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An Immunohistochemical and Immunoelectron Microscopic Study of Adhesion Molecules in Synovial Pannus Formation in Rheumatoid arthritis

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To investigate the mechanism of synovial-pannus formation in rheumatoid arthritis, using immunohistochemical and immunoelectron microscopic studies with monoclonal antibodies against the adhesion molecules, anti-CD54 (ICAM-1) anti-CD11a (LFA-1), anti-CDw49a (VLA-1), anti-CDw49b (VLA-2), anti-CDw49c (VLA-3), anti-CDw49d (VLA-4) and anti-CDw49e (VLA-5), the pattern of distribution of these molecules at the rheumatoid synovial-cartilage junction have been investigated. Treatment with anti-ICAM-1 resulted in membrane staining of most of the macrophages and fibroblasts infiltrating the synovial tissue and bordering the pannus cartilage junction. This suggests the possibility that ICAM-1 may function to facilitate the adhesion of Type A cells bearing ICAM-1 to Type B cells. ICAM-1 positive macrophages and fibroblasts were often in contact with lymphoid cells also suggest that cell-to-cell immune reaction occurs in the formation of the pannus. Our present study shows that VLA-3, VLA-4 and particularly VLA-5 are the predominant β1 integrins expressed by rheumatoid synovial pannus. Since these three integrins all function as fibronectin receptors, it is tempting to postulate that the fibronectin rich environment of the rheumatoid cartilage surface could effectively trap pannus cells expressing high levels of these molecules. VLA-5 molecule found in pericellular and interterritorial matrix distribution in the present study strongly suggests that receptor-ligand interaction between VLA-5 and cartilage matrix may occur at the early stage of pannus formation. Furthermore, the increase in β1 integrin may be necessary for the growth of the pannus and also for the upregulation of the VLA-molecules, leading secondarily to increase attachment.

Key Words

Rheumatoid pannus,
Adhesion molecules,
β1 integrins,
Electron microscopy.

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INTRODUCTION

In rheumatoid arthritis, as a part of synovial tissue reactions, proliferating synovial cells penetrate the cartilage in the form of a pannus, and cartilage destruction takes place in the zone between the cells and cartilage. The cellular origin of rheumatoid pannus has been debated by many investigators on the basis of their histologic

analysis of pannus specimens from patients with rheumatoid arthritis (1-8). It is generally accepted that fibroblast proliferation, endothelial cell proliferation, and monocyte chemotaxis are involved. In response to as yet unknown autocoids, in addition to the presence of immune complexes in the superficial cartilage (9), the proliferating synovial tissue begins to penetrate and degrade the cartilage. The mechanisms responsible for pannus formation are not fully understood but there is fairly general agreement as to the significance of marginal pannus growing over the cartilage surface and invading the cartilage matrix (1,3,4).

Although a recent study has shown that the pannus components were derived from cartilage (8), the origin of pannus has been a subject of much debate in current literatures. However, it is clear that behind the invasive front of pannus are the foci of helper T cells, immunoglobulin bearing cells and HLADR-expressing cells that are generating the immune response and the proliferative reaction that evolves from it (3,10).

In a previous study, the authors have demonstrated the recombinant human interleukin-1 (IL-1) stimulates monocytes and synovial cell attachment to rheumatoid cartilage in vitro (11). In that study, large numbers of monocytes from healthy individuals and cultured synovial cells were observed to attach to the rheumatoid articular surface in the presence of IL-1, suggesting that IL-1 generated by adherent monocytes and also from synovial cells could increase their binding to cartilage matrix protein. Furthermore, these cells attached to the cartilage surface strongly expressed intercellular adhesion molecule-1 (ICAM-1) and very late antigen-5 (VLA-5) (12). Recent studies have demonstrated that adhesion molecules of the $\beta 1$ subfamily of the integrin supergene family, made up of a series of α chains combined with the $\beta 1$ chain, to form the VLA group of receptors present on nucleated haematopoietic cells can bind to collagen, fibronectin, and laminin ligands of the connective tissue matrix (13-15). The integrin superfamily includes receptors involved in cell-to-cell adhesive interactions as well as in interactions with extracellular matrix components (13-17). Furthermore, we have recently showed that the increased expression of CDw49e (VLA-5) and CD54 (ICAM-1) at the cartilage-pannus junction may represent interaction with matrix protein (5,12,18). The results obtained in those studies confirmed the roles of adhesion molecules in the pannus formation and attachment to cartilage. In the present study, an immunohistochemical and immunoelectron microscopic investigation using immunoperoxidase staining methods was carried uot to elucidate more precisely whether adhesion molecules and ligands expressed on pannus and cartilage respectively play a role in this process.

MATERIALS AND METHODS

Twenty eight samples of rheumatoid articular cartilage covered with pannus from twenty-eight patients were obtained during synovectomy or joint replacement surgery. All patients were considered to have moderate-

to-severe active synovitis at the time of surgery. Several samples of pannus- cartilage junction from the same patients were chosen to contain the active phase of the pannus by naked eyes and were confirmed by light microscopy with haematoxylin and eosin staining. Otherwise, fibrous pannus were discarded because of the lack of cellularity. Each specimen was treated immediately after collection.

Purified anti-human-monoclonal antibodies denoted CDw49a ($\alpha 1\beta 1$, VLA-1), CDw49b ($\alpha 2\beta 1$, VLA-2), CDw49c ($\alpha 3\beta 1$, VLA-3) were obtained from T cell Diagnostic Inc. (Cambridge, MA) and CDw49d ($\alpha 4\beta 1$, VLA-4) and CDw49e ($\alpha 5\beta 1$, VLA-5) were obtained from Immunotech. (Marseilles, Cedex, France). Eack monoclonal antibody had similar specific avidities for its antigens. Purified anti-human-monoclonal antibodies denoted CD54 (ICMA-1), and CD11a (LFA-1) also were obtained from Immunotech. Purified mouse lgG was obtained from Cappel Laboratories (Chochranville, Pennsylvania). Avidin biotinylated peroxidase (ABC-kit) was obtained from Vector Laboratories (Burlingame, California), and 3-3'-diaminobenzidine was purchased from Sigma Chemical Co. (St. Louis, Missouri). Frozen sections, 4-6μm thick, were cut on a cryostat (Bright, Huntington, England) at $-20°C$, and mounted on gelatin and egg alubumin-coated glass slides. After drying at room temperature, the sections were washed with phosphate-buffered saline (PBS). Normal goat serum, diluted 1:200, was applied to the sections for 20 minutes. After washing, they were incubated with 100 to 200μl of diluted monoclonal antibody for 60 minutes. After washing with PBS, biotinylated peroxidase conjugated goat anti-mouse IgG antibody (Becton- Dickinson Monoclonal Center, Mountainview, California) was added, and this was followed by an avidin peroxidase complex. The tissue was then incubated with 3mg of 3-3'-diaminobenzidine in 10ml of Tris HCl buffer, pH 7.5, for 10 minutes. The specimens were then washed in PBS and dried at room temperature. The sections were stained with haematoxylin for background and nuclear staining of the cells. For electron microscopic examination, the sections were fixed with 1% osmium tetroxide (OsO4) for noe hour and washed in PBS, dehydrated in graded alcohol to 100%. While the sections were still wet, plastic capsules filled with Epon 812 were inverted over the sections (19). After polymerization of the Epon 812, the glass slides were heated on a hot plate and the sections were removed from the slides. Ultrathin sections were cut on an LKB microtome, and they were examined in Hitachi H-300 electron microscope without counterstaining with lead citrate.

RESULTS

A variety of cell types stained for $\beta 1$ antibodies including the synovial lining cells, mononuclear cells, and endothlial cells of the post-capillary venules (PCV). Some degree of hyperplasia of the synovial lining cells was observed. When the synovial pannus-cartilage tissue samples were stained with anti-VLA-1, anti-VLA-2, anti-VLA-3, anti-

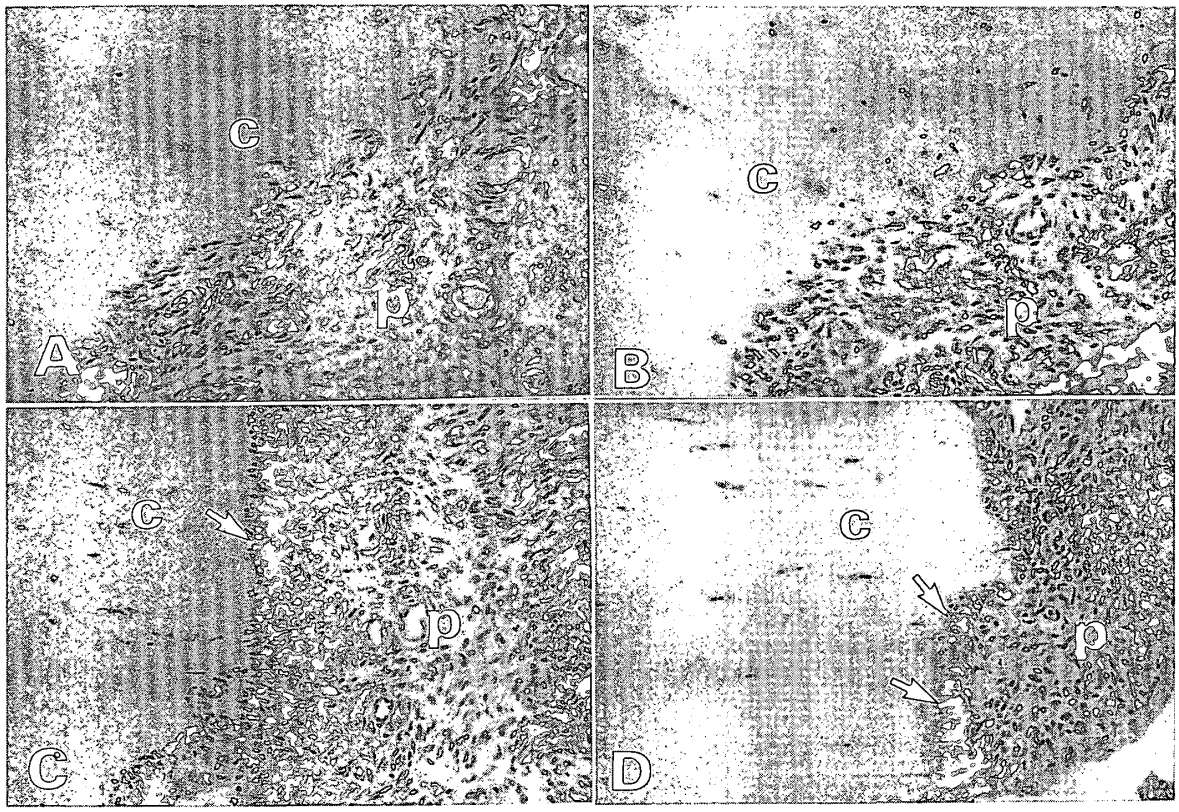

Figure 1. Staining of β1 integrins at the pannus-cartilage junction. Picture A shows VLA-1 staining, B, VLA-2 staining, C, VLA-3 Staining, and D, VLA-4 staining respectively. Weak immunoreaction is diffusely disributed at the pannus-cartlage junction. Arrows indicate the cells of peroxidase positive reactions.

VLA-4, and anti-VLA-5, almost all cells of the lining layer showed strong VLA-5 staining and lesser extent, VLA-3 and VLA-4 staining. In agreement with previous seports, the cellular component of the rheumatoid pannus varied in their numbers (2,4,6).

The intensity of staining of endothelial cells (EC) of the PCV in pannus varied, with the VLA-1, VLA-3 and VLA-5 positive EC showing more intense staining than the VLA-2 and VLA-4 EC. When the specimens were treated with anti-VLA-1 and anti-VLA-2, most of the cells located perivascularly did not show cell membrane staining, however, when the specimens were treated with anti-VLA-3, anti-VLA-4, most of the small lymphocytes and macrophages showed membrane staining (Figure 1). VLA-5 positive cells were observed in linear distribution along the border between the synovium and cartilage (Figure 2), while noly a few cells at the cartilage border showed weak staining with anti-VLA-1 and VLA-2. There were some anti-VLA-1 and strong anti-VLA-5 staining on chondrocytes at or close to the pannus cartilage junction. When the specimen was treated with anti-VLA-5, most of the cells located at

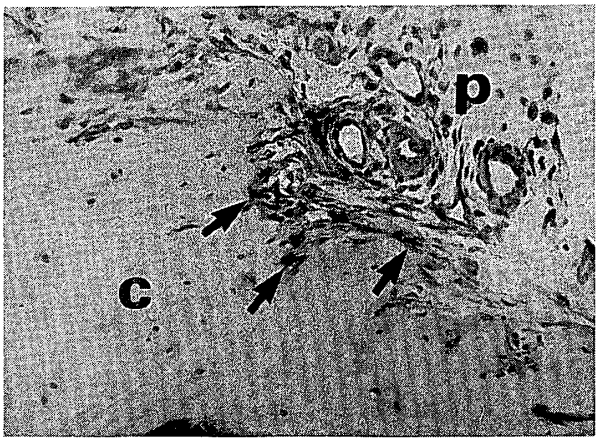

Figure 2.
Staining pattern of VLA-5 positive cells at the pannus-cartilage border.

the cartilage border showed strong staining with this antibody (Figure 2).

In the electron microscopic examination, the electron-dense materials were observed in patchy distribution on the cell membrnae and these materials were observed to be in contact with cartilage matrix (Figures 3). The cell membrane of the chondrocytes located at or close to the pannus-cartilage junction also showed strong anti-VLA-5 staining. (Figure 4).

ICAM-1 positive cells were

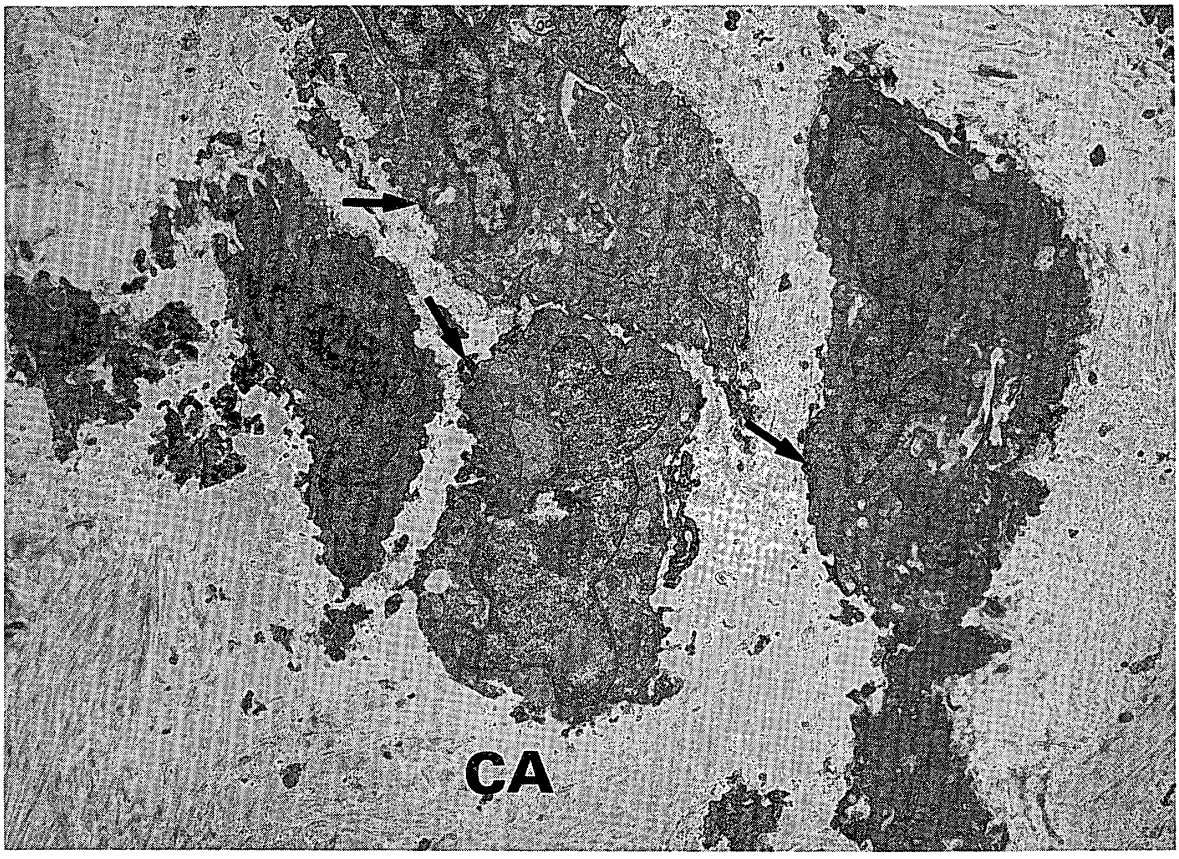

Figure 3. Electron micrograph of VLA-5 positive cells in the pannus-cartilage border. The electron-dense materials were observed in patchy distribution on the cell membrane and these materials were observed to be in contact with cartilage matrix. Arrows indicate the peroxidase positive materials. CA: cartilage matrix, Original magnification, ×4,000

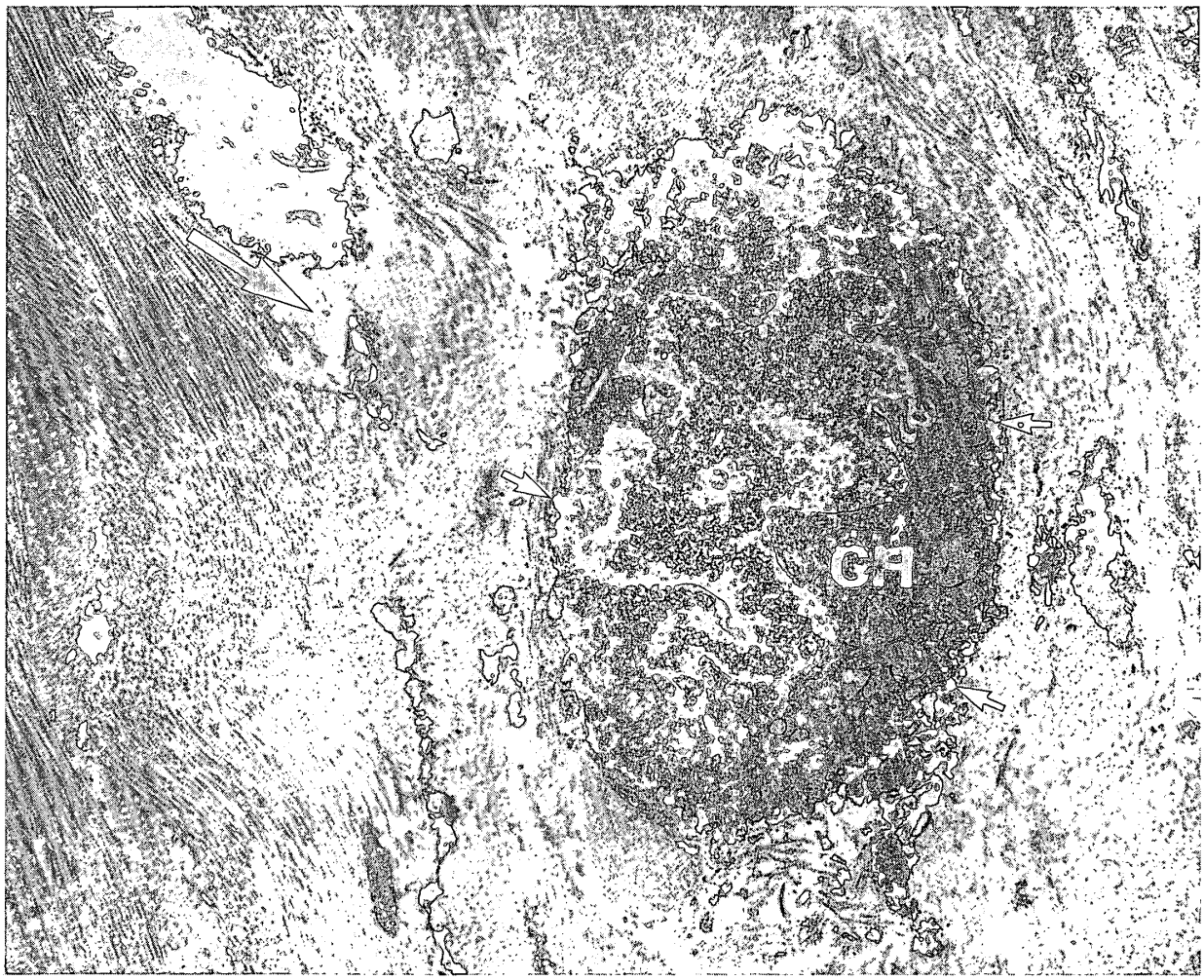

Figure 4. A chondrocyte just below the pannus-cartilage border. Short arrows indicate peroxidese positive products and large arrow indicates the direction of pannus invading the cartilage matrix. CH; chondrocyte, original magnification: ×7,000

observed in a linear distribution along the border between synovium and cartilage (Figure 5). In the electron microscope, examination of the staining of the cell membrane of these cells showed either continuous patchy staining of their cell membrane and observed to be contact with small lymphocytes and fibroblastic cells (Figures 6). However, when the specimen were treated with anti-CD11a (LFA-1), only a few cells at the cartilage border showed weak staining.

DISCUSSION

Although the rheumatoid pannus is characterized by an excessive fibroblast proliferation, the initial triggering factors contributing to pannus formation are still unclear (2-4,6,8). In a previous study, we suggested that binding of monocytes and synovial cells to cartilage in the presence of IL-1 could increase their binding to

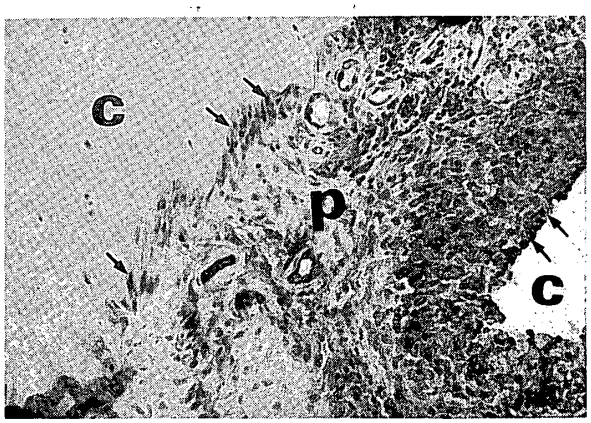

Figure 5.
Linear distribution of ICAM-1 positive cells along the border between the synovium and cartilage.
C: cartilagematrix

cartilage matrix (11). Synovial cell attachment to cartilage may be the initial step in pannus formation. In the present study, we have investigated the morphologic character and distribution of cells expressing adhesion molecules at the synovial-cartilage junction. It is likely that binding of lymphocytes, macrophages and fibroblasts in the synovial pannus to cartilage, as observed in the present observation, results from interaction between VLA receptors on these cells and cartilage matrix protein ligands.

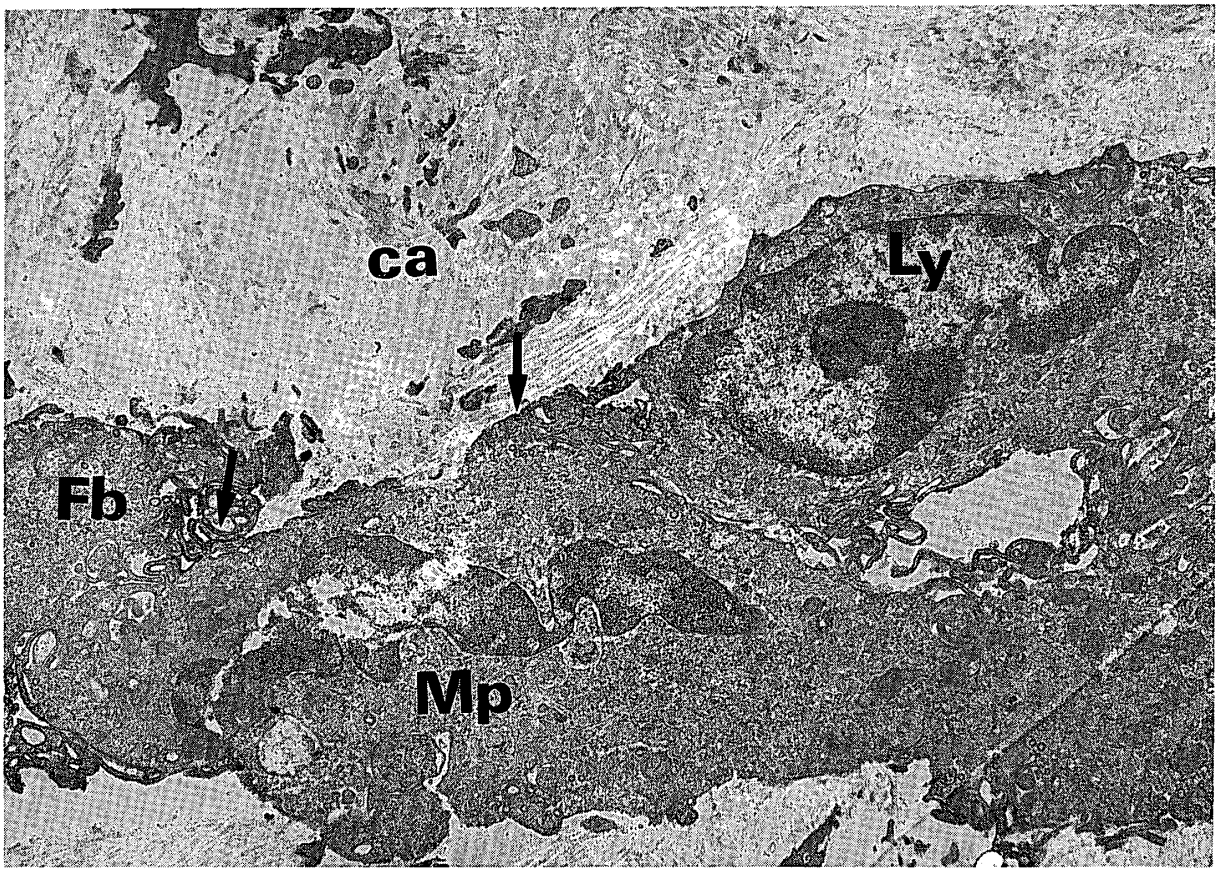

Figure 6. Electron micrograph of ICAM-1 positive cells in the pannus-cartilage junction. The cell membrane of large fibroblastic cells (Fb) and macrophage (Mp) were observed to be in contact with small lymphocyte (Ly). Original magnification: 5,000. ca: cartilage, arrows indicate the peroxidase positive products.

Staining patterns of ICAM-1, LFA-1 and VLA were somewhat different. ICAM-1 and VLA staining were widely distributed in the pannus tissue and at the border between pannus and cartilage. Since ICAM-1 binds to the β2-integrin LFA-1, which is located on mononuclear cells, its role may be limited to cell-to-cell immune reaction occurring in the formation of the pannus (20,21). In the present electron microscopic study, ICAM-1 positive macrophages and fibroblasts were often in contact with lymphoid cells. With regard to the increased expression of ICAM-1 on the cells bordering between pannus and cartilage, the evidence that this ligand may interact with β2 integrin on the endothelium.

The cells infiltrated in pannus are made up mainly of fibroblasts, macrophages and lymphocytes. In contrast to the lymphocyte-rich areas in the RA synovium (33), the pannus contained large numbers of ICAM-1 postive cells, and these cells appeared to be in contact with cartilage surface. VLA-4 positive cells and VLA-5 positive cells were present in large numbers in the pannus, and VLA-5 positive cells were usually outnumbering the VLA-4-positive cells. VLA-2 positive cells were only occasionally seen and VLA-1 positive, VLA-3 positive cells were usually small in numbers. Thus, it is likely that the tissue distribution patterns of infiltrated cells from cells from pannus PCV are influenced by the ECM of the pannus and by the ability of the cells to interact with the ECM through cell surface receptor expression. Our present study suggests that VLA-3, VLA-4 and particularly VLA-5 are the predominant β1 integrins expressed by the rheumatoid synovial pannus.

The interpretation of the increased cartilage pannus junction staining for VLA-3, VLA-4 and VLA-5 is explained as a result of β1 integrin binding to cartilage matrix leading to increased activation as well as ICAM-1 and LFA-1 interaction leading to increase β1 expression. Since these three integrins all function as fibronectin receptors (34), it is tempting to postulate that the fibronectin rich environment of the rheumatoid cartilage surface (35), could effectively trap pannus cell expressing high levels of these molecules.

The strong expression of VLA-5 on chondrocytes as observed in the present electron microscopic study would suggest that interactions between chondrocytes and fibronectin may be occurring in the pannus formation through the activation of chondrocytes by various cytokines (4,11,36). Many of the known ligand for integrins including collagen, thrombospondin, and fibronectin are present in the articular cartilage. Increased amounts of fibronectin in the pannus-cartilage junction in rheumatoid arthritis has been described (35), which may in part be related to increased *de novo* synthesis by chondrocytes (37). However, type II collagen-binding proteins including anchorin, have been identified on chondrocytes, and the role of integrins in collagen-chondrocyte interaction is as yet uncharacterized (24,38). Recent studies suggest that certain chondrocyte-ECM interaction may be mediated by integrins (17,21,34,36). Distribution of VLA-5 molecule in pericellular and also interterritorial

matrix in the present study strongly suggests that receptor-ligand interaction between VLA-5 and cartilage matrix may occur at the early stage of pannus formation. This is also suggesting the existence of activation-mediated regulatory mechanism of the VLA-fibronectin interactions at the pannus site.

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