Giant cells in arthritic synovium

Linda S Wilkinson, Andrew A Pitsillides, Jo C W Edwards

Abstract

Objectives Giant cells are commonly present in inflamed synovium, often in close association with the intimal layer. The nature of these multinucleate cells has been reassessed using new cytochemical and immunochemical techniques.

Methods Cryostat sections of non-inflamed, rheumatoid arthritic and osteoarthritic synovia were analysed for the presence of CD68 and non-specific esterase, markers associated with macrophages; activity of uridine diphosphoglucose dehydrogenase, associated with fibroblast-like synoviocytes; and tartrate resistant acid phosphatase and the vitronectin receptor subunit CD51, associated with osteoclasts.

Results Giant cells were not seen in non-inflamed tissue. In diseased tissue giant cells in the intimal layer fell into two major groups: CD68 negative or dull cells with high uridine diphosphoglucose dehydrogenase (UDPGD) activity suggestive of true synoviocyte polykaryons; and CD68 positive cells with low UDPGD activity suggestive of macrophage polykaryons. The two groups were seen in samples from patients with rheumatoid arthritis (RA) and patients with osteoarthritis (OA), but the former were more prominent in OA and the latter in RA. Most CD68 positive giant cells also showed tartrate resistant acid phosphatase activity and prominent expression of CD51. As such they were histochemically indistinguishable from osteoclasts, but their bone resorbing capacity remains unknown.

Conclusions Giant cells in arthritic synovium appear to be of two types, one related to true synoviocytes and one to macrophages.

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Giant cells are commonly present in diseased synovial tissue. Collins¹ and Grimley and Sokoloff² noted the presence of giant cells of a particular morphology within or just beneath the intimal layer in rheumatoid arthritic tissue. These cells are similar to mononuclear intimal cells in many respects and may carry processes passing up to the intimal surface.

Giant cells may derive from a range of mononuclear precursors. Macrophage derived giant cells are commonly described³ and are considered to be distinct from osteoclasts,⁴ despite a number of common features including their probable origin from bone marrow precursors.⁵ Fibroblast polykaryons

are rarely described in tissue, though they may appear in fibroblast cell cultures.

Developments in immunohistochemistry and cytochemistry have made it possible to differentiate individual synovial lining cell populations in tissue sections on the basis of metabolic activities or expression of surface or cytoplasmic markers.⁶ The aim of this study was to analyse giant cell populations in synovium in detail with particular reference to two questions: the identity of the cells described by Collins¹ and Grimley and Sokoloff² and evidence for the presence of cells showing features commonly displayed by osteoclasts.

Materials and methods

Non-inflamed synovium was obtained from the limbs of five patients undergoing amputation for localised proximal sarcomata. Tissues were taken from macroscopically normal parts of the limb and showed no histological abnormality. Inflamed synovium was obtained from 10 patients with osteoarthritis (OA) and 10 with rheumatoid arthritis (RA) (definite or classical as defined by American College of Rheumatology criteria) undergoing arthroplasty and was obtained within 20 minutes of surgical removal.

Synovial tissues were dissected into 3-5 mm pieces which were snap frozen in n-hexane at -70°C. Sections were cut at -35°C on a Bright's cryostat to a thickness of 7 µm and air dried. Sections for cytochemistry and combined staining were reacted immediately for enzyme activities. Sections for immunohistochemical staining alone were stored at -70°C until required.

Uridine diphosphoglucose dehydrogenase (UDPGD) activity has been shown to be a useful marker for fibroblast-like synoviocytes in tissue sections.⁶ The UDPGD activity was assessed using the method of Mehdizadeh *et al.*⁷ Briefly, sections and cytospin preparations were incubated at 37°C in a medium containing UDP-glucose (3 mg/ml; Sigma) and NAD (0.3 mg/ml; Boehringer) in 30% (w/v) polyvinyl alcohol (Wacker) made up in 0.05 M glycyl glycine buffer. The medium was saturated with nitrogen, the pH adjusted to pH 7.8 and, just before use, nitroblue tetrazolium (3 mg/ml) was added. In all instances the reaction time was 20 minutes. Control incubations were performed using reaction medium without UDP-glucose. After the reaction sections were washed in water, dried, and mounted in Aquamount (BDH), or, when used for further staining, rinsed in phosphate buffered saline (PBS).

Sections and cytospin preparations for double labelling were fixed in fresh acetone for five minutes before reacting for enzyme activity.

Department of Rheumatology, Faculty of Clinical Sciences, University College London, London, United Kingdom
L S Wilkinson
A A Pitsillides
J C W Edwards

Correspondence to: Dr J C W Edwards, Arthur Stanley House, Tottenham Street, London W1P 9PG, United Kingdom

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Primary monoclonal antibodies used for immunohistochemistry were EBM11 (recognising CD68, which is strongly expressed by macrophages),⁸ 23C6 (recognising CD51, part of the vitronectin receptor, strongly expressed by osteoclasts and weakly, if at all, by macrophages),⁹ and 5B5 (recognising prolyl hydroxylase which is present in collagen synthesising cells such as fibroblasts and endothelial cells).¹⁰ Sections were incubated with primary reagents for one hour at room temperature. All subsequent incubations were for 30 minutes. Incubations for immunofluorescence were in PBS at pH 7.6, the primary reagent being followed by antimouse IgG fluorescein isothiocyanate (Sera Lab) in PBS containing 20% normal human serum. For the alkaline phosphatase-antialkaline phosphatase (APAAP) technique antibody dilutions were made in TRIS buffered saline at pH 7.8. Primary monoclonal antibody incubation was followed by rabbit antimouse immunoglobulins, then APAAP (mouse) (Dako), both made up in TRIS buffered saline/20% normal human serum. Sections used for immunofluorescence were mounted in glycerol containing 1,4-diazabicyclo[2.2.2]octane (Sigma), and specimens stained with APAAP in Aquamount.

Non-specific esterase activity is a traditional marker of macrophages which clearly

distinguishes these from fibroblastic cells in synovial lining.⁶ Normal human serum activity was assessed using a standard α naphthyl acetate method.¹¹ When assessment was combined with assessment of UDPGD activity the UDPGD reaction was performed first. When combined with immunochemistry, assessment of normal human serum activity was performed first.

Acid phosphatase activity is characteristic of macrophages and osteoclasts. Acid phosphatase activity was shown using the method of Barka,¹² with naphthol-AS-BI phosphate as substrate. Tartrate resistant acid phosphatase (TRAP) activity, which detects isoenzyme-5 of acid phosphatase, present in large amounts in osteoclasts but not macrophages, was visualised by inhibition of the acid phosphatase reaction with 10 mM sodium tartrate.¹³

For double immunofluorescent staining preparations were incubated with one primary antibody, followed by a specific fluoresceinated second layer. The tissue was then subjected to a blocking incubation with 20% normal mouse serum before the addition of the second (biotinylated) primary antibody, visualised using a streptavidin conjugated reagent.

All double labelling procedures were rigorously controlled by comparison with single labelled serial sections to ensure no interference between techniques. Assessment of UDPGD and TRAP activity was combined with anti-CD68, antiprollyl hydroxylase, and anti-CD51, visualised using a fluorescein labelled second antibody.

Results

Giant cells were not seen in non-inflamed tissue. They were identified in four of the rheumatoid and five of the osteoarthritic tissues examined. This is likely to be an underestimate of their true frequency as giant cells were often only seen in a proportion of sequential sections. When present they occurred at a rate of 1–11/m² of section (up to five in a $\times 20$ objective field).

Ninety per cent of giant cells lay either among the deepest cells of the intimal layer or just deep to the intima (fig 1). These cells fell into two major groups: CD68 negative or dull cells with high UDPGD activity consistent with true synoviocyte polykaryons and CD68 positive, non-specific esterase positive cells with relatively little UDPGD activity

Figure 1 Rheumatoid arthritic synovium showing a giant cell (left of centre) close to the tissue surface (arrowed). In this instance the tissue is sectioned obliquely and the intimal cells appear as a broad band interspersed with fibrin. Bar=25 μ m.

Figure 2 Rheumatoid arthritic synovium. (A) Reacted for uridine diphosphoglucose dehydrogenase activity showing cells of high enzyme activity lining the tissue surface (uppermost) including a polykaryon (centre of field). (B) Stained for CD68 showing numerous positive cells close to the tissue surface, including many polykaryons (left of centre). Bars=50 μ m.

Figure 3 Osteoarthritic synovium showing an area close to the tissue surface containing a large number of polykaryons stained (A) for non-specific esterase, (B) vitronectin receptor, and (C) tartrate resistant acid phosphatase. Most of the polykaryons showing non-specific esterase activity also show some staining for vitronectin receptor and tartrate resistant acid phosphatase. Bars=50 μ m.

consistent with macrophage polykaryons (fig 2). Double labelling on single sections showed these groups to be distinct. The groups were seen in rheumatoid arthritic and osteoarthritic samples but the former were more prominent in osteoarthritis and the latter in rheumatoid arthritis. CD68 positive giant cells contained up to 12 nuclei and had a 'shaggy' roughly circular outline. CD68 negative giant cells had up to four nuclei and had a smaller, smoother oval cell outline with, in some instances, long broad cytoplasmic processes as seen in mononuclear synoviocytes.⁶

The CD68 positive, non-specific esterase positive giant cell group showed moderate or strong acid phosphatase activity. Most of these cells also showed TRAP activity and strong staining with antibody 23C6 directed against the vitronectin receptor subunit CD51 (fig 3). There was a high degree of codistribution of TRAP and vitronectin receptor staining.

The few giant cells seen deep in the subintimal tissue were similar to the CD68 positive cells found in the vicinity of the intima.

Discussion

It appears that Collins¹ and Grimley and Sokoloff² were describing a mixed group of giant cells in rheumatoid synovium. Most have features of macrophages, but a significant minority appear to be derived from fibroblast-like synoviocytes. In OA the latter group predominates. Moreover, there is evidence that many of the CD68 positive giant cells express one or more markers which have been associated with osteoclasts.¹⁴

The presence of synoviocyte giant cells raises the possibility that like other polykaryon forming cells the synoviocyte is an end stage

cell type which rarely, if ever, divides. Attempted mitosis in giant cells is thought to lead to cell death because of the lack of any machinery for apportioning chromatin from more than two sets of chromosomes correctly to daughter cells. Polykaryons were not found in normal tissue, however, and their presence in diseased tissue may represent an aberrant response to local factors such as cytokines¹⁵ incidental to the proliferative capacity of synoviocytes.

From the practical point of view the finding of two types of giant cell in the lining emphasises the importance of using double labelling to ensure correct identification of a potentially heterogeneous population of cells when studying properties such as enzyme activities or expression of surface markers.

The definition of osteoclasts is a matter of continuing debate. Osteoclasts may be defined as giant cells which are resorbing bone or, by proxy, as cells carrying markers which have been associated with cells resorbing bone, including vitronectin receptors and TRAP. Conceptually, osteoclasts may also be defined as cells of a lineage which have the potential to resorb bone, whether or not this potential is realised under the conditions of study. The findings of this study suggest that in the inflamed synovial intima giant cells displaying macrophage features tend also to express features associated with osteoclasts. Until the relation between osteoclasts and macrophage derived polykaryons, in terms of lineage, is clearer the relation between expression of histochemical markers and the cells' ability to degrade bone remains in doubt.

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