells are positive for lysozyme (arrow in C), and no cells in the pannus are positive for S-100 protein. A chondrocyte is strongly positive for S-100 protein (arrow in D).
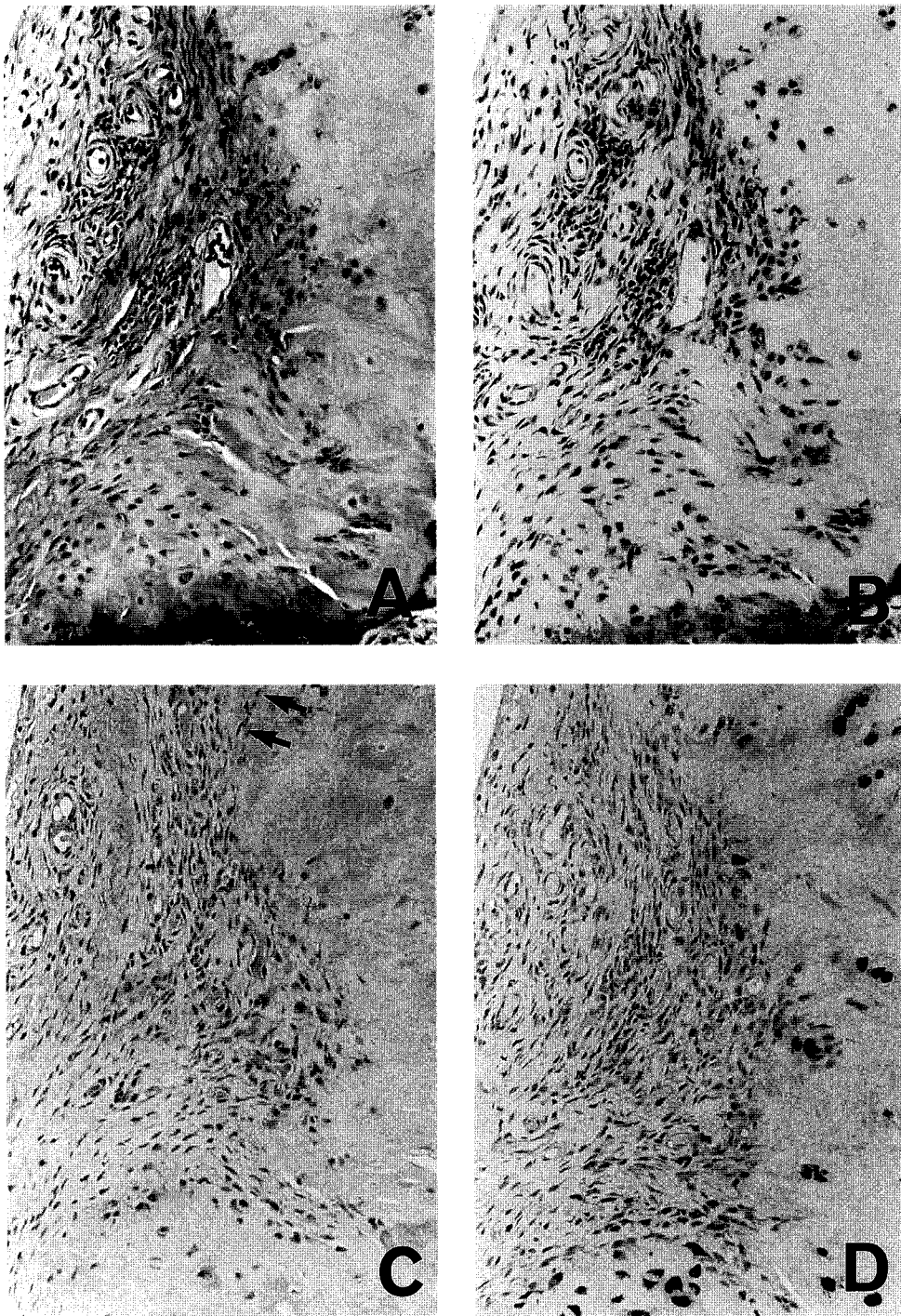
Fig. 2   Microphotographs of the junction between the cartilage and type B pannus (Case No. 10). A: Hematoxylin-eosin stain ×165. B: Safranin-O stain ×165. C: Peroxidase-antiperoxidase (PAP) stain (lysozyme) ×165. D: PAP stain (S-100 protein) ×165. Note the vascularization of the pannus, which is infiltrated with various inflammatory cells. Some lysozyme-positive cells are seen at the junction (arrows in C).
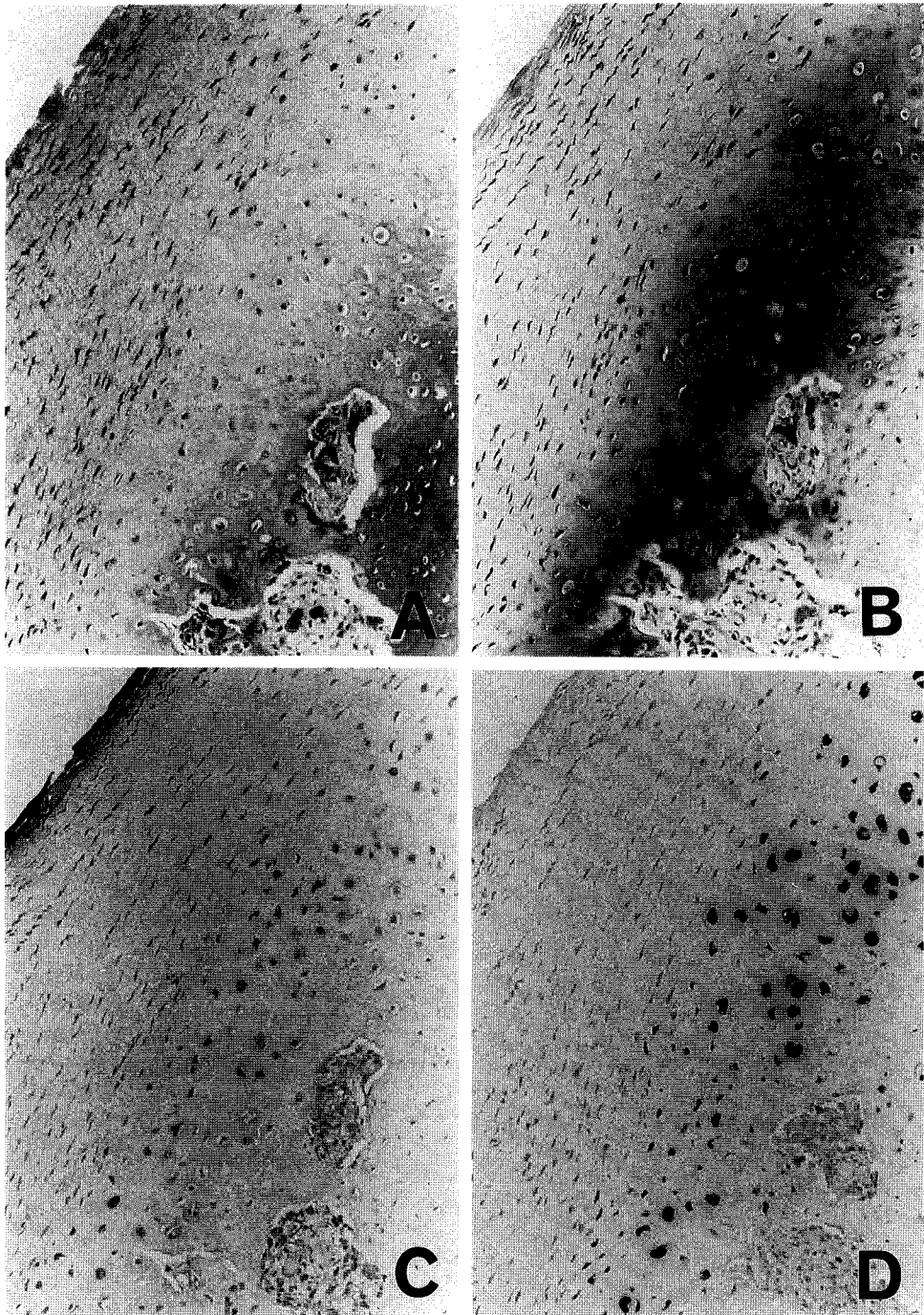
Fig. 3 Microphotographs of the junction between the cartilage and type C pannus (Case No. 6). A : Hematoxylin-eosin stain ×125. B : Safranin-O stain ×125. C : Peroxidase-antiperoxidase (PAP) stain (lysozyme) ×125. D : PAP stain (S-100 protein) ×125. The pannus consists of dense fibrous tissues and fibroblast-like cells. Note obscure junctional line. The surface of the pannus is faintly positive for lysozyme. Fibroblast-like cells are negative for lysozyme and S-100 protein.
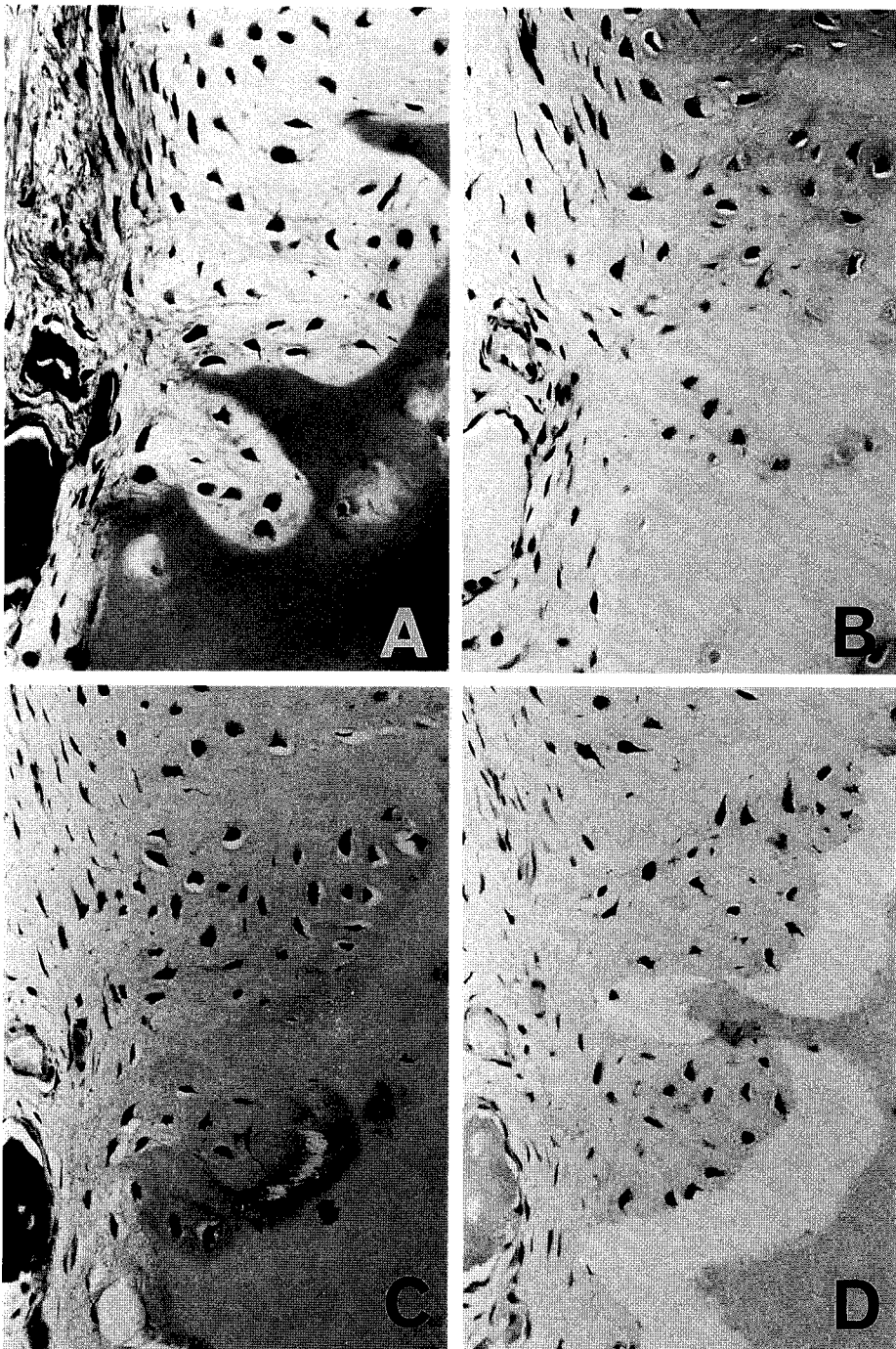
Fig. 4 Microphotographs of the junction between the cartilage and type D pannus (Case No. 14). A: Hematoxylin-eosin stain ×330. B: Safranin-O stain ×330. C: Peroxidase-antiperoxidase (PAP) stain (lysozyme) ×330. D: PAP stain (S-100 protein) ×330. The pannus has ample intercellular substance which is more homogeneous than in type C panni. The substance is stained faintly red with Safranin-O stain, and appears darker than the other areas. Cellular components of the pannus are strongly positive for lysozyme and S-100 protein.

cartilage adjacent to subchondral bone were moderately or slightly positive for S-100 protein. The strongly S-100 protein-positive chondrocytes had ample cytoplasm, compared with moderately positive cells. Most of the synovium-lining cells were moderately or faintly positive for lysozyme, although some of these cells had a strongly positive

reaction. Some of the cells in the superficial zone of the pannus tissue were also strongly positive for lysozyme.

Almost none of the cells in type A panni and granulation tissue resembling type A panni were positive for lysozyme, although a few were slightly positive. A few cell clusters under subchondral bone were posi-
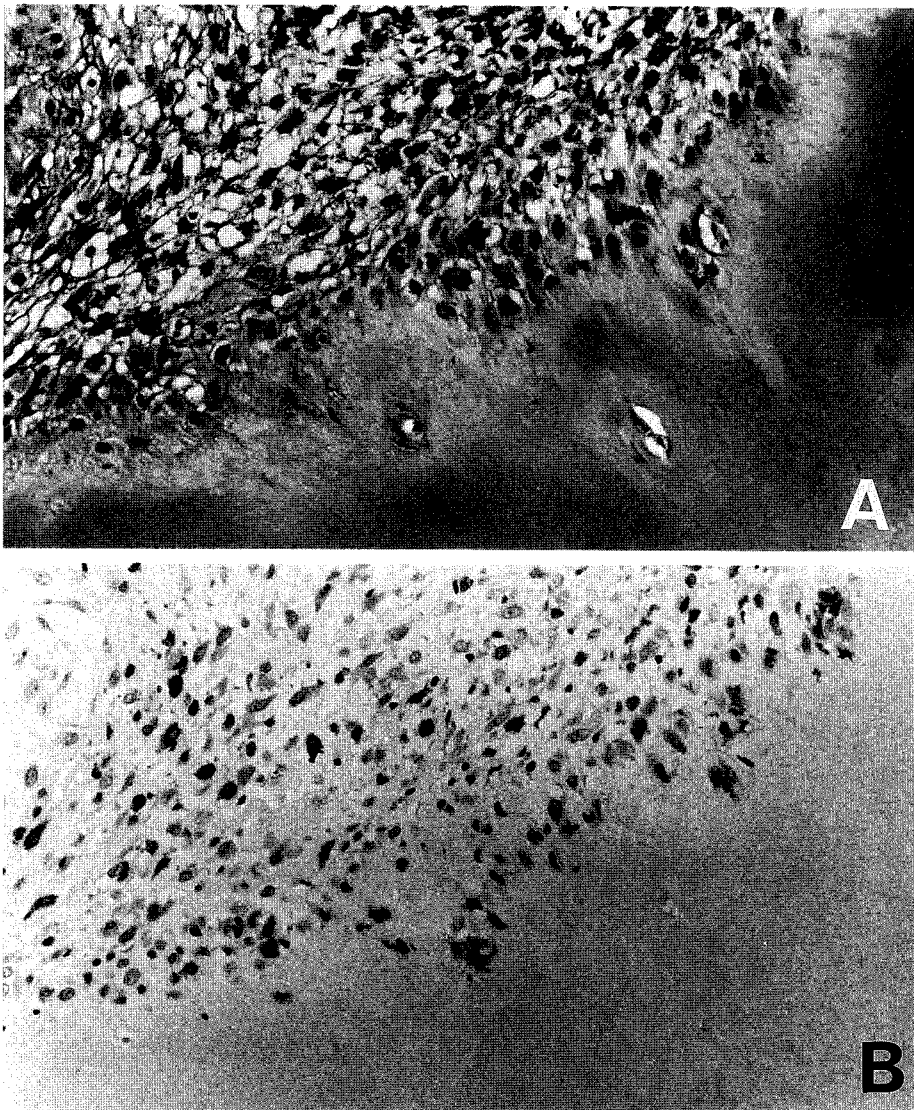

Fig. 5   Microphotographs of the junction between the cartilage and type B pannus (Case No. 7). A: Hematoxylin-eosin stain ×330. B: Peroxidase-antiperoxidase stain (lysozyme) ×330. Note the polymorphonuclear cells located at the junction, which are positive for lysozyme.

tive. There were no cells positive for S-100 protein in type A pannus tissue. In type B pannus tissue, polymorphonuclear cells were strongly positive for lysozyme, and some of them were recognized at the margin of the cartilage-pannus junction (Fig. 5).

Some of the macrophage-like cells were moderately or slightly positive for lysozyme, but fibroblast-like cells and plasma cells were negative. S-100 protein-positive cells were not recognized in type B pannus. In type C pannus, fibroblast-like cells were not posi-
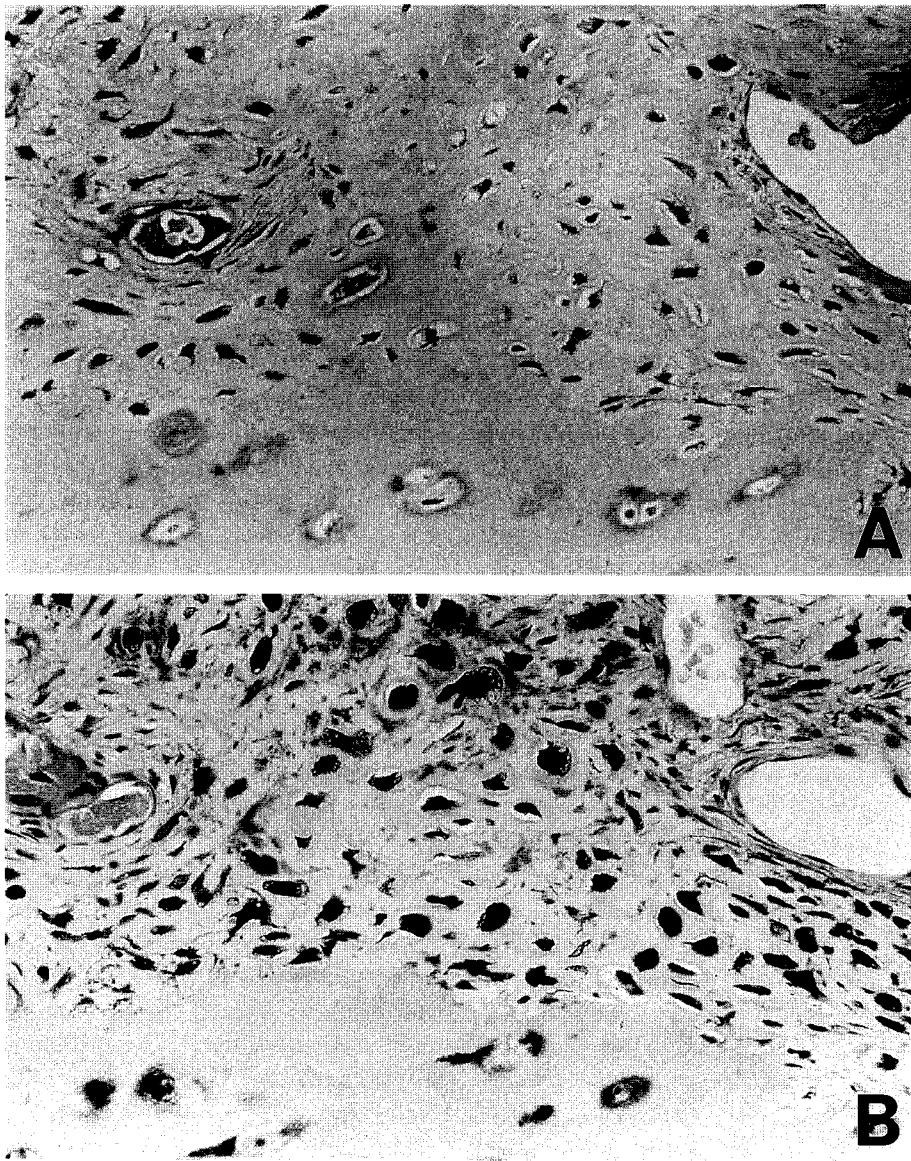

Fig. 6 Microphotographs of the junction between the cartilage and type D pannus (Case No. 15). A: Peroxidase-antiperoxidase (PAP) stain (lysozyme) ×330. B: PAP stain (S-100 protein) ×330. Oval cells in the pannus are faintly or moderately positive for lysozyme and strongly positive for S-100 protein. Elongated cells or fibroblast-like cells are negative for lysozyme and S-100 protein.

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tive for lysozyme, nor were most of the other cells. Chondrocytes in the superficial zone of the cartilage adjacent to the margin of the junction were moderately positive for lysozyme and S-100 protein. In type D pannus, major cellular components, which consisted of round or oval cells, were strongly positive for S-100 protein, and moderately to strongly positive for lysozyme. Elongated, spindle-shaped and fibroblast-like cells seen in type D pannus were negative for S-100 protein (Fig. 6).

Discussion

The cartilage-pannus junction has been studied ultrastructurally and histochemically (2, 4, 5). The immunohistochemical technique has been used by many investigators to observe the cellular activity of synovial tissue and to determine the roles of inflammatory cells in RA (8, 9). However, to apply this technique to the cartilage-synovium junction including the subchondral bone, specimens usually require decalcification, which may affect the antigenicity of the specimens.

Lysozyme is a highly cationic protein of low molecular weight which is widely distributed in the human body (15, 16). Increased levels of lysozyme in serum and/or synovial fluid have been documented in patients with RA (17, 18). More cells rich in lysozyme are found in synovial tissue of patients with RA than in synovial tissue of patients with osteoarthritis (8, 19). Lysosomal enzymes such as lysozyme are largely responsible for joint destruction in RA (8), and lysozyme is quantified to evaluate phagocytic activity in inflammation (9). The immunoperoxidase method proposed by Sternberger *et al.* (13) has been applied to identify lysozyme (14). As lysozyme is resistant to acid and heat (20), the immunoreactivity of lysozyme can be maintained even though specimens are de-

calcified by acid treatment. Recently, Matthews found that decalcification of specimens by formic acid hardly influenced the staining of IgG, IgA, IgM and lysozyme (10). Yoshimura *et al.* showed that when decalcified specimens were stained satisfactorily with H.E., those specimens were also able to be stained by immunoperoxidase method (11). However, the immunoreactivity of immunoglobulins was poor (11), as in the present study. On the other hand, lysozyme was adequately stained in the pannus tissues.

S-100 protein is known to be a specific antigen of both the central and peripheral nervous systems, and is used as a marker of varied neuroectodermal tumors (21, 22). Recently, S-100 protein was recognized in several tissues other than the nervous system, such as in cartilage cells (21, 23). S-100 protein has been detected using the immunoperoxidase method (22-24). Fibroblasts, fibrocytes in the fibrous connective tissue, and synovium-lining cells do not contain this protein (25), while dendritic cells in the epidermis and lymph nodes are positive for S-100 protein (24). No influence of decalcification on the immunoreactivity of the S-100 protein was recognized in our experiment, *i. e.*, S-100 protein was detected successfully by the immunoperoxidase method even in acid-decalcified specimens. We attempted to identify the chondrocytes at the cartilage-synovium junction using S-100 protein as a marker.

Dense fibrous pannus tissue was recognized in most of the 19 knee joints studied, and cellular pannus tissue was recognized in 5. Type A pannus and granulation tissue morphologically resembling type A tissue were seen in 4 cases. Articular cartilage and the subchondral bone adjacent to type A tissue were invaded by this type of tissue and were severely destroyed. This type A pannus may correspond to the "tumor-like mesenchymal cell clusters" described by

Fassbender (26). This tissue may be responsible for joint destruction which occurs in the early stage of pannus formation. In spite of extensive destruction of cartilage and bone adjacent to the tissue, few cell clusters had a positive reaction for lysozyme. Fritz *et al.* obtained results similar to ours in rheumatoid synovial tissues (9). Fassbender mentioned that the life span of this tissue is very short (3, 27), and that at the stage of invasion of cartilage, the cells of this tissue have already lost the lysosomes which they normally would possess (28). Thus, the period of lysozyme activity may be short in type A tissue. Case No. 4 is interesting because the three types of tissue were found side by side in a single pannus ; a small area of type A at the tip, type B in the center and type C on the synovial side. This finding suggests that type A pannus is involved in the proliferation and growth of the pannus tissue, and appears to occur in the first stage of pannus formation, followed by types B and C at later stages. In the beginning of the type B stage, small vessels may proliferate into type A pannus from the synovial tissue side. Small lymphocytes infiltrate first, and other inflammatory cells infiltrate later. With the help of such inflammatory cells, type A tissue may differentiate into fibrous granulation tissue during the proliferation of pannus tissue. Such an assumption is in agreement with the concept of Fassbender (26). However, the "mesenchymoid transformation" (27) may not always occur in whole pannus. Therefore, the cartilage and subchondral bone, which were extensively invaded by type A tissue, appear to be of the terminal stage. In type B tissue, polymorphonuclear cells were often present at the margin of the junction, were strongly positive for lysozyme, and seemed to be active in the degradation of the articular cartilage during the second stage.

Type C pannus consisted mostly of dense collagen fibers, and was found in 15 out of 19 cases. Our results indicated that type C was the most frequent type, and might occur in the later stage because the knee joints were severely destroyed as the replacement surgery was indicated. Fibroblast-like cells, negative for lysozyme, were the major cellular component in type C tissue, so that little cartilage destruction could occur in the tissue. On the other hand, type C pannus may function to maintain the pannus thickness or to repair the joint surface, since the junctional margin of type C tissue became obscure in some cases (Fig. 3).

Type D pannus resembled type C tissue, but was easily distinguished from type C tissue because of the rich homogeneous intercellular substance. As some of the homogeneous substance was stained with Safranin-O, the substance may be a residue of degraded cartilage matrix. Round or oval cells scattered in type D tissues were strongly positive for S-100 protein, which was used as a marker of chondrocytes. There is a problem as to whether or not the cells resulted from phagocytosis of degraded chondrocytes positive for S-100 protein. However, no cells positive for S-100 protein were seen at the site of cartilage erosion in other types of pannus. Therefore, these S-100 protein-positive cells are probably chondrocytes. These cells are positive for lysozyme, which is responsible for cartilage degradation. Mitrovic described fibrous metaplasia from the hyaline cartilage, and suggested that the dedifferentiation of chondrocytes into fibroblast-like cells could be involved in pannus formation (6). Cooke proposed a similar process of the dedifferentiation of chondrocytes at the pannus site in RA, and noted a lack of vessels in the tissue (29). However, Mitrovic and Cooke have not confirmed the fact that cells in the pannus are chondrocytes. Fassbender and Shiozawa oppose the view that chondrocytes are involved in pan-

nus formation (26, 30). Our observations indicated that chondrocytes positive for S-100 protein and lysozyme in type D tissue are responsible for cartilage destruction at the cartilage-synovium junction in rheumatoid joints which form pannus-like tissue. These observations firmly support the idea that chondrocytes are involved in pannus formation in RA. However, the fibroblast-like cells adjacent to the cells were negative for S-100 protein. Whether or not the chondrocytes dedifferentiate into fibroblast-like cells in RA panni remains to be elucidated.

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