Second International Meeting on Synovium

Cell Biology, Physiology and Pathology

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Consensus statement

The following is a summary, drawn up by the meeting convenors and session chairmen, of issues raised during the meeting for which a consensus has formed, together with issues for which a resolution is still needed, representing priority areas for future study.

There is general agreement that synovial intimal macrophages belong to the bone marrow derived mononuclear phagocyte system and are distinct from resident intimal fibroblasts. Doubts are still expressed on occasion in the literature, but none was raised at the meeting. The issue of overlap with intimal fibroblasts is considered below. Intimal macrophages show some functional differences from subintimal macrophages, but these differences are quantitative rather than qualitative, and reports differ in emphasis.

Most multinucleate cells within inflamed synovial intima carry macrophage lineage markers. They may show osteoclast features, but the presence of the calcitonin receptor on synovial giant cells distant from bone has been demonstrated unequivocally only in villonodular synovitis. Some rheumatoid synovial fibroblasts, both in the intima in tissue sections and in vitro, carry up to four nuclei.

Synovial intimal fibroblasts are now known to be specialised in a wide range of properties. Their high uridine diphosphoglucose dehydrogenase (UDPGD) activity is consistent with hyaluronan synthesis. They appear to be the dominant source of intimal vascular cell adhesion molecule-1 (VCAM-1). Cytoplasmic VCAM-1 is largely confined to the fibroblasts; membrane staining is strongest on fibroblasts and VCAM-1 mRNA has been found only in CD68 negative cells. Specialised components of intimal matrix are found specifically in association with the fibroblasts. The synovial lining associated epitope 67 is associated with these cells, but is also found at interesting sites in other tissue linings and haemolymphopoietic organs.

The factors controlling the intimal fibroblast state remain difficult to define and deserve further study. It is still unclear if these cells derive from subintimal cells or are a distinct population defined during fetal development. There may be several routes to the final phenotype.

There is a continuing difficulty in assessing the relative numbers of intimal macrophages and fibroblasts in normality and disease. Despite the existence of a number of new markers, technical factors still hinder a truly

representative assessment. This area of study deserves further attention.

The apparent overlap of a number of markers still raises the question of interchangeability of the two intimal populations. However, the apparent lack of specificity of markers such as CD68 and prolyl hydroxylase should not be unexpected. Moreover, different anti-CD68 antibodies have different breadths of specificity. EBM11 appears to be the most useful in synovial intima, with KP1 having too broad a specificity and PGM1 giving very restricted staining of cryostat sections unless these are trypsinised. The close proximity of cell membranes in the intima confounds interpretation of staining with membrane associated markers. Nevertheless, markers of functions which are clearly lineage related such as CD45, CD64, and high UDPGD activity, do appear to identify mutually exclusive populations under good technical conditions. Moreover, no evidence for overlap at the ultrastructural level has been found in normal tissue and the ultrastructural overlap in disease is consistent with the known convergence of macrophage and fibroblast appearances under pathological conditions. Although synovial fibroblasts express CD68 in long term culture, no other features suggest that they transform to a macrophage phenotype.

An important advance is the increasing scope for the use of markers as indicators of function, as these functions become known. The evidence that intercellular adhesion molecule-1 (ICAM-1) binds hyaluronan is an outstanding case. Technical problems are still perceived by many with regard to immunolocalisation of secretory products. Reliable methods for detecting metalloproteinases have come from years of painstaking work. Techniques for localising cytokines are still difficult to evaluate, but a consensus picture derived from the parallel use of several antibodies may evolve.

There was agreement that light microscopic morphology is not a useful guide to cell lineage. Macrophages in subintima are often elongated and intimal fibroblasts may be rounded, with or without long processes. The term 'dendritic cell' remains of operational value in the context of passageable rheumatoid fibroblast cultures. However, it is not possible to equate this population with a specific population in intact tissue, because macrophages, intimal fibroblasts, follicular dendritic reticulum cells

(DQ negative resident antigen presenting cells (APC)), interdigitating dendritic cells (immigrant DR/DQ positive expressing APC), and stromal fibroblasts may all carry long processes—that is, 'dendrites'. Thus Winchester proposed a synovial cell uncertainty principle as follows: in contexts where synovial cell identity can be established cell function is difficult to study, and where function can be studied cell origin is uncertain.

The mechanism by which the synovial intima maintains itself as a layer remains a mystery and is worthy of further work. Adhesion molecules, whether homotypic, heterotypic, or acting in a cell-matrix-cell fashion could play a part. ICAM-1, VCAM-1, fibronectin, laminin, and minor collagens, together with their integrin ligands, CD44 and hyaluronan (and epitope 67?) appear likely candidates. The multiple binding capabilities of integrins, ICAM-1 and CD44 are of particular interest, suggesting a network of complementary or competing interactions.

A chemotactic gradient encouraging cells to congregate in the intima is an interesting possibility. The role of a number of factors, including fibronectin, particulate material and soluble factors, in inducing a branching morphology in synovial fibroblasts may relate to the way in which these cells organise themselves. Intimal cells could also govern the distribution of immigrant leucocytes by generating tactic gradients, acting as a selective filter or by inducing maturation steps which alter responsiveness to tactic factors. Finally, there is the question if macrophages return into the tissue following ingestion of debris and, if so, under what influence?

Extensive evidence indicates that synovial macrophages, fibroblasts and lymphocytes show induction of gene expression, or 'activation', in disease. However, an analogy was made with a series of chords played on the piano. Each cell type expresses a range of gene products, but the 'harmonic pattern' is specific (if dissonant in disease), with important 'silent' components being expressed at basal or reduced levels. This 'tuned activity' also appears to apply to normal intimal cells, if they are considered as macrophages and fibroblasts adapted to the particular requirements of the synovial intimal environment.

Most available data on the transcription factors involved in regulating synovial cell function relate to the AP-1 system of proto-oncogenes. The AP-1 system is of interest because of its relevance to metalloproteinase regulation and also to the possible existence of cell 'transformation'. Current evidence suggests that *fos* and *jun* are expressed in rheumatoid synovial tissue, but their status in truly normal tissue is not known. Expression of AP-1 components in rheumatoid tissue is dominant in the intima, but deeper cells also express the same components. Currently, the evidence appears to be as consistent with cells responding to inflammatory stimuli as it is with a true transformed state.

The proliferative state of synovial intimal cells remains an area of debate. However, a consensus appears to be emerging. There is no evidence that intimal macrophages divide. Evidence from many sources indicates that intimal fibroblasts do divide in disease, but the rate is relatively low and is probably consistent with chronic inflammation. The evidence does not appear to support the view that a primary proliferation of resident synovial cells underlies rheumatoid disease.

The existence of a nerve supply to the synovium has moved from being an issue of ill-informed debate to one of certainty, and increasing complexity. Nerves have been shown extending to the intimal surface of normal synovium, but these disappear in rheumatoid tissue. With the recognition of the importance of both orthodromic and antidromic, and both electrical and chemical flux in nerve

fibres, the potential for a role for these fibres, not only in central appreciation of noxious stimuli, but also in local control of cell behaviour, appears considerable.

The synovial extracellular matrix is now well characterised and there is sufficient interest to ensure that as new matrix components are described their distribution in synovium is rapidly documented. There is a consensus that the synovial intima has a specialised matrix including components such as laminin, fibronectin, minor collagens, epitope 67, and hyaluronan. No basement membrane is present, but there is increasing evidence indicating that intimal matrix forms a looser, but nevertheless essential barrier to molecular flux, which may be specifically adapted to the regulation of synovial fluid composition, in conjunction with the presence of a high concentration of free hyaluronan.

Techniques are now available allowing perfusion, fluid, and solute flux to be addressed experimentally and modelled mathematically. Experimental results support a Starling based model for synovial fluid dynamics, with ultrafiltration of plasma, passage of part of that ultrafiltrate into and out of the synovial cavity via the synovial intima, and return of fluid to the circulation via reabsorption into blood vessels or lymphatic return. Macromolecules such as albumin and hyaluronan can be shown to modify fluid dynamics. Their precise role *in vivo* remains open to debate, with some apparent conflict in terms of the effect of hyaluronan that may depend on conditions of pressure and flow within joints. However, there is now good experimental and theoretical support for the suggestion that hyaluronan may inhibit rapid efflux of fluid from synovial cavities at increased intra-articular pressures, by its impaction in the synovial intimal matrix. The feasibility of simultaneous bidirectional fluid flow across the synovial surface has also been demonstrated. This provides a very neat resolution of the problem of how synovial fluid flux can occur at static synovial fluid volume without the need for cyclical changes in hydrostatic forces dependent on muscle contraction.

Changes in synovial vascularity in chronic rheumatoid disease remain an area of debate. Some studies indicate a reduced capillary density at the tissue surface, whereas others indicate vascular proliferation. Magnetic resonance imaging indicates an increase in synovial perfusion in inflammatory disease, but its spatial resolution is inadequate to address the question of the vascularity of the intimal zone. Debate may arise partly from the fact that different things are being measured in different contexts.

The primary structure of hyaluronan is well established. The nature of intermolecular interactions and domain overlap remain uncertain. A number of findings support the view that self association can occur between hyaluronan chains, but the importance of such interactions, as opposed to simple steric overlap of molecular domains, remains unclear.

The nature of hyaluronan binding proteins is becoming clearer with the characterisation of CD44 and its several splice variants, and the more recent identification of the receptor for hyaluronan mediating motility (RHAMM). The co-identity of hyaluronan receptor on liver endothelial cells (HARLEC) and ICAM-1 was disclosed at the meeting, raising considerable interest in potential new roles for hyaluronan in intercellular reactions in the immune response and cell traffic.

Data on the half life of hyaluronan in synovial cavities, approximately 12–20 hours, indicates that the molecule may pass through synovial interstitium more readily than its molecular radius might imply. The suggestion is made that diffusion may be facilitated by thermal 'wriggling' of the hyaluronan chain (reptate diffusion). There is also

evidence for degradation peripherally in addition to centrally in the liver.

Although hyaluronan may have a role in fluid film maintenance in joint lubrication and in control of synovial fluid dynamics, the continuing difficulty of demonstrating its true functional roles *in vivo* was highlighted. A role in preventing tissue ingrowth and adhesion is difficult to prove, as is a role in scavenging radicals or altered proteins.

The continuing evidence for a protein moiety closely associated with hyaluronan was highlighted. The possibility that in disease this moiety might include complement fixing IgG was explored and is worthy of further study.

Concentrations of synovial fluid solutes continue to raise interest as clinical markers of disease progression. Statistical correlations have been established. Debate continues about usefulness in individual cases. For use in analysis of pathogenesis, knowledge of distribution volumes and clearances is still needed. The origin of 'aggrecanase' activity giving rise to peptide fragments cleaved at a novel site remains uncertain. An unrecognised action of stromelysin appears to be a possibility.

The use of immunohistochemistry as an aid to prognosis and diagnostic subsets also continues to show promise. However, as with solutes, there are still some unresolved issues in terms of the scope of application to the management of individual patients. There is continuing debate about the degree of variability of histological findings, not only between patients with similar disease, but also between samples from the same joint. Useful studies clearly require careful attention to the sampling procedure.

Animal studies show that different mediators, such as cytokines, are important in different models of arthritis. However, the prospect of identifying different pathogenic pathways in different human clinical subgroups remains elusive, with as yet only some tantalising clues to possible differences between rheumatoid and psoriatic arthritis.

There is now good evidence for a role for free radicals in tissue damage. How this role compares with that of proteases needs addressing. More information is also needed on the ability of free radicals to alter cell phenotype and the role of radical (nitric oxide in particular) production by chondrocytes and fibroblasts.

New techniques are becoming available for the identification of pathogenic agents in synovium. Polymerase chain reaction techniques are of particular interest. Many of the agents detectable are relatively uncommon, emphasising the potential role of specialist referral units for diagnosis of uncommon rheumatic disorders. It is hoped that these techniques, developed in less common conditions, will also prove useful in conditions such as rheumatoid disease in the future.

Clinical interest in synovial tissue is chiefly focused on rheumatoid disease, but several groups are also interested in loosening of joint prostheses and the role of particles. The pathogenesis of loosening is still far from clear, but the development of relevant experimental models promises to identify key mediators and their interrelationships.

The scope for therapy in inflammatory rheumatic disease is expanding exponentially. The number of cytokines and other mediators has long passed 100. The strategies for interfering with each mediator are numerous. Drug targeting methods are also expanding rapidly, with techniques as diverse as conjugation of radio-nuclides to particles, intimal cell depletion using liposomes, and genetic engineering of monoclonal antibodies. Where there is no consensus is in which strategy will prove the best! The potential value of further research is self evident.

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