Synovial intimal fibroblasts

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Cells of the normal synovial intima fall into two clearly separable types, macrophages and fibroblasts.^{1,2} In diseased tissue separation on traditional criteria such as ultrastructure may be less clear cut, but recent studies using techniques for identifying specific gene products related to cell lineage suggest that this distinction remains valid.^{3,4}

The fibroblasts of synovial intima have been considered the likely source of synovial fluid hyaluronan for several decades, but direct evidence of specialisation of these cells has been difficult to obtain, because of the problems of separating intimal from other fibroblasts *in vitro*.^{5,6} Histochemical demonstration of hyaluronan in normal synovium suggests that the molecule is concentrated in the intima,⁷ but this could theoretically reflect diffusion into the intima from the synovial fluid. The first *in situ* evidence that intimal fibroblasts are specialised came from the work of Stevens and coworkers,⁸ showing that these cells, in contrast to most other fibroblasts, are associated with an unknown epitope recognised by the monoclonal antibody 67. Unfortunately, the molecule carrying this epitope has remained uncharacterised until now.

More recently, Pitsillides *et al*,⁹⁻¹¹ using a quantitative cytochemical technique developed by Mehdizadeh *et al*,¹² were able to show that intimal fibroblasts do indeed differ from other fibroblasts in their potential for synthesis of hyaluronan. The activity of the enzyme uridine diphosphoglucose dehydrogenase (UDPGD) is four to nine times greater in synovial intimal fibroblasts than in any cells of the deeper layers of the tissue. Sheets of intimal cells partially disaggregated from tissue and viewed as cytospin preparations show a clear distinction between fibroblastic cells, with high UDPGD activity, and macrophages with prominent cytoplasmic CD68.¹¹ While UDPGD activity is not a direct measure of hyaluronan synthesising ability, there are reasons for believing that, in cells which show low levels of sulphate incorporation (intimal cells), it may be the most useful cytochemical marker of this specialised activity.¹³

Knowledge of the specialisation of gene expression in intimal fibroblasts has subsequently expanded rapidly. These cells express vascular cell adhesion molecule-1 (VCAM-1) even in normal tissue, at levels rarely achieved on endothelium (judged immunochemically).¹⁴ They also show relatively prominent basal expression of the other adhesion molecules, CD44,¹⁵ and β integrins¹⁶ (chiefly in combination with $\alpha 3$, 5 and 6 chains).¹⁷ As discussed by Revell in these Proceedings, they are probably largely

responsible for the synthesis of a number of relatively specialised extracellular matrix components including types IV,¹⁸ V and VI^{19,20} collagen, laminin,¹⁸ fibronectin,¹⁸ chondroitin-6-sulphate containing glycosaminoglycans²¹ and tenascin.²²

These findings clearly establish that a functionally specialised type of fibroblast exists in synovial intima. The best term for describing these cells remains uncertain. The term synoviocyte may be useful, but unfortunately has been used in the past to include, not only intimal macrophages, but also unselected synovial cells studied *in vitro*. The ultrastructural distinction between type A and B cells¹ is less than ideal, partly because it is linked with the implication that the two cells are related in terms of ontogeny, and partly because ultrastructure is no longer considered a good gold standard for distinguishing macrophage and fibroblasts in the context of disease. The author currently favours the term synovial intimal fibroblast. Unfortunately, even this term is less than ideal because, in normal rabbit synovial intima, two fibroblast populations exist, one with high UDPGD activity and one expressing VCAM-1 (Kahn, *in preparation*). The more general term synovial fibroblast is perhaps best used for passageable cells, in tissue culture, which may be of both intimal and subintimal origin.

Recent studies in our laboratory have focussed on the epitope recognised by monoclonal antibody (MAb) 67. The epitope is expressed almost exclusively on the intima in sections of both normal and diseased synovium. Stevens *et al*⁸ have demonstrated its specific association with intimal fibroblasts. However, studies of other tissues have shown the molecule to be expressed at a range of other highly specific sites: Bowman's capsule of the glomerulus and the juxtaglomerular apparatus (JGA) in the kidney, fetal synovial intima (being present even before joint cavity formation) fetal skin, cells within lymphoid follicular germinal centres, bone marrow stromal fibroblasts, elastin fibres in some tissues, placental chorionic villi, amniotic epithelium, and weakly in adult skin stratum granulosum. There is an interesting overlap in this distribution with that of VCAM-1, which is also present in adult (but not fetal) synovial intima, Bowman's capsule (but not the JGA), lymphoid germinal centres (but on a different cell population), and bone marrow stromal cells.²³

It has proved possible to extract material from both synovium and amnion, giving a band on Western blots probed with MAb 67 corresponding to a molecular mass of about 55 kDa. This material is readily extracted

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with hyaluronidase under conditions which maintain cell integrity. Taken together with the pericellular appearance of immunochemical staining in tissue sections, this suggests that the molecule concerned is an extracellular matrix component found in close proximity to cells, which may perhaps be involved in cell matrix adhesion or signalling.

The presence of 'epitope 67' on fetal synovial intima, and at the corresponding site within fetal joints even before a cavity has formed raises interesting questions about the ontogeny of intimal fibroblasts. Other features of these cells appear at different times. CD44 expression is prominent throughout the interzone, destined to become both synovial intima and subintima, together with perichondrium, from very early on (eight weeks gestation in the human).²⁴ Differential expression of CD44 on synovial intima, as opposed to subintima, occurs after cavity formation. UDPGD activity is high in cells along the potential joint line before cavity formation,²⁴ but is not necessarily maintained on the intima immediately after cavity formation, apparently returning later. VCAM-1 is not present on synovial intima (it is present on some synovial capillaries) up to 14 weeks gestation in the human,²⁴ and as yet no evidence has been found for its expression on prenatal intima in murine or human tissue. These findings indicate that the intimal fibroblast does not take on all of its functional characteristics at a single point in time, but becomes specialised in a series of steps.

Previous reports have suggested that synovial lining will develop at adventitious sites in response to an altered mechanical environment, in addition to formation at predestined sites in joints and tendon sheaths.²⁵⁻²⁸ This has raised the suggestion that intimal fibroblasts can derive from unspecialised connective tissue fibroblasts in response to local conditions at a tissue surface. A number of experiments have been carried out to try to define the conditions required for the expression of intimal fibroblast features.²⁸ Both UDPGD activity and VCAM-1 expression have been seen on cells lining ectopic spaces in rheumatoid nodules. Attempts to induce these features on cells lining adventitious cavities created experimentally have met with mixed success. Rodent air pouches show no cells with high UDPGD activity.²⁹ Tissue lining a plastic implant placed subcutaneously in the author's forearm for five weeks and subjected to movement showed a layer of cells of high UDPGD activity, but minimal VCAM-1 expression. Tissues lining loosened orthopaedic implants show much more convincing features of intimal fibroblasts, with data from our own and from Revell's group showing UDPGD activity, VCAM-1 expression, laminin, type IV collagen, and epitope 67.^{28 30}

In vitro studies of regulation of intimal fibroblast features are still at an early stage. UDPGD activity, VCAM-1 expression, and epitope 67 expression all decline in tissue culture.^{28 31 32} VCAM-1 expression is relatively

easy to maintain by modifying the culture medium conditions (work in preparation by Croft *et al*). UDPGD activity appears more capricious. One significant observation is that synovial, and not dermal, fibroblasts in culture will continue to show high UDPGD activity and VCAM-1 expression if in contact with mineral particles, such as glass or hydroxyapatite.³³ Only the cells in direct contact with particles retain these features, other cells in the same culture being negative. Although it is most unlikely that particles determine the behaviour of synovial cells in situ in normal tissue, it may be that particle contact mimics some sort of tactile stimulus relating to a discontinuity in the physical environment of the cell. This could be perceived through receptors such as CD44 and integrins binding to hyaluronan or the Arg-Gly-Asp sequence on matrix components associated with surrounding structures.

The control of intimal fibroblast function is currently an area of intense investigation. Preliminary evidence suggests that hyaluronan, VCAM-1, CD44, and integrins may form a network of interacting signal systems which may control both fibroblast and macrophage function in normal synovial intima. Modulation of these interactions may contribute to cytokine production by macrophages and activation of several mechanisms promoting immigration of leucocytes in disease.

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