A monoclonal antibody (Mab 67) marks type B synoviocytes

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Summary. The functionally important lining cells of the synovium (types A and B synoviocytes) are the subjects of much study but have presented problems with their characterization and microscopical identification, particularly at the light level. Type A (macrophage-like) synoviocytes, however, are more easily localized than the type B (fibroblast-like) variety because of the greater availability of antimacrophage antisera. We describe, using light and electron microscopy, a monoclonal antibody which in the synovial intimal layer is specific for type B synoviocytes.

Key words: Synovium – Synoviocytes – Monoclonal antibody – Immunohistochemistry – Electron microscopy

Introduction

The synovium is lined by a layer of intimal cells which have been classified on ultrastructural criteria into type A and type B synoviocytes [1, 2]. It has been suggested in the past that these cells belong to a single population and that they assume type A or B morphology under appropriate conditions [3]. The description of an intermediate cell type by some workers favours this concept [2]. However, type A synoviocytes resemble macrophages by electron microscopy while type B cells have abundant endoplasmic reticulum and are somewhat like fibroblasts.

Recent studies have shown that a large proportion of the intimal cells label with monoclonal antibodies which are macrophage markers and that they also express HLA class II molecules [4–8]. Fibronectin has been localised to the intimal cell layer by light microscopy [9] and shown by immunoelectron microscopy to be produced by type B synoviocytes [10]. A monoclonal antibody (Mab 67) which delineates certain cells of the intimal layer in all synovial samples has also been described [6]. It was not possible to localise precisely the antigen marked with this antibody to a particular cell type in frozen sections at the light microscope level. However, the technique adequately demonstrated that macrophages or fibroblasts in the subsynovial connective tissue or cellular infiltrate of inflamed synovia were not marked with Mab 67. In order to establish the cell type being labelled by Mab 67, we performed further studies using resin embedded sections for high-power light and electron microscopical examination.

Materials and methods

Frozen specimens. Standard indirect immunoperoxidase staining was applied to sections of fresh frozen synovium obtained at synovectomy during joint replacement. Fifteen such specimens were screened using Mab 67 as the primary antibody. The samples came from 7 rheumatoid arthritis (RA) patients (1 male, 6 female) and 9 osteoarthritis (OA) patients (1 male, 8 female), aged between 36 and 70 years.

Resin embedded specimens. Small blocks of synovium (approx. 1 mm³) from 1 OA and 2 RA patients were fixed in 0.1% glutaraldehyde in cacodylate buffer for 12 min at 4°C or in periodate-lysine-paraformaldehyde (PLP) for 15 min at 4°C. The specimens were washed thoroughly in cold 0.1 M TRIS-buffered saline (TBS) then incubated at 4°C with Mab 67 supernatant overnight on a rotator, washed again and then similarly incubated with rabbit anti-mouse peroxidase conjugate (Dako) diluted 1:80 in TBS for 1 h. After 30 min washing in three changes of cold TBS, binding sites were reacted with 3'3-diaminobenzidine with CoCl₂. The specimens were then fixed overnight in 2.5% glutaraldehyde, post-fixed (1 h) in osmium tetroxide, dehydrated and embedded in araldite resin. Semi-thin (~0.5 μm) resin sections were stained with toluidine blue and examined using optical microscopy to a maximum magnification of x1000. Thin sections (~90 nm) were stained using Reynolds's lead citrate and uranyl acetate and examined by electron microscopy using a Hitachi 500.

Results

Frozen sections. Examination of frozen sections of both inflamed (RA) and non-inflamed (OA) synovium showed the presence of labelling in the intimal cell layer with the Mab 67 (Fig. 1). Deep connective tissue fibres resembling the elastin of skin and vascular tissue were also labelled.
Fig. 1. a A low power light micrograph of a frozen section of rheumatoid synovium stained using monoclonal antibody (Mab 67) with an immunoperoxidase technique. Intense staining of a population of cells in the intimal layer is indicated by the arrows (× 126).

b At high power it can be seen that the staining is confined to some of the lining cells (arrowheads) and that certain more superficially located cells do not exhibit Mab 67 immunoreactivity (arrows; × 252)

Table 1. Characteristics of labelling with monoclonal antibody 67

<table>
<thead>
<tr>
<th>Target cells</th>
<th>Reference</th>
<th>Comments</th>
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<tbody>
<tr>
<td>1. Synovial intimal cells</td>
<td>Palmer et al. [6]</td>
<td>Not codistributed with fibronectin; not abolished by elastase treatment</td>
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<td></td>
<td>This paper</td>
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<tr>
<td>Type B synoviocytes</td>
<td>Palmer et al. [6]</td>
<td>Abolished by elastase treatment</td>
</tr>
<tr>
<td>2. Elastin fibres in skin and blood vessels</td>
<td>Palmer et al. [6]</td>
<td>Not abolished by elastase treatment</td>
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<tr>
<td>3. Large cells with dendritic morphology in germinal centres of tonsil and spleen</td>
<td>Revell and Lalor (unpublished findings)</td>
<td></td>
</tr>
<tr>
<td>4. Large cells with dendritic morphology in lymphoid follicles of rheumatoid synovium</td>
<td>Revell and Lalor (unpublished findings)</td>
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as were occasional cells in germinal centres of lymphoid follicles. There was no labelling of fibroblastic cells in the subintimal region.

Resin sections. Light microscopical examination of pre-embedding Mab 67-labelled synovia clearly showed the presence of peroxidase localised to cells in the intimal layer (Fig. 2). These cells were evenly distributed among other cells where the surface layer was one or two cells thick in the uninflamed synovia. Where there was an increase in the thickness of the intima in the inflamed rheumatoid synovia, the Mab 67-labelled cells were situated in the deepest part of this layer. No label was seen in fibroblasts in the subintimal connective tissue. Electron microscopical examination showed the morphology of the labelled cells to be that of type B synoviocytes, as shown by the presence of abundant endoplasmic reticulum and well-developed Golgi apparatus with a notable absence of cytoplasmic vesicles and vacuoles. Immunoperoxidase label was localised to the surface of these cells (Fig. 3). Label was also present on collagen fibres immediately adjacent to the type B synoviocytes (Fig. 4). No other cell or structure was labelled with Mab 67 in the intimal layer.

Discussion

The monoclonal antibody, 67, has been shown previously to label cells of the synovial intimal layer [6], but the cell type marked has not been previously recognised. We have now demonstrated that this antibody localises to those cells having abundant endoplasmic reticulum and showing the ultrastructural characteristics of type B synoviocytes. These cells have recently been shown to be situated in the deeper part of the synovial lining layer in the normal rat joint [11] and to have a superficial covering of macrophages [12]. The present results show that the human synovium also has a more orderly arrangement to