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Ultrastructure of synovial changes in rheumatoid disease and in seronegative inflammatory arthropathies

C. Hollywell, C.J. Morris, M. Farr, and K.W. Walton
Department of Investigative Pathology, Rheumatism Research Wing, The Medical School, University of Birmingham, Birmingham B 15, Great Britain

Summary. Synovial tissue has been examined by electron microscopy from patients suffering from either sero-positive or sero-negative inflammatory arthropathies to allow direct comparison of the ultrastructural changes found in these groups and to confirm and extend observations previously made in a smaller group of sero-negative arthropathies. Both groups have been compared with material from healthy controls.

The sero-positive group comprised 13 cases of 'definite' or 'classical' rheumatoid arthritis. The sero-negative group consisted of 9 cases of arthritis secondary to Crohn's disease (3); Reiter's syndrome (2); Whipple's disease (1); Behcet's disease (1); Wegener's granulomatosis (1) and ankylosing spondylitis (1). The control tissue was obtained from 6 non-arthritic subjects undergoing surgery for non-inflammatory conditions.

Confirmation was obtained of changes previously reported in subcellular organelles, especially in synovial B cells, in all forms of inflammatory arthritis as compared with controls. Attention is now drawn to other intracellular changes in B cells and intermediate cells which included: a marked increase of intermediate filaments and microfilaments: and proliferation of pinocytotic vesicles and rough endoplasmic reticulum. These changes were often accompanied by the presence, in the immediate environment of these cells, of extracellular microfibrillary masses but little or no accumulation of intermediate filaments.

It was confirmed that synovial A cells were reduced in number but showed changes suggestive of increased phagocytic activity and also exhibited proliferation of cytoskeletal elements.

Differences in these structural changes between seropositive and seronegative arthritis were of degree rather than of kind and no 'specific' or diagnostic differences were observed between the various forms of seronegative athropathies. The possible significance of the structural changes observed is discussed.

Offprint requests to: C. Hollywell at the above address

Key words: Cytoplasmic filaments – Synovial cells – Rheumatoid arthritis – Seronegative arthropathies

Electron microscopic (EM) studies have shown that the cytoplasm of eukaryotic cells contains an extensive *filamentous cytoskeleton*. The filaments concerned are customarily divided into three groups according to their diameters: *microfilaments* (5–6 nm); *intermediate filaments* (8–12 nm); and *microtubules* (20–25 nm).

Intermediate filaments (IF) are further subdivided according to their composition and the cell-type in which they occur. IF in all forms of smooth muscle are composed of *desmin*; those in mesenchymal cells (including fibroblasts and synoviocytes) contain *vimentin*; while epithelial cells secrete IF containing *keratins* (Lazarides 1980). Since the filamentous cytoskeleton is thought to determine cell rigidity, cell movement and interaction of the cell with the surrounding matrix or adjacent cells, alterations in the relative proportions of the fibrillar components and/or their disposition in the cell might effect alterations in the mechanical properties of the cell occurring in response to disease.

In this study, therefore, we have examined changes in the ultrastructure of synovial cells, with particular reference to the cytoskeleton and cell organelles, occurring in response to inflammatory synovitis arising from various clinically defined conditions as an extension of our previous observations on the ultrastructure of synovium in seronegative arthropathies (Morris et al. 1983).

Materials and methods

Clinical material

The 13 patients diagnosed as suffering from rheumatoid disease conformed to the 'definite or classical' categories of rheumatoid arthritis (RA) as judged by the criteria of the American Rheumatism Association (1959) and all were seropositive with Rose-Waaler titres varying between $^1/_{32}$ and $^1/_{512}$. In 11 of the 13 patients, closed synovial biopsies were obtained using the biopsy needle of Williamson and Holt (1966). In the remaining 2 cases whole synovia were available from the lower end of the ulna after its removal during replacement surgery.

Closed (needle) biopsies of synovium were examined from 9 patients with seronegative arthropathies (mainly knee effusions) associated with clinical diagnoses of Crohn's disease (3 cases); Reiter's syndrome (2 cases); and 1 case each of Whipple's disease, Behcet's disease, ankylosing spondylitis and Wegener's granulomatosis. The tests performed to establish these diagnoses and the diagnostic criteria employed have been previously described (Morris et al. 1983).

As controls, synovial tissue was examined from specimens obtained from 6 non-arthritic individuals undergoing orthopaedic surgery for non-inflammatory conditions (mainly menisectomies).

Preparation of material for electron microscopy

Small blocks (1 mm^3) of synovial membrane were fixed for electron microscopy in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4) at 4° C as soon as possible after removal of the tissue. Specimens were post-fixed in 2% osmium tetroxide, dehydrated in graded alcohols and embed-

ded in Spurr resin (Spurr 1969). Ultra-thin sections were cut using a Reichert OM U2 ultramicrotome, mounted on copper grids, and stained with uranyl acetate and lead citrate (Reynolds 1963).

Sections were viewed in a Siemens Elmiskop 102 electron microscope at an accelerating voltage of 80 K.V.

Results

Observations on control tissues

In confirmation of previous descriptions, the synovial cells in healthy membranes were found to be divisible, on an ultrastructural basis, into two principal types, the Type A (phagocytic cells) and the Type B (secretory cells) with only occasional cells of an intermediary character (Barland et al. 1962). The Type A cells in our control material contain scanty lysosomes and show no evidence of ingestion of electron-dense fibrin-like material. The Type B cells contain elements of rough endoplasmic reticulum (allowing identification of the secretory function of these cells) but there is no structural evidence to suggest an abnormal level of such functional activity. Both types of cell contain only sparse intermediate filaments and microfilaments while the amount of extracellular filamentous and fibrillary material is minimal.

Synovium in rheumatoid and seronegative arthropathies

Certain of the ultrastructural features of rheumatoid synovium described by other authors (Barland et al. 1962 and 1964; Wyllie et al. 1966; Norton and Ziff 1966; Ghadially and Roy 1967) are again observed. These include: changes in the ratio of synovial A to B cells and an increase in intermediate cells; increased cellularity; and changes in structure of intracellular organelles. As we have previously noted (Morris et al. 1983) these features appear not to be in any sense specific or diagnostic of RA but seem rather to be the morphological responses in synovial tissue to inflammatory changes in general since the same changes, in varying degree, are seen in the seronegative (SN) group of arthropathies.

Some authors have also noted the occurrence in rheumatoid synovium of intracellular microfibrillar material in both A and B cells (Norton and Ziff 1966; Hirohata and Kobayashi 1964) or in B cells alone (Ghadially and Roy 1967 and 1969). Observations on a limited range of SN arthritides previously suggested that these changes are also seen in inflammatory arthritis regardless of the underlying cause. For this reason, a wider range of such cases has now been examined and compared directly with cases of seropositive RA.

With one exception (referred to in more detail later) the results in the present study are similar to those previously obtained but particular attention has now been given to the fibrillar and filamentous components seen in RA and SN synovium.

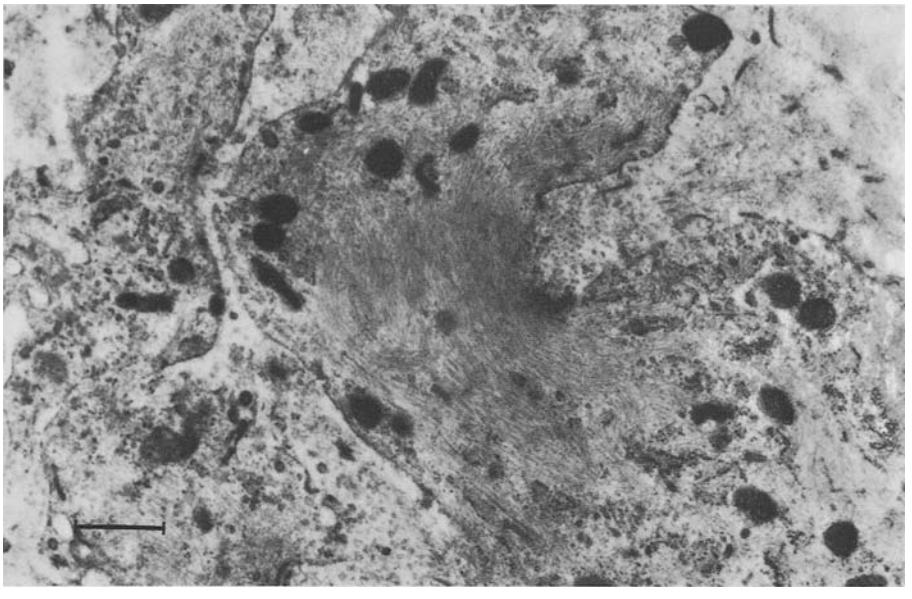

Fig. 1. Electron micrograph of a synovial B cell (Reiter's syndrome). Intermediate filaments (IF) are arranged in bundles along the long axis of the cell, displacing the cell organelles. Surrounding main IF bundles is a fine network of microfilaments. (*scale line* = 750 nm)

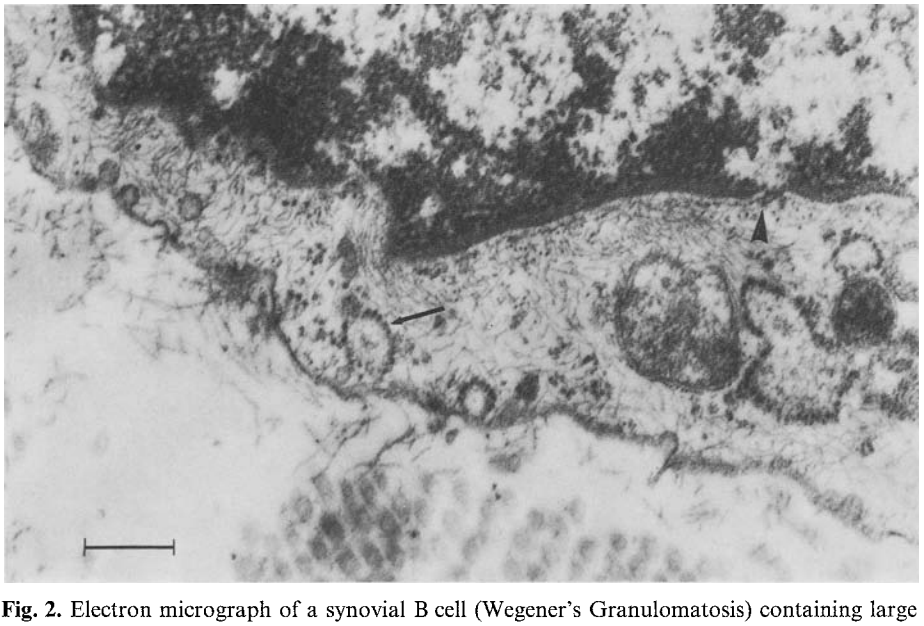

Fig. 2. Electron micrograph of a synovial B cell (Wegener's Granulomatosis) containing large numbers of filaments. The thickened nuclear membrane is coated with microfilaments (MF) and has large nucleopore complexes associated with MF tufts (*arrowhead*). The numerous micropinocytotic vesicles associated with the plasma membrane contain granular material, occasional larger vesicles are coated with polyribosomes (*arrow*). (*scale line* = 250 nm)

Changes in microfibrillar and filamentous components in B and intermediate cells

In the present study, as in preceding ones, an increased cellularity of the synovium is noted in the SN group as in RA. This is accompanied by a relative preponderance of B cells and an apparent increase of intermediate cell types. Both the B cells and intermediate cell types show the presence of microfilaments and intermediate filaments as a prominent feature. The intermediate filaments (IF) often predominate, displacing cell organelles (Fig. 1) and being arranged in bundles along the long axis of the cell.

Surrounding the main bundles of IF, a fine network of microfilaments (MF) is frequently seen arranged in a random manner. These MF are commonly associated with abnormal mitochondria, sometimes filling these organelles and being apparently attached to their cristae. In synovial cells containing large amounts of microfilamentous material, the nuclear membrane is often thickened and coated with MF showing large nucleopore complexes associated with tufts of MF (see Fig. 2).

Other changes in inflammatory arthropathies in B cells

Numerous micropinocytotic vesicles, occurring in association with the plasma membrane, are also common features of the B cells in both RA and SN arthropathies. The vesicles often contain granular material. Occasionally, larger pinocytotic vesicles are noted, the walls of which are associated with polyribosomes (Fig. 2).

In the immediate vicinty of cells showing these features, extracellular microfibrils are observed as large electron-dense deposits which, at points of contact with the cell membrane, are related to even more electron-dense amorphous material (Fig. 3). Such material is not limited to the plasma membrane but is present also in the cytoplasm of the cell. These areas are often associated with high concentrations of micropinocytotic vesicles (Fig. 3).

In both RA and SN arthritis, synovial B cells show thickening and prominence of the rough endoplasmic reticulum (RER) with large numbers of associated polyribosomes. In some cells the RER is expanded and dilated into large numbers of ribosome-coated vesicles (Fig. 4) which contain electron-dense granular material similar to that seen in some pinocytotic vesicles, as mentioned above. The secretory B cells also often display prominent centrioles, usually in conjunction with microtubules radiating throughout the cell (Fig. 5).

Changes in A cells

In the biopsies examined from both RA and SN arthritis, A cells are less numerous than B cells and show less fibrillar change. However, the A cells show an increase in their content of dense bodies (free lysosomes) and occasional cytolysosomes, allowing identification of their phagocytic nature

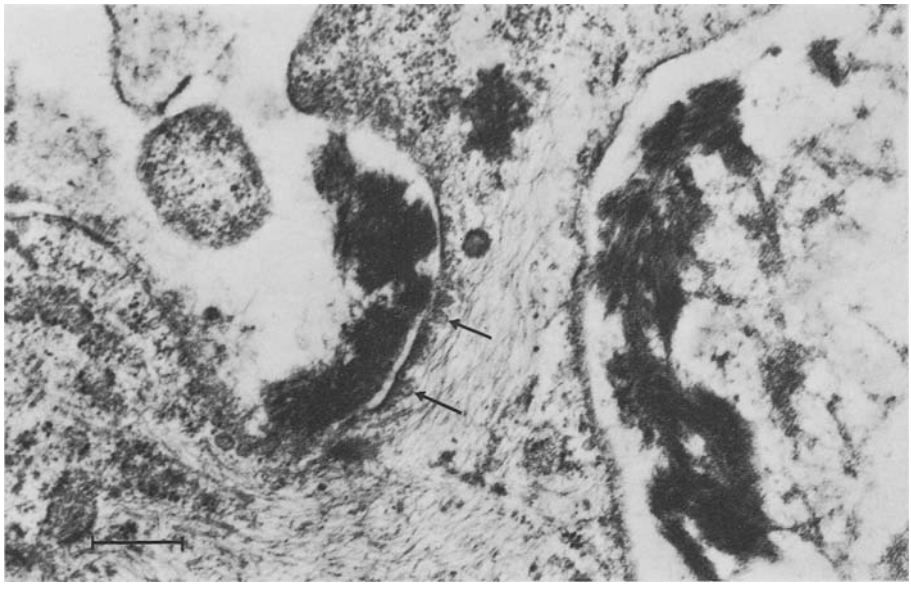

Fig. 3. Electron micrograph of a synovial cell (Reiter's syndrome) showing large electron dense deposits of extracellular microfibrils which, at points of contact with the plasma membrane, are related to plaques of highly electron dense amorphous material. These areas are associated with numerous micropinocytotic vesicles (*arrows*). The intermediate filaments appear parallel to these plaques, arising and terminating in the cytoplasm. (*scale line* = 300 nm)

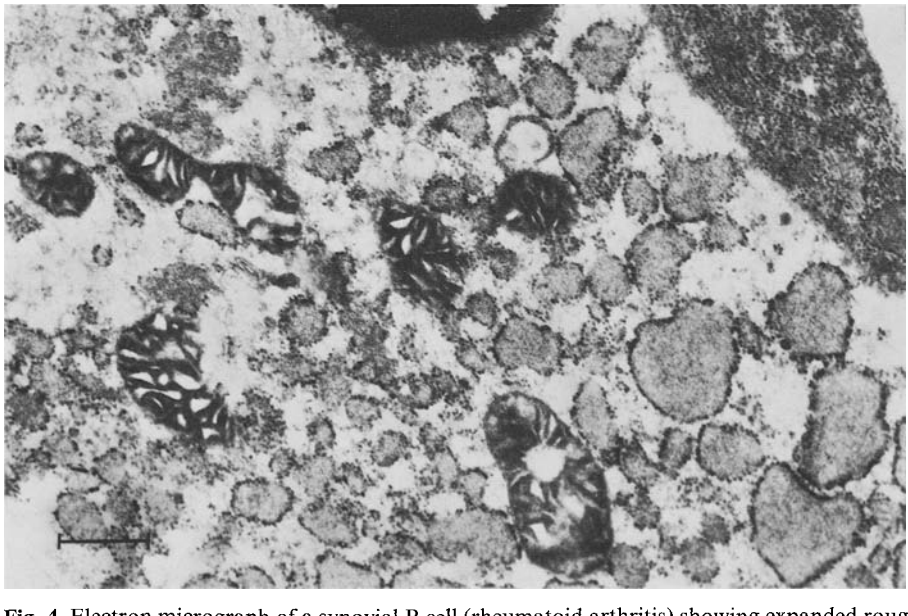

Fig. 4. Electron micrograph of a synovial B cell (rheumatoid arthritis) showing expanded rough endoplasmic reticulum (RER) formed into ribosome coated vesicles containing an electron dense granular material similar to that seen in some pinocytotic vesicles. (*scale line* = 500 nm)

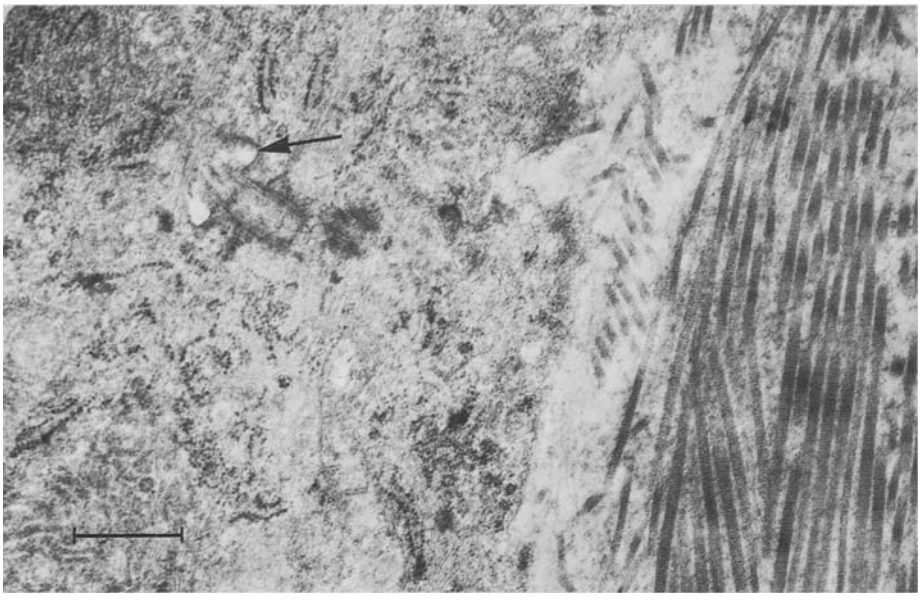

Fig. 5. Electron micrograph of a secretory B cell (rheumatoid arthritis). The abnormal centriole protrudes into a vacuole (*arrow*) giving it the appearance of the base of a cilium. Prominent microtubules radiate from the centriole throughout the cytoplasm. (*scale line* = 500 nm)

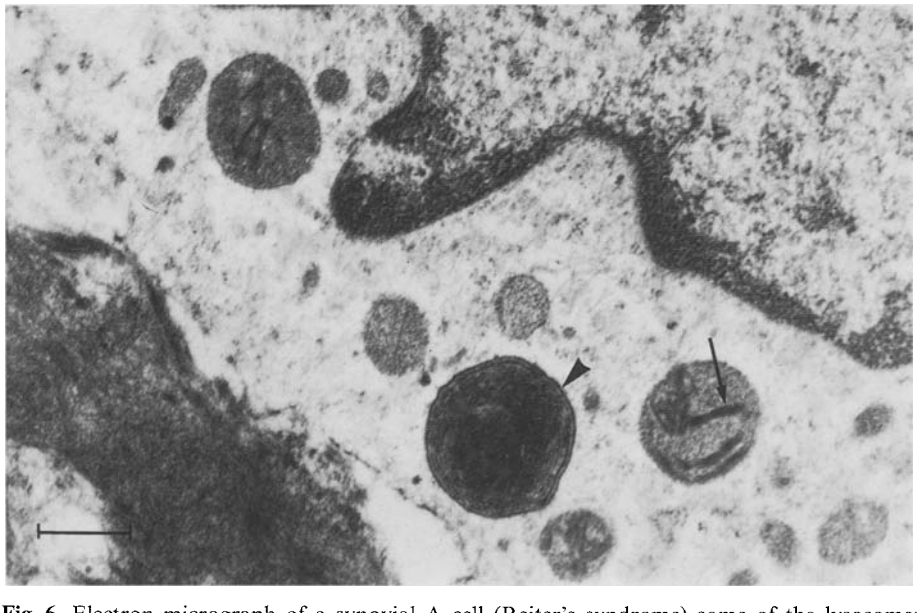

Fig. 6. Electron micrograph of a synovial A cell (Reiter's syndrome) some of the lysosomes contain electron dense material (*arrow*) a cytolysosome is present (*arrowhead*). (*scale line* = 300 nm)

(Fig. 6). Some of the lysosomes contain electron dense material, considered possibly to be fibrin ingested by the cells.

Abnormal extracellular deposits

One of the cases included in the present study, a patient with long-standing Whipple's disease affecting the bowel and associated with arthritis affecting the knees, presented many of the features described above. In addition, the synovial biopsy showed the presence of extracellular fibrillar material with the 'chopped straw' appearance characteristic of *amyloid fibres* (Farr et al. 1983). It has been reported that microdeposition of amyloid in joints is an asymptomatic phenomenon forming part of the degenerative processes of senescence (Goffin 1981). On the other hand, generalised amyloidosis is a relatively common complication of long-standing and severe rheumatoid arthritis.

However, in the present series, no instance of localised amyloid deposition was seen in the sero-positive biopsies examined and the case mentioned above was the sole instance of such deposition encountered among the seronegative arthropathies.

Apart from this single instance of amyloid deposition, one other extracellular feature which appeared worthy of note was the occurrence of extracellular ribosome-coated spherical bodies containing fibrillar material. In our earlier observations, these were seen only in biopsies from our seronegative cases. However, in the present study similar appearances were seen in the deeper levels of the two whole-thickness rheumatoid synovia examined so it would seem that these changes are also non-specifically related to inflammatory change in general. Their possible functional significance is discussed below.

Discussion

In this study we were struck by the increase of both intra- and extracellular filamentous and fibrillary material present in the type B cells and intermediate cells of the tissues we were examining, in addition to the changes in intracellular organelles which we had previously noted and reported (Morris et al. 1983). Initially, the fibrils and filaments were observed in additional examples of sero-negative arthropathies but a closer examination of the seropositive biopsy material showed that this change was not confined to our seronegative arthropathies. Reference to the literature makes it clear that other authors (e.g. Norton and Ziff 1966; Ghadially and Roy 1967 and 1969) have also noted similar changes in rheumatoid synovium (an increase of cytoplasmic 100 Å filaments) although no undue emphasis has been placed on this structural feature.

The functional significance of these changes is debatable. Changes of a broadly similar character, observed in leukaemic cells, have been linked with defective mobility of such cells (Felix and Sträuli 1976). This is not

supported by studies on normal fibroblasts in which intermediate filaments are not thought to be concerned with cell movement or division since the filaments lag behind microtubules and actin during cell locomotion and remain coiled in the periphery of the cell during mitosis (Jorgensen et al. (1976), Hynes and Destree (1978)).

Another possibility that has been considered is whether intermediate filaments play a structural role in anchoring the nucleus. It has been shown by Lehto et al. (1978) and by Small et al. (1978), using detergent-extracted cytoskeletons, that intermediate filaments terminate at both the nuclear membrane and at adhesion plaques on the plasma membrane. However, in our material we were unable to show these terminal connections. But the membrane did show electron-dense plaque areas, often in association with extracellular microfibrillar material. In our hands, the intermediate filaments appeared to run roughly parallel to such areas (Fig. 3), arising and terminating in the cytoplasm. The electron dense plaques were often accompanied by numerous micropinocytotic vesicles, many of which contained granular material. It is not possible to say whether such vesicles are engulfing or secreting the material they contain.

Cells containing increased numbers of intermediate filaments show other features suggesting high metabolic activity. They contain many mitochondria, some abnormal in appearance, the rough endoplasmic reticulum is prominent and frequently becomes so dilated that it splits into ribosome-coated vesicles. The latter may also be found extracellularly. Such cells show active micropinocytosis and often contain a mat-like arrangement of microfilaments (50–60 Å in diameter) which are known to be actin-like in nature (Ishikawa 1969). Since a link has been suggested between 100 Å filaments and actin (Lazarides and Balzer 1977; Buckley et al. 1977) it is possible that the microfilaments which we have seen to be attached to the nuclear membrane thus serve to connect the nucleus to the intermediate filament network rather than the latter serving directly as an anchor for the nucleus.

The association of the increase in cytoskeletal filaments and fibrils with other features indicative of increased cellular activity leads us to favour the possibility that filamentous and fibrillar proliferation are simply a non-specific response to the venous stasis and microvascular disturbances of an inflammatory process in synovium, as suggested by others (eg Goldie 1969; Dryll et al. 1977). The increase in IF and MF is often such as to alter the overall appearance of a synovial cell so as to make it difficult to 'type' the cell with certainty. This presumably accounts for the altered A to B cell ratio and the increase in intermediate cells which we and others have noted.

Proliferation of cytoskeletal elements is nevertheless a feature also of synovial A cells in response to inflammation. The most characteristic feature of these cells is the increase of dense bodies and lysosomes along with occasional cytolysosomes and lysosomes containing fibrin-like material which is not seen in control (healthy) synovia. It is even possible that the apparent increase in lysosomes in the cells of RA and SN arthritis derives

from alterations in internal cell movement created by the increased numbers of MF and IF. These would restrict movement of lysosomes formed within the cell and give a visual impression of crowding of the cytoplasm, in some areas, by the dense bodies.

The centrioles of synovial A cells in our material, were often very prominent and were associated with microtubules which radiated out from the centrioles to other parts of the cell. The centrioles are occasionally seen in conjunction with vacuoles into which they protrude giving them the appearance of the base of a cilium (cf Fig. 5). Similar appearances have been noted in fibroblast cultures derived from RA synovia in a study reported by Wynne Roberts and Castor (1972).

Lastly, accumulations of microfibrils were also seen extracellularly, usually close to plasma membranes but often dispersed throughout the tissue. The nature of this microfibrillar material awaits further investigation by immunohistochemical methods but it appears likely to originate from the adjacent cells.

We therefore conclude that: microfibrillar and filamentous proliferation is a major ultrastructural feature of synovitis regardless of whether this is due to rheumatoid or to seronegative inflammatory arthritis; the process mainly affects B and intermediate cells but is also seen in A cells and is associated with extracellular deposits; and that the precise role of these cytoskeletal elements in determining the course of inflammatory synovitis still remains to be determined.

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References

Barland P, Novikoff AB, Hamerman D (1962) Electron microscopy of the human synovial membrane. J Cell Biol 14:207–220

Barland P, Novikoff AB, Hamerman D (1964) Fine structure and cytochemistry of the rheumatoid synovial membrane, with special reference to Lysosomes. Am J Pathol 44:853–866

Barland P, Novikoff AB, Hamerman D (1964) Lysosomes in the synovial membrane in rheumatoid arthritis: a mechanism for cartilage erosion. Trans Ass Am Physicians 77:239

Buckley IK, Raju TR, Stewart M (1977) Some intermediate sized filaments in cultured chick embryo fibroblasts contain F-actin. J Cell Biol 75a, MF 583

Dryll, Lansaman J, Cazalis P, Peltier AP, De Seze S (1977) Light and electron microscopic study of capillaries in normal and inflammatory human synovial membrane. J Clin Pathol 30:556–562

Farr M, Morris CJ, Hollywell CA, Scott DL, Bacon PA, Walton KW (1983) Amyloidosis in Whipple's arthritis. J Roy Soc Med (in press)

Felix M, Straüli P (1976) Different distribution pattern of 100 Å filaments in resting and locomotive leukaemia cells. Nature 261:604–606

Ghadially FN, Roy S (1967) Ultrastructure of synovial membrane in rheumatoid arthritis. Ann Rheum Dis 26:426–443

Ghadially FN, Roy S (1969) Ultrastructure of synovial joints in health and disease. Butterworths, London

Goffin YA, Thona Y, Potvliege PR (1981) Microdeposition of amyloid in the joints. Ann Rheum Dis 40:27–33

Goldie I (1969) The synovial microvascular derangement in rheumatoid arthritis and osteoarthritis. Acta Orthop Scand 40:751–764
Hirohata K, Kobayashi I (1964) Fine structure of synovial tissues in rheumatoid arthritis. Kobe J Med Sci 10:195
Hynes RO, Destree AR (1978) 10 nm filaments in normal and transformed cells. Cell 13:151–163
Ishikawa H, Bischoff R, Holtzer H (1969) Formation of arrowhead complexes with heavy meromyosin in a variety of cell types. J Cell Biol 43:312–328
Jorgensen AO, Subrahmanya L, Turnbull C (1976) Localisation of the neurofilament protein in neuroblastoma cells by immunofluorescent staining. Proc Nat Acad Sci USA 73:3192–3196
Lazarides SE (1980) Intermediate filaments as mechanical integrators of cellular space. Nature 283:249–256
Lazarides E, Baltzer D Jr (1977) Heterogeneity of desmins between mammalian and avian muscles and their co-isolation with a specific isoelectric variant of actin. J Cell Biol 75a, MF 427
Lehto VP, Virtanen I, Kurki P (1978) Intermediate filaments anchor the nuclei in nuclear monolayers of cultured human fibroblasts. Nature 272:175–177
Morris CJ, Farr M, Hollywell CA, Hawkins CF, Scott DL, Walton KW (1983) Ultrastructure of the synovial membrane in seronegative inflammatory arthropathies. J Roy Soc Med 76:27–31
Norton WL, Ziff M (1966) Electron microscopic observations on the rheumatoid synovial membrane. Arthritis Rheum 9:589–610
Pollard TO, Weihing RR (1974) Actin and myosin and cell movement. CRC Crit Rev Biochem 2:1–65
Reynolds ES (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol 17:208–212
Small JV, Cellis JE (1978) Direct visualization of the 10 nm 100 Å filament network in whole and enucleated cultured cells. J Cell Sci 31:393–409
Spurr AR (1969) A low viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26:31–43
Williamson N, Holt JP (1960) A synovial biopsy needle. Lancet 1:799
Wynne-Roberts CR, Castor CW (1972) Ultrastructural comparison of rheumatoid and non rheumatoid synovial fibroblasts grown in tissue culture. Arthritis Rheum (1972) 15:65–83
Wyllie JC, Haust MD, More RH (1966) The fine structure of synovial lining cells in rheumatoid arthritis. Lab Invest 15:519–529

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