Membrane properties and esterase activity of synovial lining cells: further evidence for a mononuclear phagocyte subpopulation

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SUMMARY Recent work in our laboratory with mouse radiation chimeras suggests that the macrophage type of synovial lining cell is derived from bone marrow. Further evidence for including this cell type in the mononuclear phagocyte system is now presented. Rosette formation with IgG coated sheep red cells, immunofluorescent staining for surface Ia antigens, and nonspecific esterase staining all show the presence in the synovial lining of a subpopulation of cells carrying these markers. The proportion of such cells in the lining is very variable, as is the proportion of 'type A' cells recognised with the electron microscope.

The mononuclear phagocyte series, including monocytes, macrophages, and their precursors in the bone marrow, is now generally accepted as a distinct and irreversibly differentiated cell line.¹ The type A synovial lining cell is morphologically similar to other mononuclear phagocytes,² but its inclusion in this series has until now carried a question mark, since the literature on membrane properties and histochemical reactions of lining cells is conflicting,^{3–8} and bone marrow derivation had not been established. Disagreement may largely reflect different methods of isolation and identification of lining cells. In a previous paper⁹ we presented evidence for bone marrow derivation of type A lining cells. In the present paper an attempt has been made to reassess the evidence for the presence of other mononuclear phagocyte markers as defined by van Furth.¹

Materials and methods

Animal material was obtained from 2 strains of mice, Gruneberg Brown (haplotype H2k) and BALB/C (H2d), and from Wistar rats. Synovium was collected by anterior synovectomy of the knee under a dissecting microscope. Haematoxylin and eosin (H and E) stained sections showed that specimens contained both synovial lining cells and subintima.

Synovial cultures were set up from mouse synovial tissue, collagenase being used for disaggregation.

Synovial fragments were incubated for 90 minutes at 37°C in a 1% solution of collagenase (Sigma type II) in Hanks's balanced salt solution (HBSS). Every 30 minutes fragments were passed up and down through a Pasteur pipette. The disaggregated material was washed once with HBSS and transferred to culture wells containing glass coverslips. Cultures were maintained in medium 199 with 10% newborn calf serum, 1% glutamine, 100 µg/ml of penicillin and 100 µg/ml of streptomycin. Cultures were washed with phosphate buffered saline (PBS) after 6 days and the adherent cells incubated with opsonised sheep red cells prior to fixation in methanol and staining with H and E.

Peritoneal cells were obtained from mice and rats by peritoneal lavage with PBS. The cells were washed once and transferred to culture wells containing glass coverslips. Cultures were maintained in medium as above for 18 hours, washed in PBS, and the adherent cells used as control material for sheep red cell studies or immunofluorescence.

Synovial material for frozen section studies was snap frozen in isopentane cooled with solid carbon dioxide. Specimens were stored in liquid nitrogen. Sections were cut 5–7 µm thick.

Human material was collected from patients with acutely painful knees undergoing arthrotomy for suspected meniscal tears. Such material was considered as nearly normal as could be ethically obtained. No patient had evidence of coexistent inflammatory joint disease. Synovium was snap frozen in isopentane containing solid carbon dioxide and

stored in liquid nitrogen. Sections were cut 5–7 μm thick.

Sheep red cell rosetting was performed on synovial cultures (with peritoneal adherent cells as positive control) and on frozen sections of synovium (with spleen sections as control). Whole sheep blood in Alsever's medium was centrifuged and the cells washed 4 times in HBSS. Unsensitised cells (E) were prepared by adding 100 μl of packed cells to 1 ml of HBSS. For sensitised cells (EA) 10 μl of purified rabbit antisheep red cell IgG (Cordis) was added. Both preparations were incubated for 30 minutes at 37°C and washed 4 times in HBSS. The cells were resuspended in 10 ml of HBSS. With these preparations high rates of EA rosetting were obtained with peritoneal cells without significant E adherence, and good zonal adherence was obtained for both E and EA on spleen sections.

Immunofluorescence for Ia antigens was performed by a standard indirect method. Frozen sections were air dried and fixed in ethanol for 10 minutes at 4°C. Anti-Ia antibodies used were of 2 types. A mouse antimouse IAk monoclonal antibody was kindly provided by Dr D. Katz in the form of tissue culture supernatant. A mouse antirat Ia mono-

clonal antibody was obtained from Seralab (clone OX4) also in the form of tissue culture supernatant. A goat antimouse IgG Fc fragment serum conjugated with fluorescein was used as the second layer reagent. A goat antimouse IgG Fab fragment fluorescein conjugated serum was also used in a limited number of experiments. Both reagents were supplied by Nordic Laboratories.

Esterase staining was performed on frozen sections by a standard alpha naphthyl acetate method. Rat kidney was used as a positive control.

Results

TISSUE CULTURE OF MOUSE SYNOVIUM

After disaggregation with collagenase suspensions of synovial material contained large numbers of free cells, but also clusters of up to 100 cells with traces of matrix material. After 6 days in culture such cells had emigrated to form a more or less uniform monolayer. Six-day-old cultures were examined after incubation with either E or EA and staining with H and E.

The most striking feature of the cultures was the

Fig. 1 Frozen section of human synovial villi from a 22-year-old male with a torn meniscus. Extensive adherence of immunoglobulin coated sheep red cells (EA) is seen. (H and E, $\times 1000$).

extreme variation in cell morphology. Large elongated cells with pale nuclei and no attachment of E or EA were thought to be fibroblastic. Rounded cells, with small dense nuclei, many bound EA (but not E), and frequently stellate processes, were considered to be mononuclear phagocytes. EA rosetting cells formed 5–10% of the total. Many cells were unclassifiable. Rounded, stellate, and elongate forms of both EA positive and EA negative cells were seen. Although approximately 50% of cells in the original specimen were lining cells (judged by H and E sections) these could not be distinguished in culture.

SHEEP RED CELL ROSETTING ON FROZEN SECTIONS

Widespread adherence of EA was seen on sections of human synovial lining (Fig. 1). Occasional rosettes also formed on subintimal cells. Adherence of E was minimal. Clusters of EA were also seen on sections of mouse and rat synovial lining but were difficult to interpret on such tiny tissue fragments.

ESTERASE STAINING

Human and rat tissue stained for esterase gave similar appearances. The majority of synovial cells, both

lining and subintimal, were negative, but a significant minority showed moderate esterase activity similar to that seen in macrophages elsewhere but less than that seen in renal tubular cells (Fig. 2). The esterase positive cells in the lining were as few as 1 in 20 in flattened areas but in more cellular areas made up the majority of lining cells. Esterase positive cells were often branched. In unfixed specimens activity appeared diffuse throughout the cytoplasm, but after formalin fixation the activity appeared to be confined to distinct granules.

IMMUNOFLOUORESCENT STAINING FOR IA ANTIGENS

With the antimouse IAK reagent on mouse tissue, cell membrane staining was seen in sections from H2k mice but not from H2d mice as expected, confirming the reagent specificity. Moreover, staining was not seen when the second layer reagent was directed at the Fab fragment of IgG, only occurring with the anti-Fc fragment reagent. This suggests that fluorescence due to binding to Fc receptors is unlikely to have interfered significantly. Membrane staining was seen on 10–30% of peritoneal adherent cells, with only about 10% showing strong fluorescence.

Fig. 2 Frozen section of human synovial villus from a 30-year-old male with a torn meniscus. Irregular darkly stained esterase positive cells can be seen in the lining layer. (Alpha-naphthyl acetate with light counterstain of haematoxylin, $\times 1000$).

Synovial tissue showed scattered membrane stained cells in the subintima and occasional membrane stained cells in the lining. These made up 2% of lining cells as judged by serial H and E sections. Positive cells were with one exception absent from the surface layer, occurring in the deeper cells layers where the lining was more than 1 cell thick.

With the antirat Ia reagent very similar results were obtained on rat synovium and peritoneal cells. Stained cells were again almost totally absent from the surface cell layer, but where the lining was thickest stained cells occurred at regular intervals in the deeper layers.

Discussion

A number of authors have tried to identify synovial lining cells in tissue culture and have made differing observations on their ability to bind opsonised sheep red cells.³⁻⁸ In the present study cultures contained both lining and subintimal cells, and this appears to have been the case in most previous studies other than those of Barratt *et al.*,³ who selectively removed lining cells from pig synovium by trypsinisation and scraping of the surface. In the present study lining cells could not be specifically identified. We would agree with the conclusions of Krey⁴ in that only 2 subpopulations of cells could be recognised with confidence—mononuclear phagocytes and fibroblastic cells. The presence of stellate or dendritic forms of both EA positive and EA negative cells suggests that the use of such morphological terms alone to identify cell subgroups (such as collagenase secreting cells¹⁰) may be confusing.

Barratt *et al.*³ found a very high proportion of EA rosetting cells in their lining cell cultures. They concluded that all lining cells could express Fc receptors and were mononuclear phagocytes. While providing good evidence for a mononuclear phagocyte subpopulation, these findings are open to alternative interpretation. Differential death rates for types A and B cells may have enriched the Fc receptor bearing cell content. In our experience fibroblasts co-cultivated with macrophages tend to remain compact or stellate for a week or more before taking on their more familiar ribbon-like appearance. This may partly explain the absence of fibroblasts noted by Barratt *et al.* in early cultures. Barratt *et al.*³ supported their hypothesis by observations on organ culture of villi. The upper lining cells took on a macrophage morphology and the lower cells a 'type B' morphology. This apparent transformation may, however, reflect migration. In our experience co-cultures of macrophages and fibroblasts take up such a stratified arrangement spontaneously.

Our observations on frozen sections confirm the presence of EA rosette forming cells in the lining. Esterase positive cells have also been demonstrated. No indication of cell numbers can be derived from the rosetting technique, but the esterase method suggests that the proportion of positive cells varies from as little as 5% in flattened areas to as much as 50% in more cellular areas. Similar variability is seen in the distribution of type A cells under the electron microscope. The suggestion that lining cells are esterase negative⁸ may relate to the extreme paucity of positive cells in flattened nonvillous synovium.

Ia antigens are found on a variety of cell types, including B lymphocytes and some mononuclear phagocytes.¹¹ They are not thought to be present on tissue fixed cells such as fibroblasts. Endothelial cells may, however, show weak staining with immunofluorescent techniques. Mature macrophages from peritoneal fluid show low positivity rates, but monocytes are more uniformly positive. Ia antigens are not, therefore, specific mononuclear phagocyte markers, but in the present context provide a method for distinguishing mononuclear phagocytes from fibroblastic cells. Ia antigens also have the advantage over 'macrophage specific' antigens at present in having a known functional significance. Ia positive cells have been detected in the lining, but in smaller numbers than esterase positive cells. This would be consistent with the relatively low positivity rates for macrophages, as opposed to monocytes, elsewhere. The virtual absence of Ia positive cells from the tissue surface may indicate that loss of Ia expression relates to some local maturation process.

In summary, within the limits of available techniques it appears that a proportion of synovial lining cells are positive for each class of marker suggested by van Furth¹ as criteria for inclusion in the mononuclear phagocyte system.

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