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The synovial lining micromass system: Towards RA in a dish?

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A characteristic feature of rheumatoid arthritis (RA) is the hyperplasia of the synovial lining. In RA, the synovial tissue undergoes a marked transformation, due to the influx of immune cells including monocytes, lymphocytes, mast cells, neutrophils, as well as increased angiogenesis, and synovial fibroblast hyperplasia, resulting in the destruction of cartilage and bone. The synovial lining, which consists of synovial fibroblasts and macrophages derived from circulating monocytes, is attached at the bone-cartilage junction and is the site at which joint damage in RA begins. In normal individuals the synovial lining is 1–3 cell layers thick, but increases to as much as 13 cell layers in patients with RA. The hyperplasia of the synovial lining is a result of an increased number of synovial fibroblasts and macrophages, which occurs due to enhanced proliferation, recruitment, and/or survival. The normal function of synovial fibroblasts is to produce extracellular matrix and contribute to the lubrication of joint by secreting molecules such as hyaluronan and lubricin. Recently, a three dimensional synovial lining type membrane was generated in culture using synovial fibroblasts and matrigel and this lining behaved similar to a normal synovial tissue (1,2). In the current issue of *Arthritis & Rheumatism* Kiener et al (3) furthered these studies by demonstrating that synovial fibroblasts uniquely orchestrate the incorporation of monocytes into the cultured synovial lining.

The mechanisms by which synovial fibroblasts contribute to the pathogenesis of RA have been the focus of studies for many years. Synovial fibroblasts from patients with RA have been shown to display anchorage independence, enhanced invasiveness into cartilage, increased proliferation, and decreased cell death as compared to synovial fibroblasts from patients with osteoarthritis or disease free controls. Further, RA synovial fibroblasts spontaneously secrete numerous cytokines, chemokines, and matrix-metalloproteinases (MMPs) that promote inflammation and joint destruction through autocrine and paracrine mechanisms (4). Thus, the RA synovial fibroblasts do not appear to be innocent bystanders in the pathogenesis of RA, but rather active and crucial participants.

Despite these insights, the techniques employed to characterize the mechanisms by which synovial fibroblasts contribute to the pathogenesis of disease may not always reveal the whole story accurately (5). *In vitro* cell culture employing synovial fibroblasts adherent to plastic culture dishes following 3 or more passages, is the most commonly used approach. This system relies on examining a spontaneous or induced function, such as expression of a cytokine, transcription factor, survival factor or proliferation by RA synovial fibroblasts. While the ease and accessibility of this system is appealing for researchers to conduct their studies, it has become clear that the behavior of synovial fibroblasts may change once they were placed on “plastic” compared with the *in vivo* observations made by the direct examination of synovial tissue (6). For example, spontaneous differences between RA and control synovial fibroblasts

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may become less apparent compared to studies examining intact synovial tissues by immunohistochemistry (7). Further, treatment with cytokines such as TNF α or IL-1 β or even synovial fluid itself may not be sufficient to reproduce the differences between RA and controls that were observed when examining whole synovial tissue. This was clear for studies examining expression of Bcl-2, which showed increased expression of Bcl-2 in RA compared with osteoarthritis synovial tissue, while no difference was observed employing isolated RA or OA synovial fibroblasts (8). Similarly, increased levels of the phosphorylated isoform of MAP kinase kinase 3 and 6 (MKK3, MKK6) was readily detected in RA as compared to OA synovial tissue, but these differences are not observed in cultured RA or OA synovial fibroblasts, even following stimulation with cytokines.

The differences observed between studies that employ cultured RA and OA synovial fibroblasts compared to studies that examine them in the intact synovium may be due to the lack of a three dimensional structure to the synovial fibroblasts and to lack of interaction with macrophages, which co-exist with synovial fibroblasts in the synovial lining. It may also be due to the absence of the multitude of factors, such as IL-1, IL-6, IL-17, TNF α , or TGF β that constantly bath these cells *in vivo*. Thus, the system developed by Kiener and colleagues may provide the necessary structure or matrix for synovial fibroblasts to revert to their active phenotype that is observed in intact RA synovial tissue.

Supporting the notion that structure is important, studies that have examined synovial fibroblast invasiveness consistently show differences between RA and osteoarthritis synovial fibroblasts. Employing either *in vitro* or *in vivo* systems, RA synovial fibroblasts have consistently been more invasive compared with disease free controls osteoarthritis synovial fibroblasts or dermal fibroblasts. The matrigel transwell assay (9,10) or the matrix associated transepithelial resistance invasion assay (11) have shown the invasive potential of RA compared to osteoarthritis synovial fibroblasts. Further, the RA SCID model is a potent system for examining the invasiveness of synovial fibroblasts *in vivo* (12). In this model, synovial fibroblasts are co-implanted with cartilage under the renal capsule of SCID mice, which lack an intact immune system thereby preventing the rejection of the transplant. Previous studies have shown that only RA but not osteoarthritis synovial fibroblasts migrate, attach to, and destroy the adjacent cartilage (12). One of the strengths of these studies is that the synovial fibroblasts may be modified by siRNA or infected with vectors such lentivirus, retrovirus, or replication defective adenovirus to delete or over-express genes prior to transplant. However, these studies are limited since they do not examine the interaction between synovial fibroblasts and the various immune cells in the joint such as macrophages and lymphocytes. Taken together, these studies suggest that providing a matrix or scaffold for the RA synovial fibroblasts may recreate, in part, the environment of the synovium and thereby maintain RA synovial fibroblasts in active and invasive state, allowing investigators to more faithfully characterize the differences between RA and controls.

To date, the essential role of synovial fibroblasts in contributing to inflammatory arthritis in rodent models remains elusive. While many animal models of inflammatory arthritis show activation of synovial fibroblasts, the inability to delete these cells or to directly modulate them *in vivo* without affecting other cells is a barrier. Further, one of the central difficulties that has plagued researchers that focus on synovial fibroblasts is the lack of markers to identify them. The earliest way to identify synovial fibroblasts relied of their morphology, i.e. stellate morphology, increased rough endoplasmic reticulum, and no vacuoles (13). These studies were extended to include negative identification, employing antibodies to all hematopoietic derived immune cells (CD45) or specifically to macrophages (CD68) (5). Thus, the CD45, CD68 negative populations were considered synovial fibroblasts. Additional markers including CD55, VCAM-1, UDPGD, Thy-1, propyl-4hydroxylase have shown some specificity, ranging from 25–95% positive staining for synovial fibroblasts (5). The differences in staining may

reflect the heterogeneous nature of synovial fibroblasts in tissue and in isolated single cell populations. Recently, the identification of cadherin 11 as a marker for synovial fibroblasts has gained substantial attention (14). Cadherin 11 is not only a reputable marker for synovial fibroblasts but is also required for their ability to maintain the architecture of the synovial lining. Mice lacking cadherin 11 display a hypoplastic synovial lining and are refractory towards K/BxN serum transfer-induced arthritis (2). Further, anti-cadherin 11 antibody or cadherin 11-Fc fusion protein abrogates disease development and moderately ameliorates established disease in the K/BxN serum transfer model (2). Studies that isolate the promoter of cadherin 11 and identify the elements that are crucial for expression in synovial fibroblasts will transform the field of synovial fibroblast biology as researchers will then be able to specifically target synovial fibroblasts to manipulate their function.

To further understand the role of cadherin 11 in synovial fibroblasts, Lee et al and Kiener et al developed a three dimensional synovial fibroblast organ culture system (1,2). In this system synovial fibroblasts are resuspended in a matrigel matrix or a collagen matrix, which was allowed to gel prior to addition of culture medium. Overtime these cells form a synovial lining type membrane that produces an organized extracellular matrix including reticular fibers at the interface of the matrix and fluid phase. The synovial lining micromass is responsive to TNF α and requires cadherin 11 (1,2). Disruption of cadherin 11 or elimination of cadherin 11 prevents the formation of the synovial lining type membrane, while ectopic expression of cadherin 11, but not cadherin E, in L cells is sufficient for the generation of a synovial lining type layer in the matrigel. Although these studies provided a structure resembling a synovial lining, they did not incorporate macrophages, which are also found in the synovial lining. However, in this issue of *Arthritis & Rheumatism*, Kiener and colleagues have now generated a three-dimensional synovial lining micromass culture model which includes monocytes/macrophages (3). They show that the synovial fibroblasts are responsible for supporting the survival and co-compaction of monocytes/macrophages into the three dimensional culture micromass. Further, while normal dermal fibroblasts failed to organize into a synovial lining type membrane, they did support monocytes/macrophage survival. Additionally, the authors extended previous studies that characterized the three-dimensional synovial lining type membrane by demonstrating that this membrane displays normal synovial lining function, producing lubricin, and is readily transformed into a hyperplastic synovial lining, through the addition of pro-inflammatory cytokines.

Due to the work by Kiener et al it is now conceivable to propose studies that examine the interaction of synovial fibroblasts and macrophages, within a structural environment that is seen *in vivo*. Since these two cell types are at the leading edge of the pannus that invades cartilage and bone, future studies will be required to assess how these cells interact to cause joint destruction. Additionally, studies may be designed to examine differences between RA and control synovial fibroblasts in the micromass system and to examine the effects of RA synovial macrophages on synovial fibroblasts from patients with osteoarthritis or disease free controls. Further, mechanistic studies may be performed by using genetically altered synovial fibroblasts or macrophages, which would be accomplished through traditional means prior to addition to the matrigel. Alternatively, synovial fibroblasts and bone marrow macrophages from normal and arthritic mice, deficient in various components from the cell cycle, apoptotic, or signal transduction machinery may be isolated and co-cultured to demonstrate the role of these factors on maintaining activation, survival, and cell number in the synovial lining. These types of studies will provide the basis for better understanding the complex relationship between synovial fibroblasts and macrophages.

Nonetheless, certain issues remain to be determined employing the micromass organ culture system using synovial fibroblasts and monocytes/macrophages. Earlier studies have demonstrated that the co-culture of synovial fibroblasts and monocytes results in increased

monocyte and synovial fibroblast activation (15). In the current article by Kiener et al, it is not clear whether the combination of cells leads to increased spontaneous release of cytokines and chemokines. The contribution of cytokines and chemokines by each cell type, both spontaneously and following stimulation with TNF α also remains to be shown. Further, the differences in synovial lining development and the production of proinflammatory factors using cells from RA and controls needs to be defined. Although complicated because of the need for HLA matching, it would be informative to know how other cell types such as lymphocytes or endothelial cells would affect the structure and function when present in the sublining region of the micromass cultures. Taken together, the strength of this system is that it provides a structure or scaffolding for the development of a synovial lining consisting of both synovial fibroblasts and macrophages which may mimic *in vivo* situations and that it allows for monitoring the synovial cellular phenotype over an extended period of time.

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