CONCISE REPORT

Distribution of lymphatic vessels in normal and arthritic human synovial tissues

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Objectives: To investigate the distribution of lymphatic vessels in normal, rheumatoid arthritis (RA) and osteoarthritis (OA) synovium.

Methods: Synovial tissues from 5 normal controls, 14 patients with RA, and 16 patients with OA were studied. Lymphatic vessels were identified by immunohistochemistry using antibodies directed against the lymphatic endothelial hyaluronan receptor (LYVE-1) and recognised blood vessel endothelial markers (factor VIII, CD34, CD31).

Results: Lymphatic vessels were found in all zones of the normal, OA, and RA synovial membrane. Few lymphatic vessels were seen in the sublining zone in normal and OA synovium which did not show villous hypertrophy. However, in both RA synovium and OA synovium showing villous hypertrophy and a chronic inflammatory cell infiltrate, numerous lymphatic vessels were seen in all zones of the synovial membrane, including the sublining zone of the superficial subintima.

Conclusions: Lymphatic vessels are present in normal and arthritic synovial tissues and are more numerous and prominent where there is oedema and an increase in inflammatory cells in the subintima, particularly in RA. This may reflect increased transport of hyaluronan and leucocyte trafficking in inflamed synovial tissues.

The synovial membrane covers the inner, non-weightbearing surfaces of diarthrodial joints. The synovial membrane is highly vascular and contains numerous anastomosing small blood vessels which are responsible for the transfer of small and large molecules to and from the blood into synovial tissues. The nature and distribution of lymphatic vessels in the synovial membrane is less well defined. Lymphatic vessels have been identified in the synovial membrane by various techniques, including lymphangiography, electron microscopy, histochemistry, and immunohistochemistry.[1–3] These markers, however, are not specific for lymphatic vessels and have yielded conflicting results regarding the presence/absence and distribution of lymphatic vessels in specific arthritic conditions. Fujiwara et al showed using 5'-nucleotidase staining that lymphatic vessels are present in normal osteoarthritis (OA) and rheumatoid arthritis (RA) synovium.[1] Wilkinson and Edwards, however, found that lymphatic vessels are absent in RA synovium.[2]

The largest concentration of hyaluronan (HA) is found in the musculoskeletal system, and HA is a major component of synovial fluid. Lymphatic vessels transport extracellular matrix components such as HA from the interstitial fluid to lymph nodes, where it is degraded into small fragments. Recently, a new HA receptor, LYVE-1 (lymphatic vessel endothelial HA receptor), has been shown to be a highly specific marker of lymphatic endothelial cells in lymphatic vessels and lymph node sinuses.[4] In this study we used LYVE-1 expression to examine the distribution of lymphatic vessels in normal, RA, and OA synovial tissues.

PATIENTS AND METHODS

Five samples of normal synovium were obtained from the unaffected knee joints of legs amputated for bone/soft tissue sarcomas. Fourteen RA and 16 OA synovial tissues were obtained at the time of knee joint arthroplasty surgery. The samples were fixed in formalin then embedded in paraffin wax. Sections (5 μm) were cut, dewaxed, and rehydrated by successive immersion in xylene, graded ethanol, and water, followed by microwave treatment (700 W, 2×4 minutes) in Target Retrieval Solution (Code No S1700, DAKO). Endogenous peroxidase was blocked by 0.3%(vol/vol) hydrogen peroxide and protein block serum (Code No X0909, DAKO). The sections were incubated with a mouse-anti-human LYVE-1 monoclonal antibody generated against the LYVE-1 ectodomain Fc fusion construct (residues 1-232), the sequence for which is detailed by Banerji et al[4]; this monoclonal antibody displays the same specificity for lymphatic vessel endothelium as the original polyclonal antiserum.[5–8] The antibody was diluted 1:40 in phosphate buffered saline and the antigen detected by incubation with labelled polymer and diaminobenzidine (Envision+ kit; DAKO). The sections were then counterstained with haematoxylin, dehydrated, cleared, and mounted.

Antibodies directed against a number of other vascular endothelial cell markers (factor VIII, CD31, CD34, and CD68, a macrophage associated antigen) were also used for staining by the same protocol as that described above for LYVE-1.

RESULTS

Lymphatic vessels in normal, RA, and OA synovium were identified by LYVE-1 staining in all samples of synovium studied. The LYVE-1+ lymphatic vessels were thin walled and lined by flattened endothelial cells; these vessels were distinguished from blood vessels and capillaries as they did not contain red blood cells and were negative for CD34 and factor VIII. Endothelial cells lining small and large blood vessels were negative for LYVE-1, confirming the specificity of this marker for lymphatic vessels.[4]

Lymphatic vessels were seen in adipose, areolar, and fibrous types of the normal synovial membrane. In general, fewer lymphatic vessels were noted in the fibrous type of synovial membrane. In normal synovium, relatively few lymphatic vessels were found in the superficial subintima, particularly in the sublining zone immediately beneath the intima (fig 1A). In normal synovium, most lymphatic vessels

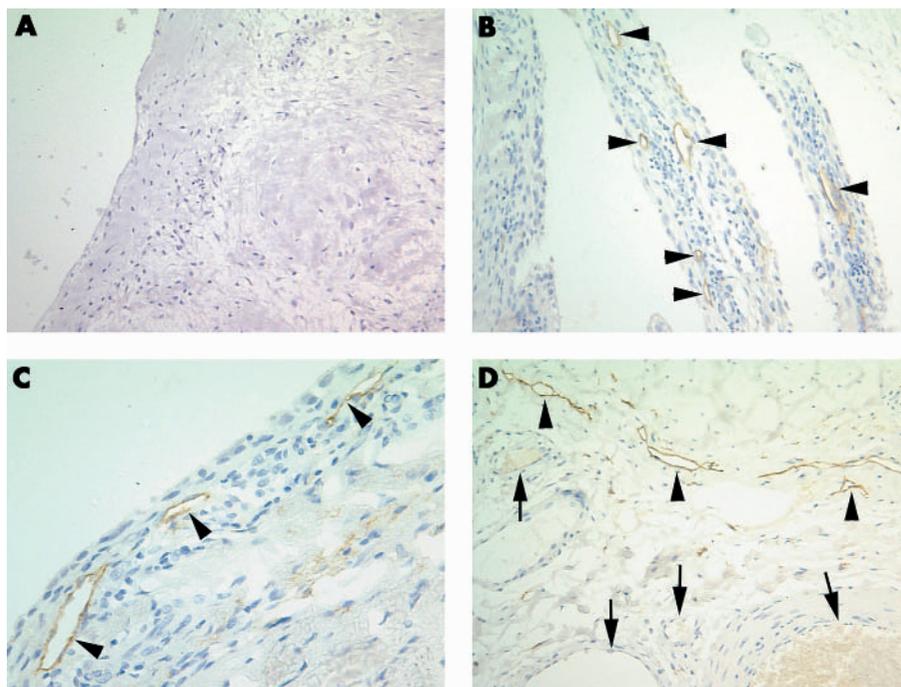...

Figure 1 LYVE-1 staining of normal synovium (A), and superficial (B, C), and deep (D) zones of RA synovium showing LYVE-1+ lymphatic channels (arrowhead) in RA synovium. Endothelial cells lining blood vessels (arrowed) are negative for LYVE-1. (Immunoperoxidase: magnification A, B, D ×200; C ×400).

were found in the deep subintima and underlying fibrous tissue of the joint capsule. In OA joints, lymphatic vessels were seen in all regions of the synovial membrane, including the superficial, mid-, and deep zones of the subintima. Where the OA synovium was not oedematous or inflamed and did not show villous hypertrophy, the distribution of lymphatic vessels was similar to that seen in normal synovium. However, where OA synovium was thickened, oedematous, and contained scattered chronic inflammatory cells, lymphatic vessels were generally more numerous in all zones of the subintima and particularly prominent in the sublining zone. Some of these vessels were dilated, whereas others were compressed and had a slit-like lumen. Histomorphometric evaluation (after examination of at least 20 random high power fields) for LYVE-1 stained vessels in the superficial, mid-, and deep zones of the synovium showed mean values of 6.65, 2.61, and 3.25 vessels respectively in inflamed OA synovium and 2.01, 2.16, and 2.82 LYVE-1 stained vessels in the corresponding zones of non-inflamed OA synovium.

In RA synovial tissues, lymphatic vessels were numerous and found in all zones of the synovial membrane, including the sublining, superficial and deep subintima as well as in capsular connective tissue (figs 1B, C). In areas of villous proliferation, numerous small lymphatic vessels, which had a dilated or slit-like lumen, were found in the sublining and superficial subintima. In mid- and deep zones of the subintima and underlying capsular tissue, there were also numerous lymphatic vessels; many of these were found near small blood vessels, around which they formed a network of lymphatic channels (fig 1D).

DISCUSSION

Lymphatic vessels transport and recirculate extracellular matrix components from the interstitial fluid and are involved in trafficking of leucocytes to and from the lymph nodes and secondary lymphoid tissues. HA is an extracellular matrix component of synovial connective tissue and a constituent of synovial fluid. In tissues it undergoes constant turnover and is thought to regulate lymphocyte migration.[9] The breakdown products of HA have potent proinflammatory effects. For this reason it is thought that relatively little HA is degraded in situ and that most enters the lymphatic system and is degraded in lymph nodes.[10] Most proteins that interact with HA belong to the Link superfamily, whose members contain a conserved HA binding domain termed the Link module. LYVE-1 is a new Link superfamily HA receptor on lymphatic endothelium, which has sequence homology with the inflammatory leucocyte homing receptor CD44.[4 11 12] Although CD44 is a primary receptor for HA on migrating leucocytes, it is (unlike LYVE-1) absent from lymphatic vessels[13 14]; in contrast, LYVE-1 is expressed on lymphatic but not vascular endothelial cells.

Using LYVE-1 to identify lymphatic vessels in normal, OA, and RA synovium, we noted an increase in the number of small lymphatic vessels in inflamed synovial tissues, particularly in RA. This increase in lymphatic vessels may reflect the increased turnover of HA/increased trafficking of leucocytes that occurs in the RA inflammatory microenvironment. The LYVE-1 receptor is equally exposed on both the lumenal and ablumenal surfaces of lymphatic vessels and it is likely that LYVE-1 is involved in the transport of HA from the extracellular matrix to the lumen of lymphatic channels.[12] LYVE-1 may also have a role in regulating leucocyte migration, the attraction of leucocytes towards lymphatic vessels being regulated by chemokines such as secondary lymphoid-tissue chemokine (SLC) (CCL21) released by lymphatic endothelium.[15] HA, which is sequestered by LYVE-1 on the ablumenal surface of lymphatic endothelium, promotes the adhesion and migration of CD44+ leucocytes across the vessel wall; interaction with LYVE-1-HA complexes on the lumenal surface would facilitate subsequent transcytosis and migration to lymph nodes. Increased lymphangiogenesis is another means whereby an increase in the number of lymphatic vessels might occur in inflammatory OA and RA. It has been shown that LYVE-1+ vessels co-express the receptor (VEGFR-3) for vascular

endothelial growth factor C (VEGF-C), a potent stimulator of lymphangiogenesis which has been identified in the hypertrophic synovium of arthritic joints.[16]

Our finding that lymphatic vessels are abundant in RA synovium is in contrast with the results of previous studies, which reported the presence of few if any such structures in rheumatoid synovial tissues[1 2 16]; these studies relied largely on differential staining with blood vessel endothelial markers to identify lymphatic vessels, whereas our study used positive staining of lymphatic vessels for LYVE-1, a marker whose specificity for lymphatic vessels has been validated in numerous tissues.[4 11 12] The levels of HA are known to be increased in both the synovial tissues and serum of patients with RA.[18 19] An increase in the number of LYVE-1^{+} lymphatic vessels in RA synovium is in keeping with the increased turnover of HA and increased trafficking of leucocytes that is likely to occur in this inflammatory condition. It is also worth remarking that the failure of mechanical drainage of synovial fluid into lymphatic vessels has been proposed as a cause of villous proliferation of synovial tissues in RA[1 2]; if this is the case then this is more likely to be due to overloading of existing lymphatic channels with HA-rich extracellular fluid and leucocyte trafficking to lymph nodes rather than a lack of lymphatic vessels.

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