RAPID REPORT

Adhesion of rheumatoid peripheral blood and synovial fluid mononuclear cells to high endothelial venules of gut mucosa

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Abstract

Mononuclear cells isolated from paired blood and synovial fluid of seven patients with rheumatoid arthritis showed cytoadherence to porcine Peyer's patch high endothelial venules using the Stamper–Woodruff method. A significantly greater proportion of binding was found among the synovial fluid lymphocytes. These would appear to be a population of cells that share adherence characteristics with cells known to be of gut mucosal origin, suggesting that in rheumatoid arthritis some lymphocytes derived from mucosa migrate to joints.

The well recognised association of seronegative arthritis with chronic inflammatory bowel disease has prompted the hypothesis that in this condition, and in rheumatoid arthritis, there may be an abnormal trafficking of cells. This could lead to inappropriate migration to synovial membrane by lymphocytes of mucosal origin, for which the term 'lymphocyte iteropathy' has been suggested.¹

Chronic inflammation is characterised by extravascular collections of mononuclear cells, comprising lymphocytes, macrophages, and plasma cells. Central to our understanding of this process is the concept of controlled emigration of cells from the vascular tree. This requires cell-endothelium interaction. Emigration takes place through portals termed high endothelial venules and is brought about by adhesion-ligand interactions, which are reversible, permitting de-adhesion and onward migration into specific sites. At least two mutually exclusive populations of lymphocytes have been described, with a tendency to migrate primarily to lymph node or mucosa—Peyer's patch high endothelial venules.² An *in vivo* correlate of the binding phenomenon is the Stamper–Woodruff cytoadherence technique, in which lymphocytes are layered under controlled conditions onto cryostat sections of tissues bearing high endothelial venules.³ We used this method to examine the binding of peripheral blood and synovial fluid lymphocytes from patients with rheumatoid arthritis onto high endothelial venules of mucosal origin.

Materials, methods, and results

Seven patients (three female, four male) who

satisfied the modified criteria for the diagnosis of rheumatoid arthritis were studied.⁴ All had active disease at the time of sampling (at least one painful swollen joint, a raised plasma viscosity, and an increased C reactive protein). Their average age was 65 years (range 38–79). Disease duration ranged from five months to 12 years. Six were already receiving second line agents.

Mononuclear cells were obtained by gradient centrifugation from the paired samples. This gave yields of greater than 90% lymphocytes. Cells (5×10^6) were applied to each section of porcine Peyer's patch,⁵ and the sections stained with haematoxylin and eosin. High endothelial venules were readily identifiable as circular structures with a characteristically plump endothelium. Binding by overlaid lymphocytes could clearly be seen in a slightly different plane of focus (fig 1). Enumeration was carried out by one observer using bright field illumination (magnification $\times 250$). A minimum of 10 fields was counted on each of five slides for each sample. The intraobserver coefficient of variation was 3.9%. Results were expressed as specific binding (SB) where:

$$SB = \frac{\text{No of cells adherent in HEV rich zones}}{\text{No of cells adherent in HEV poor zones} \times \text{No of HEVs}} \times 100$$

(HEV = high endothelial venule)

Figure 2 depicts the specific binding for the samples, showing that the binding was greater

Figure 1 Adherence of synovial fluid mononuclear cells to Peyer's patch high endothelial venules.

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Figure 2 Specific binding values of paired lymphocytes from patients' synovial fluid (SFL) and blood (PBL) ($n=7$), and porcine lymphocytes from Peyer's patch (PPL) ($n=16$), lymph node (LNL) ($n=7$), or blood ($n=16$), to porcine Peyer's patch high endothelial venules.

in all cases for synovial fluid cells ($p<0.002$). Corresponding data for porcine lymphocytes from blood, peripheral lymph node, and Peyer's patch are also given.

Discussion

Our results indicate that although both circulating peripheral blood lymphocytes and synovial fluid lymphocytes include cells which bind to Peyer's patch high endothelial venules, the proportion so doing is significantly greater in synovial fluid. It does not, however, tell us anything about their lineage.

It may well be that many lymphocytes found in inflamed rheumatoid synovium are attracted by inflammatory mediators. Therefore, attachment of lymphocytes to high endothelial venules present in *inflamed* synovium is likely to involve ligands modified by inflammation.⁵ Thus in regard to the origin of these lymphocytes, deductions that are based on *in vitro* binding to rheumatoid synovial high endothelial venules

and inhibition by various monoclonal antibodies may be misleading. The observation that a mucosa specific B lymphoblastoid cell line (KCA) failed to bind to synovial high endothelial venules from patients with inflammatory arthritis, and that binding of human peripheral blood or mouse mesenteric node lymphocytes to synovial high endothelial venules was not inhibited by the monoclonal antibody MEL-14 (which did inhibit binding to lymph node high endothelial venules), led to the proposition that synovial membrane lymphocytes represent a third population of lymphocytes with specific homing to synovium, as distinct from those populations that home to peripheral lymph nodes or mucosal tissues.⁶

By contrast, our findings, based on the use of mixed T and B cell populations and non-inflamed high endothelial venules, imply that a proportion of cells that have actually been in contact with and passed through rheumatoid synovial membrane display adhesion to mucosal high endothelial venules. This would be in keeping with the hypothesis that in rheumatoid arthritis at least a proportion of the mononuclear cells found within the joints share adherence characteristics with lymphocytes of the gut, from where they might have originated.

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