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\( \beta 1 \)-Integrin Expression in the Rheumatoid Synovial-Pannus Formation

Hitoshi Ishikawa, Soichiro Hirata, Takashi Isobe, Yasurou Nishibayashi, Hitoshi Kubo, Masahiro Nannbae, Natsuko Nakagawa and Yoshihiro Andoh

In order to investigate the mechanism of synovial pannus formation in rheumatoid arthritis, using an immunohistochemical staining technique with monoclonal antibodies against adhesion molecules, anti-CDw49a (VLA-1), CDw49b (VLA-2), CDw49c (VLA-3), CDw49d (VLA-4) and CDw49e (VLA-5), the pattern of distribution of these molecules at the rheumatoid synovial cartilage junction has been investigated. Twelve samples of rheumatoid articular cartilage covered with pannus were examined. Treatment with purified anti-human-monoclonal antibody CDw49a resulted in membrane staining of most of the endothelial cells of the postcapillary venules in pannus, and of few of the cells infiltrating the synovial tissue and bordering the pannus cartilage junction. When the specimen was treated with purified anti-human-monoclonal antibodies CDw49c anti-(VLA-3), CDw49d anti-(VLA-4) and CDw49e anti-(VLA-5), most of the cells in cartilage pannus junction stained, but there were few staining cells against purified anti-human-monoclonal antibody CDw49b anti-(VLA-2). There were some anti-VLA-1 and anti-VLA-5 staining of the chondrocytes at or close to the junction. These results show that the specific adhesion molecules of \( \beta 1 \) integrin tested may play a role in rheumatoid pannus formation and increased expression of VLA-3, VLA-4 and VLA-5 at the cartilage pannus junction may represent interaction with matrix protein. Thus VLA molecules stained in the cartilage-pannus junction provide appropriate anchorage, and achieve the variety of immune reaction. Furthermore, the increase in \( \beta 1 \) integrin may be necessary for the growth of the pannus and also for the upregulation of the VLA molecules, leading to secondarily to increase attachment.

**Key Words**
- \( \beta 1 \) integrins,
- Adhesion molecule,
- Rheumatoid arthritis,
- Pannus formation,
- Cell-extracellular matrix interaction.

**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 (1-4). 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. It is generally accepted that fibroblast...
proliferation, endothelial cell proliferation, and monocyte chemotaxis are involved (1–6). 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 (3,4,7). Although recent study has shown that the pannus components were derived from cartilage (8), the origin of pannus has been subject of much debate in current literature. In a previous study, the authors have demonstrated that recombinant human interleukin-1 (IL-1) stimulated monocyte and synovial cell attachment to rheumatoid cartilage in vivo (9). In that study, large numbers of monocytes 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. Recent studies have demonstrated that adhesion molecules of the β1 subfamily of the integrin supergene family, made up of a series of α chains combined with the β1 chain, to form the VLA (very late antigen) group of receptors present on nucleated haematopoietic cells can bind to collagen, fibronectin, and laminin ligands of the connective tissue matrix (10–12). The integrin superfamily includes receptors involved in cell-to-cell adhesive interactions as well as in interactions with extracellular matrix components (10–14). 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 (15–17). The results obtained in those studies were confirmed the roles of adhesion molecules in the pannus formation and attachment to cartilage. In the present study, an immunohistochemical investigation using the immunoperoxidase staining methods was carried out to determine whether β1 adhesion molecules and ligands expressed on pannus and cartilage respectively play a role in this process.

MATERIALS AND METHODS

Twelve samples of rheumatoid articular cartilage covered with pannus from twelve 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 microscope with hematoxylin 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 (α1β1, VLA-1), CDw49b (α2β1, VLA-2), CDw 49c (α3β1, VLA-3) were obtained from T cell Diagnostic Inc. (Cambridge, MA) and CDw49d (α4β1, VLA-4) and CDw49e (α4β1, VLA-4) and CDw49e (α5β1, VLA-5) were obtained from Immunotech (Marseilles, Cedex, France). Purified anti-human mouse monoclonal antibody CD54 (intercellular adhesion molecule-1; ICAM-1) was also
purchased from Immunotech. Each monoclonal antibody had similar specific avidities for its antigens. Purified mouse IgG 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, 4-6 μm thick, were cut on a cryostat (Bright, Huntington, England) at −20°C, and mounted on gelatin and egg albumin coated 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 200 μl 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 avidine peroxidase complex. The tissue was then incubated with 3mg of 3-3'-diaminobenzidine in 10 ml of Tris HCl buffer, pH 7.5, for 10 minutes. The specimen were then washed in PBS and dried at room temperature. The sections were stained with haematoxylin for background and nuclear staining of the cells.

RESULTS

Staining with anti-CD54 (ICAM-1) resulted in membrane staining of most of the macrophage-like cells and small lymphocytes present at the cartilage pannus junction. A variety of cell types stained for β1 antibodies including the synovial lining cells, mononuclear cells, and the endothelial cells of the PCV. The staining patterns of the β1 integrins are shown in Table 1. Histological grading scores were given as follows; the mean percentage of cells stained are represented: none stained (−); 1-5% (+); 6-25% (+ (+)); and more than 51% (+ + +). Some degree of hyperplasia of the synovial lining cells were observed in twelve samples examined. When 12 RA synovial pannus tissue samples were stained with anti-VLA-1, anti-VLA-2, anti-VLA-3, anti-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 (Figure 1). In agreement with previous reports, the cellular components of the rheumatoid pannus varied in their numbers (1-4). The intensity 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 (Figure 2). The degree of β1 staining cells of different adhesion molecules in pannus cartilage junction varied. The reasons for such variation was specimen size difference and a wide variation in cellularity. When the specimen 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 and anti-VLA-5 showed membrane staining of small lymphocytes and most of the macrophage-like cells, with the
Figure 1. $\beta_1$ adhesion molecules expressed in the rheumatoid synovial pannus tissue. Staining for intercellular adhesion molecule-1 (ICAM-1) is also shown. Staining patterns of $\beta_1$ integrins are different from lymphocytes aggregates, endothelial cells, and lining layer (Original magnification, ×100).

VLA-5 positive cells showing most intense staining than the VLA-4 positive cells. VLA-3, VLA-4 and VLA-5 positive cells were observed in a linear distribution along the border between synovium and cartilage (Figure 2), while only a few cells at the cartilage border showed weak staining with anti-VLA-1 and anti-VLA-2. There were some anti-VLA-1 and strong anti-VLA-5 staining on chondrocytes at or close to the
Table 1. β1 integrin molecule expression on rheumatoid synovial pannus junction

<table>
<thead>
<tr>
<th>β1 integrin</th>
<th>Synovial tissue</th>
<th>Synovial Pannus</th>
<th>Chondrocytes</th>
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<tr>
<td></td>
<td>EC</td>
<td>Lym</td>
<td>Lining cells</td>
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<tr>
<td>VLA-1</td>
<td>+++</td>
<td>++</td>
<td>±±</td>
</tr>
<tr>
<td>VLA-2</td>
<td>+</td>
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<td>±</td>
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<tr>
<td>VLA-5</td>
<td>+++</td>
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Table 1. The mean percentage of cells stained are represented as follows: none stained (−); 1–5% (+); 6–25% (+ +); 26–50% (+ + +) and more than 51% (+ + + +). Abbreviations used represent as follows: EC; endothelial cells of postcapillary venules and Lym; lymphocytes infiltrated in synovial tissue.

DISCUSSION

The integrins are a family of ubiquitous cell-surface extracellular matrix (ECM) adhesion receptors whose members mediate many cellular processes central to tissue morphogenesis, homeostasis, and repair (18–19). Recent studies (10,11) have demonstrated an interaction between adhesion molecules of β1 group of the integrin supergene family and fibronectin, collagen and laminin of the ECM, and stimulation of synoviocyte proliferation by a reaction between membrane bound VLA-5 and fibronectin has also reported (12, 20, 21).

Rheumatoid synovial tissues are enriched for lymphocyte expressing high level of integrins (21–23). Furthermore, in a previous study we described the possibility that VLA-5 may facilitate the growth of the pannus by virtue of its ability to react with fibronectin secreted by proliferating synoviocytes of the pannus (15,16). Synovial cell attachment to cartilage may be the initial step in pannus formation. Adhesion molecules are critically important for binding of inflammatory cells to ECM such as fibronectin and collagen via the β1 integrins (24,25). Thus in the present study, the authors have investigated the morphologic character and distribution of cells expressing β1 adhesion molecules at the synovial-cartilage junction.

Our immunohistochemical study describes the in situ staining patterns of members of β1 integrin family of molecules by EC of the PCV, mononuclear cells, synovial lining cells, and the cells bordering between the pannus and the cartilage surface.
The endothelium of the PCV in the pannus stained intensely for VLA-1, VLA-3 and VLA-5. This suggests that density of the $\beta_1$ integrins on the EC is relatively high in agreement with previous reports (21,26,27). Our observation that endothelial staining for $\beta_1$ was more intense in cellular rich pannus areas compared to fibrous pannus is in keeping with regional differences in the expression of this molecule by the endothelium. The cells infiltrated in pannus are made up mainly of small lymphocytes, macrophages and fibroblasts. In contrast to the lymphocyte-rich areas in

Figure 2. $\beta_1$ staining of the cartilage-pannus junction, and strongly ICAM-1 positive cells are also observed in the synovial tissue at the cartilage-pannus border. Diffuse and weakly positive VLA-3, VLA-4 cells and strongly positive VLA-5 cells are observed (Original magnification, $\times 100$).
the RA synovium, pannus contained large numbers of ICAM-1 positive cells (Figure 2), 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 in these areas. VLA-2 positive cells were only occasionally seen and VLA-1, VLA-3 positive cells were usually small in numbers. Thus, it is likely that the tissue distribution patterns of infiltrated cells from pannus PCV are influenced by the ECM of the pannus and 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 $\beta$1 integrins expressed by 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 $\beta$1 integrin binding, such as VLA-3, VLA-4 and VLA-5, to cartilage matrix leading to increased activation as well as ICAM-1 and LFA-1 interaction leading to increased $\beta$1 expression. Since these 3 integrins all function as fibronectin receptors (28), it is tempting to postulate that the fibronectin rich environment of the rheumatoid cartilage surface (29) could effectively trap pannus cells expressing high levels of these molecules. The strong expression of VLA-5 molecule, and weak VLA-1 molecule expression on chondrocytes as observed in the present study would suggest that interactions between chondrocytes and fibronectin may be occurring in the pannus formation through the activation of chondrocytes by various cytokines.

Many of the known ligand for integrins, including collagen, thrombospondin, and fibronectin, are present in articular cartilage. Increased amounts of fibronectin in the pannus-cartilage junction in rheumatoid arthritis has been described (30), which may in part be related to increased de novo synthesis by chondrocytes (31). However, type II collagen-binding proteins, including anchorin II, have been identified on chondrocytes, and the role of integrins in collagen-chondrocyte interaction is as yet uncharacterized (19,32). Recent studies suggest that certain chondrocyte-ECM interaction may be mediated by integrins (19,33,34). VLA-5 molecule found in pericellular and also 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. This is also suggesting that existence of activation-mediated regulatory mechanism of the VLA-5-fibronectin interactions at the pannus site. It is also worthy to mention that VLA molecules stained in the pannus cartilage junction provide appropriate anchorage for the achievement of the variety of immune response.
REFERENCES


