Int. J. Exp. Path. (1993) **74**, 113–115

Brief communication

Comparison of surface fibroblastic cells in subcutaneous air pouch and synovial lining: differences in uridine diphosphoglucose dehydrogenase activity

L.S. WILKINSON, A.R. MOORE*, A.A. PITSILLIDES, D.A. WILLOUGHBY* AND J.C.W. EDWARDS

*Departments of Rheumatology Research, University College and Middlesex School of Medicine and *Experimental Pathology, St Bartholomew's Hospital Medical College, London, UK*

Received for publication 5 February 1992
Accepted for publication 21 August 1992

Summary. In contrast to synovial tissue, rat subcutaneous air pouch lining was found to lack cells showing high activity of uridine diphosphoglucose dehydrogenase, an enzyme involved in hyaluronan synthesis. This indicates that the properties of cells on the surface of synovium are not determined simply by tissue cavitation. Shearing forces may be more important in inducing the specialized behaviour of synovial surface fibroblasts.

Keywords: air pouch, synovium, joint development, hyaluronan

In 1981 it was reported that a structure with the essential features of synovial lining could be generated by the injection of air into rodent subcutaneous tissue (Edwards *et al.* 1981). The suggestion was made that, although air pouch and synovial lining differ in detail, no major difference could be identified in terms of cell origin, structure or function (Edwards *et al.* 1987). This led to the hypothesis that tissue splitting was the critical stimulus which determines synovial differentiation, whether during embryonic development or subsequently in the formation of adventitious bursae or pseudarthroses.

We have recently demonstrated that fibroblast-like or type B synovial lining cells show high activity of uridine diphosphoglucose dehydrogenase (UDPGD), an enzyme involved in the formation of one of the monosaccharides of hyaluronan (HA) (Pitsillides & Blake 1992). This enzyme activity clearly distinguishes type B lining cells from the intermingled macrophages (type A cells) and all cells in the deeper subintimal tissue (Wilkinson *et al.* 1992). In the light of these findings air pouch lining was reevaluated.

Correspondence: Dr J.C.W. Edwards, Arthur Stanley House, Tottenham Street, London W1P 9PG, UK.

Materials and methods

Dorsal subcutaneous air pouches were formed as described previously (Edwards *et al.* 1981) in 12 rats and excised either 6 ($n=6$) or 12 ($n=6$) days after induction. Animals were killed by cervical subluxation under ether anaesthesia. Air pouch and knee synovial tissues ($n=4$) were excised, snap frozen in isopentane at −70°C and cryostat sections cut. Sections were assayed for

(a) UDPGD activity, using a modification of the method of Mehdizadeh *et al.* (1991). Briefly, sections were incubated for 20 minutes at 37°C in an atmosphere of nitrogen with 5.3 mM uridine diphosphoglucose as substrate, 0.45 mM NAD as coenzyme and 3.7 mM nitrobluetetrazolium in 30% polyvinyl alcohol made up in 0.05 M glycylglycine buffer at pH 7.8 and saturated with nitrogen. Sections were then washed and mounted in Aquamount (BDH). Control sections were incubated with substrate-free reaction medium to ensure specificity of the dehydrogenase activity demonstrated.

(b) HA distribution, using a modification of the method of Ripellino *et al.* (1985). Briefly, sections were incubated with a biotinylated probe derived from carti-

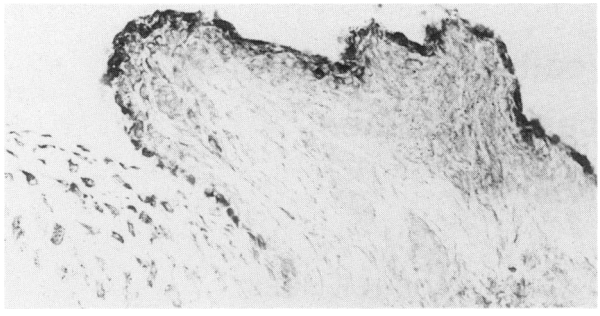

Figure 1. Cryostat section of rat knee synovium with the luminal surface uppermost reacted for UDPGD activity showing high activity in the surface layer of cells. The micrograph includes the chondrosynovial junction and moderate activity is also seen in chondrocytes, to the left. Final magnification × 200.

Figure 2. Cryostat section of 12 day air pouch tissue with the luminal surface uppermost reacted for UDPGD activity showing no cells of high activity. Modest activity, indicated by scattered granules of reaction product, is seen in cells throughout the inflamed tissue. Final magnification × 200.

lage core protein HA binding region and developed using streptavidin alkaline phosphatase complexes and fast red.

(c) Non-specific esterase (NSE) activity, using a standard α-naphthyl acetate technique.

Results

A surface layer of cells with high UDPGD activity was found in all samples of knee synovium as expected (Fig. 1), but no such layer was seen in either 6 or 12-day air pouches (Fig. 2) despite the presence of comparable numbers of cells. Cells with low levels of UDPGD were seen scattered in air pouch tissue. Staining for NSE activity showed the cells on the surface of both tissues to be of two populations, one of high NSE activity, consistent with macrophages (or type A cells in synovium), and one of absent NSE activity consistent with fibroblasts (or type B cells in synovium). In synovium double labelling showed the cells of high NSE activity to be a separate cell population from those with high UDPGD activity. Moderate staining for HA was found throughout the air pouch lining tissue with no preferential staining of the superficial tissue. In contrast synovial tissue showed a distinct surface band of stronger staining for HA.

Discussion

UDPGD is involved in synthesis of HA and other glycosaminoglycans and it seems likely that high activity in type B or fibroblastic synovial lining cells relates to specialization in terms of production of synovial fluid HA. Currently, it is probably the most useful marker of specialized type B cell function in synovial tissue. The findings of this study indicate that the formation of a synovial lining layer containing cells with the characteristics of type B cells is dependent on stimuli other than tissue cavitation alone.

Type B lining cells may belong to a self replicating population which differentiates irreversibly during embryogenesis. This is, however, hard to reconcile with the suggestion that type B cells are continually replaced from the subintima (Henderson 1987). Moreover, it is inconsistent with the development of adventitious bursae, pseudarthroses and other ectopic or acquired forms of synovium at sites which would not be expected to carry such a predetermined synovial cell population.

Alternatively, the presence of a surface layer of fibroblasts with the specialized behaviour of type B synovial cells may reflect the effects of surface shearing forces on fibroblasts which are continually replaced from a pool of unspecialized fibroblastic cells deeper in the tissue. Such shearing forces could explain the development of a lining capable of producing a glairy HA rich fluid in acquired synovial spaces.

The subcutaneous air pouch remains a useful system for the study of inflammation for simple practical reasons. It provides large quantities of homogeneous tissue with a fluid phase for biochemical and cytological analysis. Access is easy and humane. By subjection to potential mechanical and biochemical inducers of UDPGD activity, the air pouch may also help delineate the conditions necessary for type B synovial lining cell differentiation.

Acknowledgement

The financial support of the Arthritis and Rheumatism Council is gratefully acknowledged.

References

EDWARDS J.C.W. (1987) Functions of synovial lining. In *The Synovial Lining in Health and Disease*. Eds B. Henderson & J.C.W. Edwards. London: Chapman & Hall. pp. 41–74.

EDWARDS J.C.W., SEDGWICK A.D. & WILLOUGHBY D.A. (1981) The formation of a structure with the essential features of synovial lining by subcutaneous injection of air. *J. Pathol.* **134,** 147–156.

HENDERSON B. (1987) Cell division and tissue maintenance. In *The Synovial Lining in Health and Disease*. Eds B. Henderson & J.C.W. Edwards. London: Chapman & Hall. pp. 75–102.

MEHDIZADEH S., BITENSKY L. & CHAYEN J. (1991) The assay of uridine diphosphoglucose dehydrogenase activity: discrimination from xanthine oxidase activity. *Cell Biochem. Funct.* **9,** 103–110.

PITSILLIDES A.A. & BLAKE S. (1992) Uridine diphosphoglucose dehydrogenase activity in synovial lining cells in the experimental antigen induced model of rheumatoid arthritis; an indication of synovial lining cell function. *Ann. Rheum. Dis.* **51,** 992–995.

RIPELLINO J A., KLINGER M.M., MARGOLIS R.U. & MARGOLIS R.K. (1985) The hyaluronic acid binding region as a specific probe for the localisation of hyaluronic acid in tissue sections. *J. Histochem. Cytochem.* **33,** 1060–1066.

WILKINSON L.S., PITSILLIDES A.A., WORRALL J.G. & EDWARDS J.C.W. (1992) Light microscopic characterisation of the fibroblast-like synovial intimal cell (synoviocyte). *Arthritis Rheum.* **35,** 1179–1184.