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Ultrastructural localisation of muramidase in the human synovial membrane

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SUMMARY The synovial intimal cell layer comprises two morphological types of cell, A and B, with an intermediate type also postulated. Type A cells show features in common with other cells of the mononuclear phagocyte system, while type B cells appear similar to fibroblasts and are assumed to have synthetic activity. Muramidase is a marker of mononuclear phagocytic cells, and we have investigated the synovial membrane for the presence of this enzyme in cells by an immunogold labelling technique. Muramidase was localised within intracytoplasmic vacuoles in subintimal macrophages and type A synoviocytes. This finding provides further evidence that type A cells are closely related to macrophages.

Key words: electron microscopy, synoviocytes, lysosomes, enzyme production.

The surface of the synovial membrane is covered by a layer of intimal cells (synoviocytes), which are increased in number in various pathological conditions. Two main types of synovial intimal cell are recognised by electron microscopy, types A and B, though an intermediate cell, sometimes called AB, has also been described.[1] The type A cell is characterised by prominent Golgi apparatus, many vesicles and vacuoles, and sparse rough endoplasmic reticulum, whereas the type B cell is well endowed with rough endoplasmic reticulum, but Golgi complexes, vesicles, and vacuoles are not abundant.

Hyperplasia of the intimal cell layer in conditions such as rheumatoid arthritis (RA) is considered to be due to an increase in type B and AB cells,[2 3] although others believe that type A cells predominate.[4] The manner in which the number of synoviocytes is increased is not yet understood. Synoviocytes may be derived from a common precursor which multiplies locally,[5–7] but evidence has been presented more recently for a bone marrow origin for type A cells.[8 9]

The monocyte/macrophage lineage of cells contains significant amounts of the enzyme muramidase (EC 3.2.1.17). The synthesis and secretion of the enzyme by mononuclear phagocytes in vitro has been demonstrated by Gordon and colleagues.[10] The enzyme has subsequently been used as a marker for cells of monocyte/macrophage lineage in immunohistochemical studies.[11–13] Reports of the ultrastructural distribution of muramidase are few and relate to tissues where the concentration of the enzyme is very high, for example, in Paneth cells of the gastric mucosa.[14]

Muramidase has been employed in the present study as a marker of macrophages in the synovial membrane in order to examine further the possibility that synovial intimal cells are linked to the monocyte/macrophage system, on the basis of shared enzyme expression with these cells.[15] Although muramidase is also present in polymorphonuclear leucocytes (PMNs), these may be readily distinguished on a morphological basis from other cell types. We have studied the distribution of cells containing muramidase in synovial lining with electron microscopical methods in order to determine which type of synoviocyte contains the enzyme.

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Materials and methods

Synovial specimens from 11 patients, eight with rheumatoid arthritis and three with osteoarthritis (see Table 1 for clinical details) were fixed in 1% glutaraldehyde in 0·1 M phosphate buffer for two hours at room temperature and washed in phos-

Table 1 *Summary of the clinical details of those patients whose synovial membranes were studied using immunoelectron microscopy*

Patient No	*BJRU No*	*Age (years)*	*Sex*	*Joint*	*Source*	*Diagnosis*
1	70/84	58	M	Knee	S*	RA
2	91/84	76	F	Knee	S	RA
3	104/84	51	M	Knee	S	RA
4	109/84	66	F	Knee	S	RA
5	119/84	U*	M	Ankle	S	RA
6	131/84	59	F	Knee	S	RA
7	132/84	55	F	Knee	S	RA
8	86/85	76	F	Knee	S	RA
9	73/84	60	F	Knee	S	OA
10	87/84	48	F	Hip	S	OA
11	85/85	U	F	Hip	S	OA

*U=unknown; S=surgery.

phate buffered saline (PBS) twice for 15 minutes. Excess reactive glutaraldehyde was neutralised by exposing blocks to 0·5 M ammonium chloride in PBS for four hours. The blocks were washed again in PBS and partially dehydrated in graded concentrations of methanol, the temperature being decreased to −30°C as the concentration of methanol was increased. The blocks were subsequently transferred to Lowicryl K4M resin and polymerised at −30°C by ultraviolet light. Silver coloured sections were mounted on Pioloform coated nickel grids and stained for immunoelectron microscopy by the immunogold method. Grids were floated on 1% bovine serum albumin (BSA) in PBS containing 0·1% Tween 20, alone for five minutes and subsequently with rabbit antihuman muramidase antibody (DAKO) (1:8 or 1:16 titrated to optimal dilution) in the BSA/PBS Tween 20 diluent for two hours at room temperature. The grids were then washed twice in PBS and probed with a goat antirabbit–gold conjugate of 15 nm diameter (Jannsen) for one hour at room temperature. Grids were washed again in PBS and then distilled water, and counterstained with 5% aqueous uranyl acetate. Control sections were performed by omission of the primary antibody and by inclusion of irrelevant rabbit antibody, in this case rabbit anti-*Mycobacterium paratuberculosis* (DAKO).

Results

Examination of the intimal cell layer in all cases showed the localisation of muramidase (seen as gold particles) to intracytoplasmic vacuoles in cells which were morphologically type A synoviocytes (Fig. 1). In contrast, the type B cells showed no evidence of the presence of the enzyme (Fig. 2). Preservation of the fine detail was somewhat variable in these surface cells, but the vacuoles containing gold particles in the better preserved intimal cells appeared to be primary lysosomes (Fig. 3), and these did not appear in any of the type B cells. The degree of preservation did not allow clear visualisa-

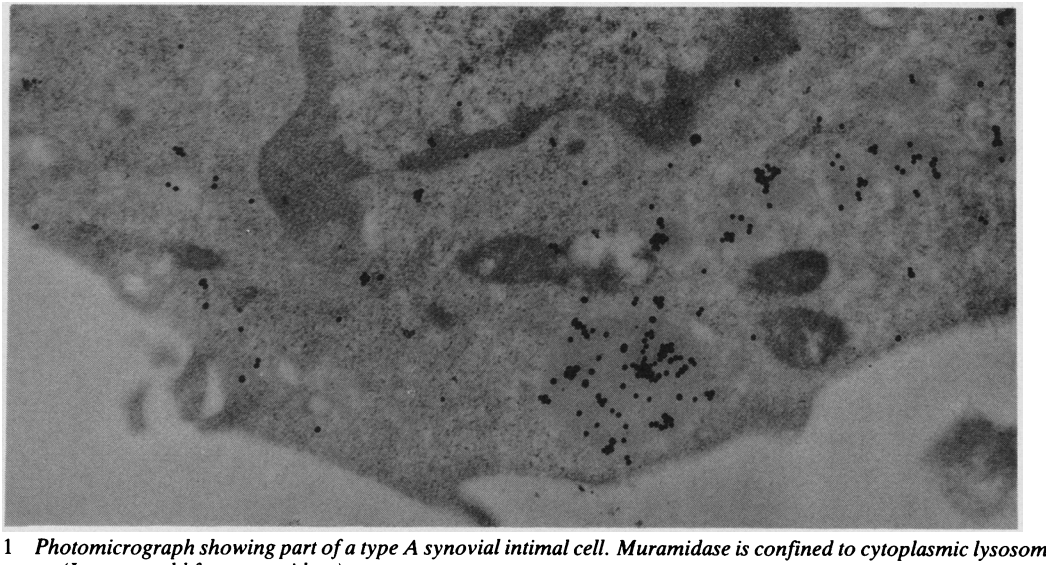

Fig. 1 *Photomicrograph showing part of a type A synovial intimal cell. Muramidase is confined to cytoplasmic lysosomal structures. (Immunogold for muramidase).*

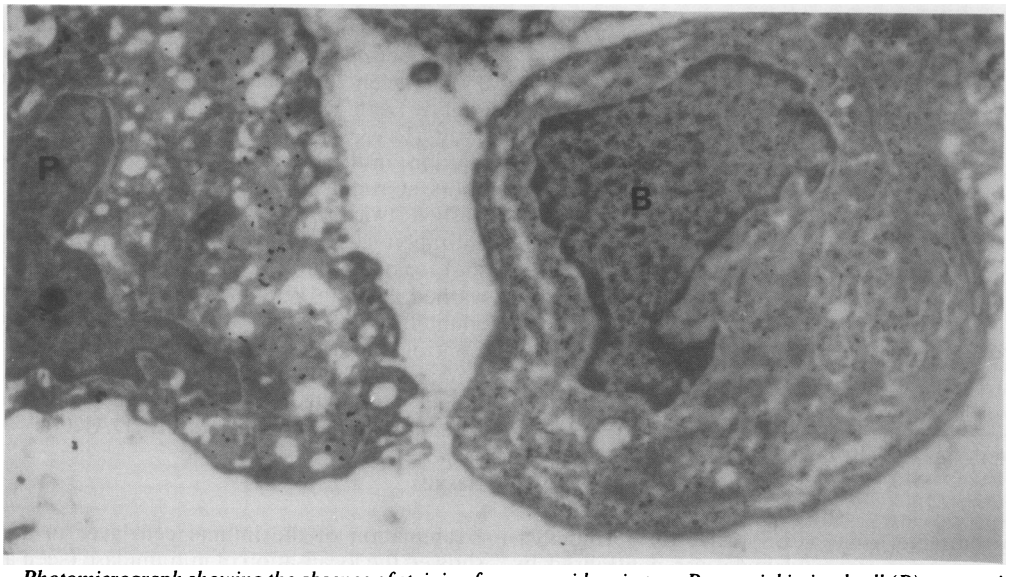

Fig. 2 *Photomicrograph showing the absence of staining for muramidase in type B synovial intimal cell (B) contrasting sharply with adjacent polymorphonuclear leucocyte (P). (Immunogold for muramidase).*

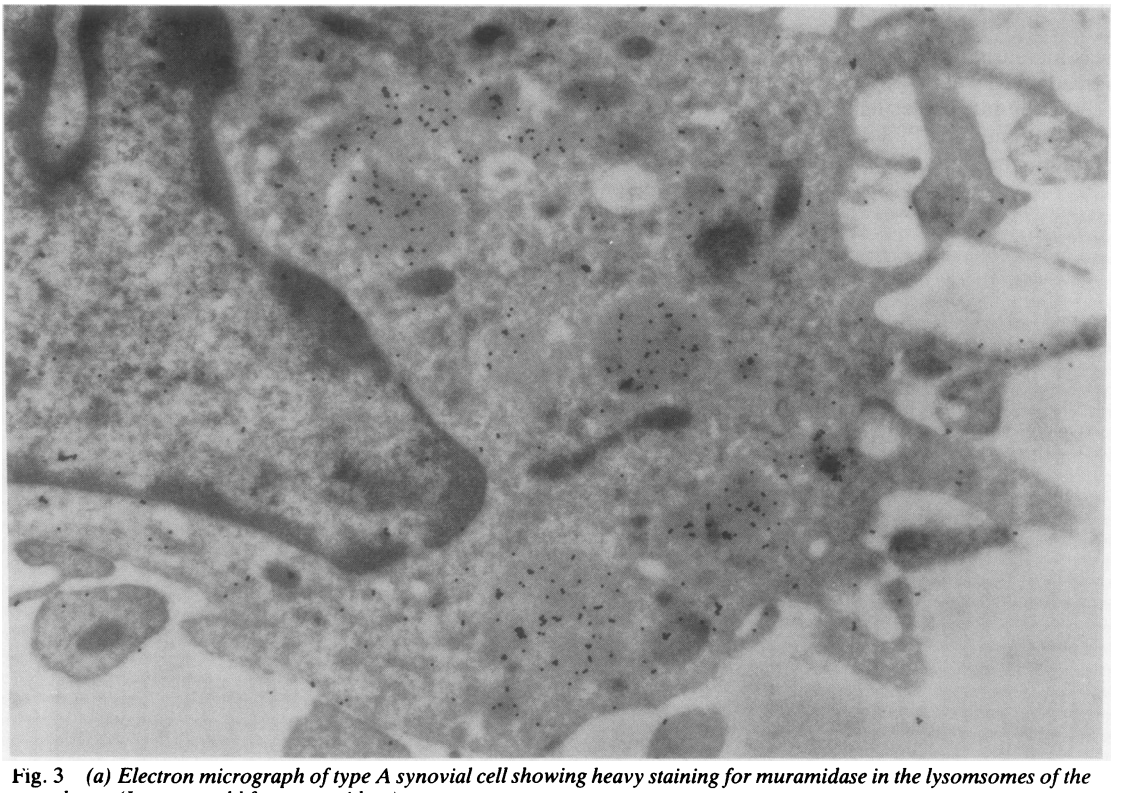

Fig. 3 *(a) Electron micrograph of type A synovial cell showing heavy staining for muramidase in the lysomsomes of the cytoplasm. (Immunogold for muramidase).*

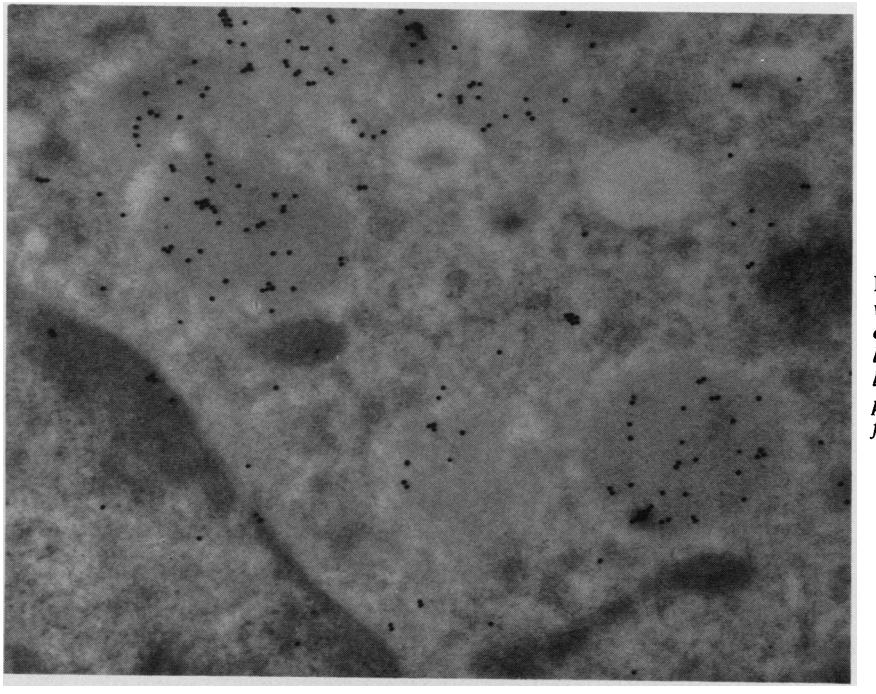

Fig. 3 *(b) High power view of type A synovial cell showing individual lysosomes with localisation of gold particles. (Immunogold for muramidase).*

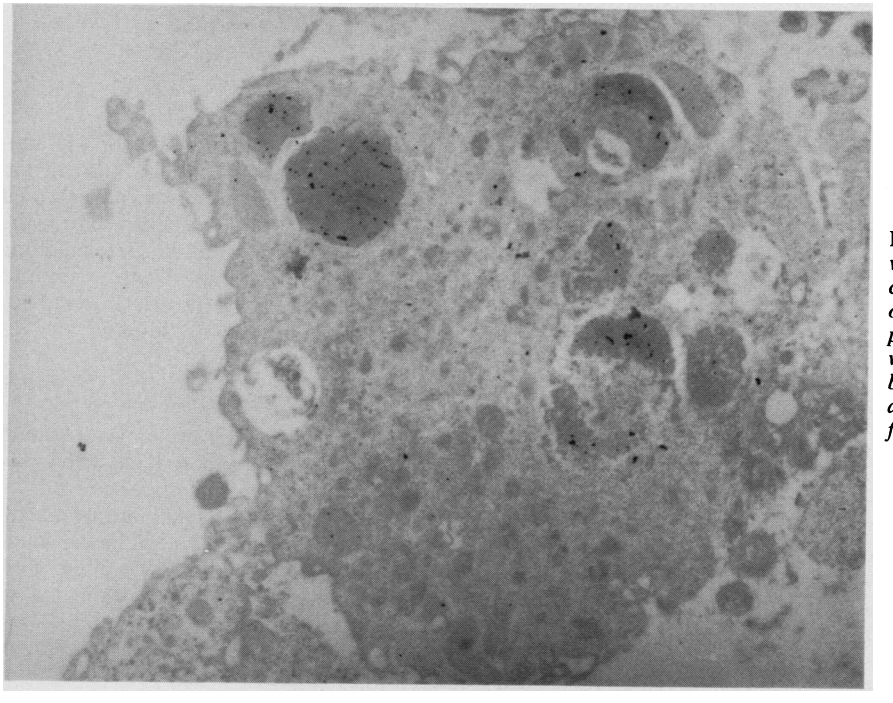

Fig. 4 *Phagocytic vacuoles in type A lining cell containing remnants of erythrocytes. The presence of iron in the vacuoles was confirmed by x ray microprobe analysis. (Immunogold for muramidase).*

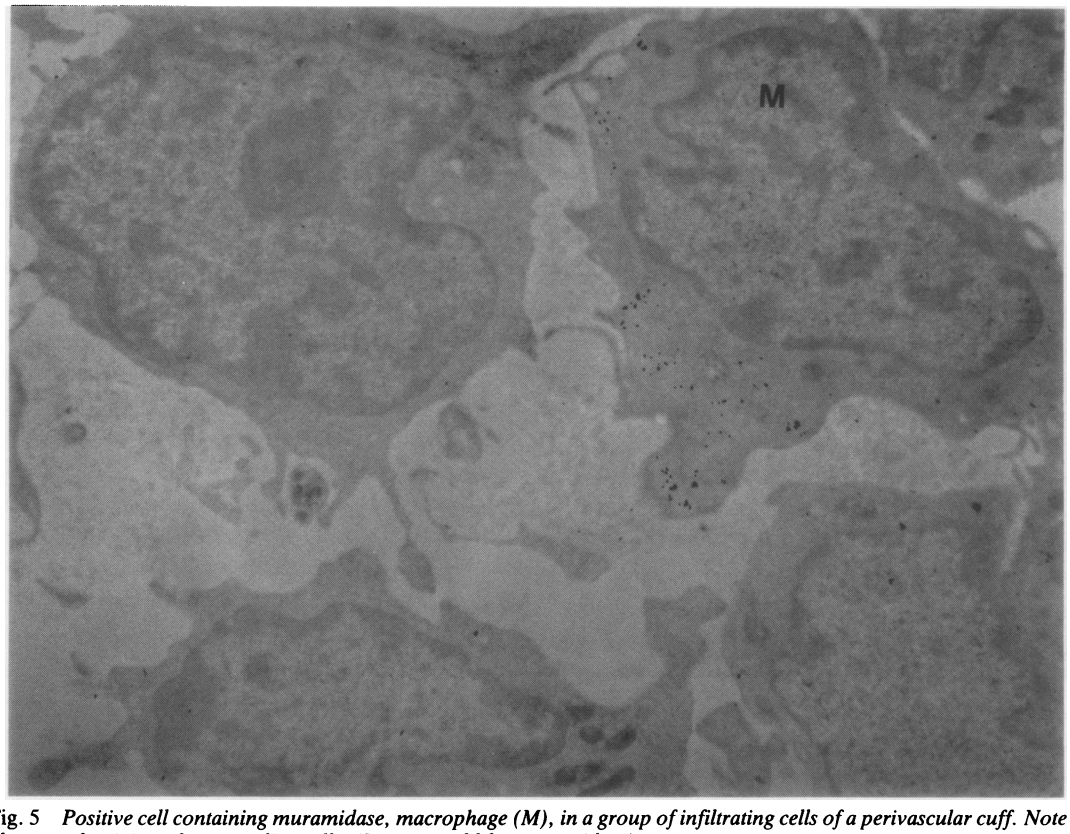

Fig. 5 *Positive cell containing muramidase, macrophage (M), in a group of infiltrating cells of a perivascular cuff. Note absence of staining of surrounding cells. (Immunogold for muramidase).*

tion of components such as Golgi apparatus. Localisation of gold probe to lysosomes would imply the production of the enzyme by the type A cells. The type A cells all showed expression of muramidase, but in some specimens many empty cytoplasmic vacuoles were observed, and label for the enzyme was markedly decreased in these sections. Muramidase was often localised to phagocytic vacuoles containing iron, the presence of which was confirmed by *x* ray microprobe analysis (Fig. 4).

Two types of subintimal cell were labelled, namely infiltrating macrophages and PMNs. The macrophages were closely associated with other infiltrating cells which showed no expression of the enzymes (Fig. 5) and which were presumably lymphocytes. Closer examination of the subintimal macrophages also showed localisation of the gold probe to discrete vacuoles within the cytoplasm having morphology consistent with primary lysosomes (Fig. 6).

In addition to the subintimal macrophages the PMNs also showed strong expression of muramidase, both within vessels (Fig. 7) and also in the subintimal tissues. Marking for muramidase showed a surprising number of PMNs to be present in the synovial tissue, and they were often seen engulfing electron dense fibrous material. Control sections performed with rabbit anti-*Mycobacterium paratuberculosis* antibody showed no staining.

Discussion

The distribution of muramidase in synovial tissue of patients with osteoarthritis and RA has been previously described at the light microscope level.[16 17] Muramidase was found in PMNs, subintimal macrophages, and a proportion of the cells in the lining layer.[16] Their data, however, did not allow clear identification of the cell type which stained positively. We confirmed these findings[17] but were unable to show any difference in the distribution of cells containing muramidase in RA, ankylosing

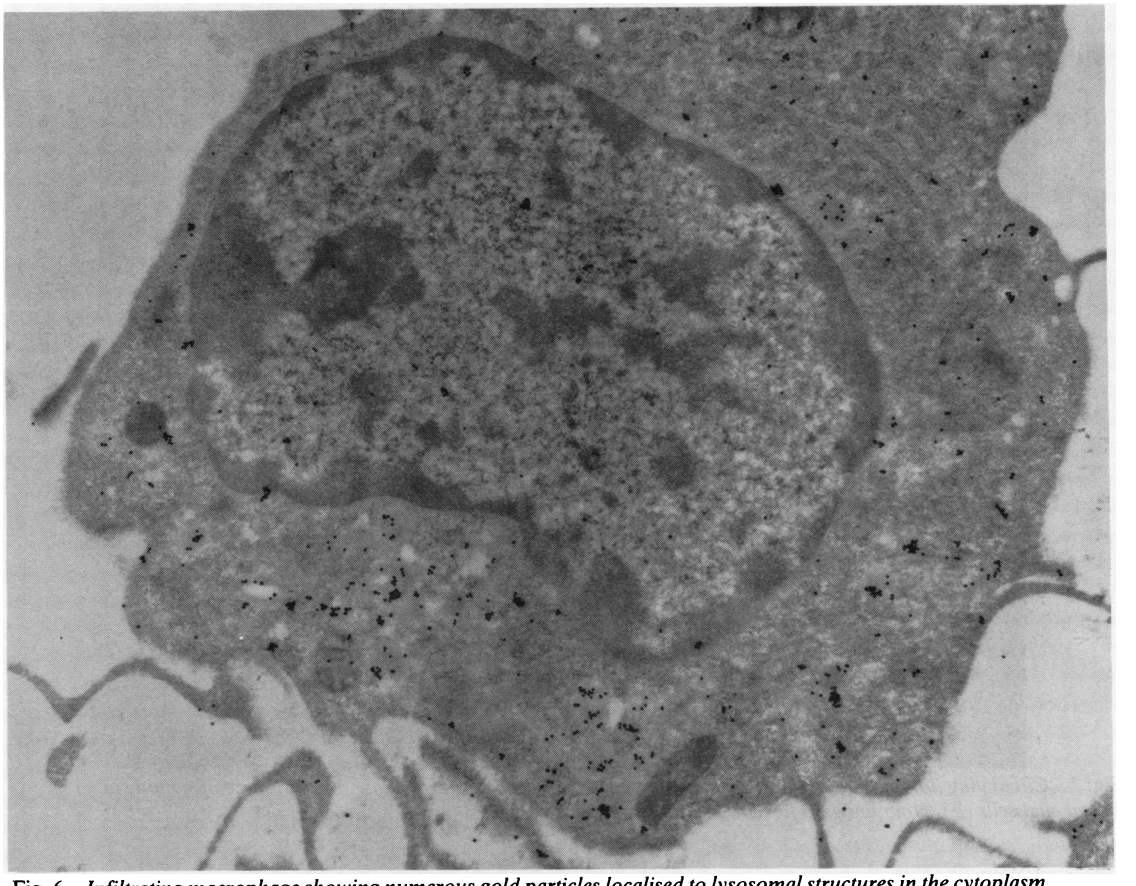

Fig. 6 *Infiltrating macrophage showing numerous gold particles localised to lysosomal structures in the cytoplasm. (Immunogold for muramidase).*

spondylitis, psoriatic arthritis, or Reiter's syndrome. Using the peroxidase-antiperoxidase technique[18] and routinely processed formaldehyde fixed tissue, intracellular staining of infiltrating macrophages appeared to be part granular and part cytoplasmic.

Further studies suggested that the proportion of lining cells which stained positively for muramidase might be dependent on disease activity, though these authors were also unable to identify the positive lining cells morphologically.[19]

The use of the immunogold technique has allowed the identification of the type A synovial intimal cells as the cells containing muramidase. In the specimens studied we found muramidase positive intimal cells in all cases. It is clear, however, that in some cases there was considerable extraction of cell contents during processing as evidenced by extensive vacuolated areas where no electron opaque material could be observed. This probably accounts for the failure of a small proportion of type A intimal cells to stain positively for muramidase. Muramidase having a molecular weight of only 14 kD would be prone to leaching,[20] particularly at the intimal cell surface, by contact with the aqueous fixatives as indicated by Reitamo.[21] Despite this, we believe we have provided evidence for the presence of muramidase, a macrophage marker, in type A synoviocytes. The possibility of secondary uptake of the muramidase by the synovial type A cells seems unlikely since the enzyme was localised to primary rather than secondary lysosomes.

Briggs and colleagues surveyed all blood and bone marrow cells and showed that of the cell types examined, not only the monocytes, but also polymorphonuclear neutrophils (PMNs) contained muramidase activity.[22] Baggiolini and colleagues

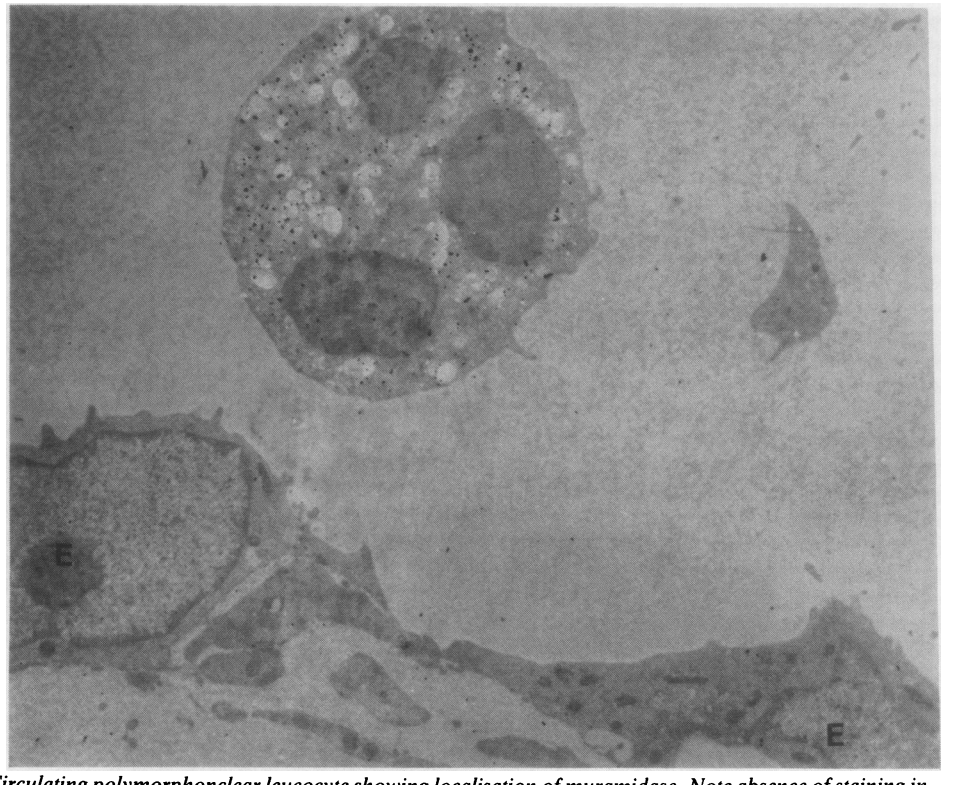

Fig. 7 *Circulating polymorphonclear leucocyte showing localisation of muramidase. Note absence of staining in endothelial cells (E). (Immunogold for muramidase).*

showed that in PMNs the enzyme was located in both the primary and secondary granules.[23]

In the subintimal tissue muramidase was localised to PMNs and macrophages as has been shown previously by light microscopy.[24] As expected from these studies the concentration of muramidase in the PMNs was particularly high and clearly shown in sectional fractions of PMNs as well as whole cells. The number of PMNs thus demonstrated in the subintimal tissue was considerable, particularly in the rheumatoid specimens. This observation is consistent with the large number of polymorphs found in the inflammatory fluids in RA,[25] which must migrate from the circulation, through the synovial tissue, to the joint space, and the demonstration of PMNs by light microscopy using monoclonal antibodies.[26]

Macrophages in the subintimal tissue were often in close apposition to other cells in 'perivascular cuffs'. The presence of these cells in this position would indicate their arrival from the circulation. These cells are thought to be active in the presentation of antigen to activated lymphocytes.

It has been suggested by experimental studies that the synovial intimal cell layer contains macrophages.[8 9] Immunohistochemical studies have shown positive staining of a proportion of these cells for HLA-DR antigen[27–31] and macrophage monoclonal antibody markers.[31 32] All these studies have been performed at light microscope level and, therefore, have not enabled the identification of the cells as type A or B synoviocytes, or as other cells which are infiltrating the synovial surface cell layer.

The ultrastructural demonstration of the enzyme muramidase in both the subintimal macrophages which have presumably migrated from the circulation, and in the lysosomes of type A synoviocytes offers further evidence for a monocyte/macrophage lineage for the type A synoviocyte, though it is still not possible to state whether one cell (the macrophage) becomes the other (the synoviocyte).

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