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Inflammatory arthritis

Mesenchymal precursor cells

M Corr, N J Zvaifler

What are mesenchymal precursor (stem) cells doing in rheumatoid arthritis joints?

Genes implicated in limb development and bone and joint formation,[1 2] particularly members of the segment polarity family that encode components of the hedgehog and wingless/Wnt signalling pathways, have recently been identified in inflamed synovial tissues,[3 4] prompting speculation about their role in the pathogenesis of rheumatoid arthritis (RA) (this issue, page 6). In the limb bud of the embryo these genes are associated with primitive mesenchymal cells, which can be identified by their expression of heterodimeric surface membrane molecules that bind members of the transforming growth factor β super family, including bone morphogenetic protein receptors (BMPR), endoglin, anaplastic lymphoma kinase 1, and transforming growth factor β receptors.[5 6] Postnatal bone marrow has similar mesenchymal progenitor cells (MPC) that provide the reticular stroma which supports haemopoiesis, and when appropriately stimulated MPC can give rise to bone, cartilage, fat, muscle, or fibrous tissues.[6 7] RA synovium also contains cells with phenotypic and functional characteristics of MPC.[8 9]

ORIGINS OF SYNOVIAL MPC

The primordial appendicular skeleton begins as a condensed rod of primitive MPC that develop into articular structures.[1] Among isolated normal rabbit and human synovial fibroblasts there is a minority population of cells that can be induced into osteogenic, chondrogenic, and adipogenic pathways, suggesting that a few undifferentiated MPC are normally present in synovial tissues.[10 11] Their numbers are greatly increased in RA synovial tissues; 5–10 times more BMPR+ cells are identified in the intimal lining,[12] and specific members of the Wnt family (5a and 13) are preferentially expressed in suspensions of whole synovium.[3] Such findings may reflect either an expansion of a local population of MPC or a migration of MPC from the marrow into the inflamed joint. Both scenarios assume that the disease process is already established. Inflammatory mediators might influence the growth of resident MPC, but which of the numerous factors present in the RA synovium stimulate or retard growth of MPC remains to be clarified. The alternative scenario is an extension of a conventional paradigm of RA pathogenesis—namely, inflammatory mediators, like tumour necrosis factor α, alter the endothelium of synovial blood vessels and facilitate entry of blood cells into the joint. The recent demonstration that MPC are normally present in the circulation of humans[13 14] supports this hypothesis. Thus through either expansion or ingress, mesenchymal progenitors might participate in perpetuation of synovial disease. But how could they play a part in the initiation of RA?

ROLE OF MPC IN THE INITIATION OF RA

A number of recent papers have speculated about the onset of RA: when and how it begins, and whether clinical synovitis is preceded by an asymptomatic innate immune reaction in the joint. Although difficult to confirm in humans, there is considerable support for this idea from animal models of arthritis (reviewed by Firestein and Zvaifler[15]). Injection of an arthritis prone strain of mice (DBA/1) with complete Freund's adjuvant results in increased numbers of activated cells in the juxta-articular epiphysial bone marrow, increased inflammatory cytokines in the bone marrow, and enlargement of small vestigial channels (called cartilage canals) that traverse from the bone marrow into the joint through the "bare area".[16] These changes are seen many days before the appearance of arthritis. At the same time, large cells expressing BMPR are present in the marrow, within the cartilage canals, and in synovial tissues. These mesenchymal progenitors antedate the appearance of either neutrophils or lymphocytes.[16]

> "What are the origins of RA? Do mesenchymal precursor cells have a role?"

MOLECULAR SIGNALS IN THE DEVELOPING LIMB

Limb bud formation begins at an early stage in embryogenesis at a time when fibroblast growth factor (FGF) in the lateral plate mesoderm indirectly signalling through Wnt molecules in the overlying ectoderm induces a condensation called the apical ectodermal ridge (AER; fig 1).[17] The AER interacts with primitive, undifferentiated mesenchymal cells in the underlying progress zone. The production of FGF proteins in the AER instructs the growth and differentiation of MPC to expand in a proximal-distal orientation to become limbs. A separate signalling region, called the zone of polarising activity, is responsible for development in a cranial (thumb) to caudal (little finger) orientation. Sonic hedgehog (Shh) protein, made in the zone of polarising activity, maintains FGF-4 production and together they activate HoxD gene expression and sustain cell division in the progress zone. Simultaneously, Shh induces the BMPs required for chondrogenesis and subsequent osteogenesis. Continued induction of Shh is controlled by reciprocal interactions with Wnt7a, FGF-4, and, possibly, retinoic acid (fig 1). Less is known about the downstream effector genes that interpret these signals.

EVIDENCE FOR EXPRESSION OF EMBRYONIC GENES AFTER BIRTH

If molecular programmes that regulate skeletal development are recapitulated in tissue regeneration and repair then they might be present in a diseased joint. Amphibians (*Axolotl* and *Xenopus*) can completely replace amputated limbs. Gene expression is the same during regeneration as in the embryo.[18 19] For instance Msx genes, transcription factors expressed in the AER and progress zone that maintain embryonic tissues in an undifferentiated and proliferative state, are re-expressed within hours after either limb amputation or wound healing in adult *Axolotl*.[20] Fractured bone provides a good model for postnatal analysis of genes involved in repair of mammalian tissues. Molecular signals for osteogenesis (transcription factor cbfa-1, Indian hedgehog (Ihh), and osteocalcin), chondrocyte maturation (Ihh, transcription factor gli-1, and collagen type 2), and vascular invasion (matrix metalloproteinase (MMP)-9 and -13, and vascular endothelial growth factor) are the same in fetal development and adult repair.[21] However, there are two important differences. Firstly, the origin of the MPC that participate in the repair process is not known. They may derive from the bone marrow or periosteum, from MPC resident in the surrounding tissues, or within the bleeding that accompanies the fracture.[22] Secondly, both fracture healing and wound repair needs an initial inflammatory process.[23] This latter requirement may be more relevant to events in the rheumatoid synovium. The cells that accumulate at the site of injury elaborate cytokines and growth factors, activate clotting, and induce proteolytic digestion of fibrin, which are all essential for producing the scaffold and matrix that supports the subsequent tissue regrowth, scarring, and remodelling.[23] Inflammation appears to regulate the expression of certain critical developmental genes. For instance, Wnt genes are expressed in mouse skin within hours of wounding and Wnt4 production is greatly enhanced in cultured mouse fibroblasts by trauma and fibrinolytic fragments.[24] At the site of fractured bone Ihh and its receptors, Smoothened and Patched, are expressed rapidly.[25] Thus the presence of these same developmental genes in RA synovial tissues may reflect inflammation, regeneration, or tissue repair, or a combination of these. But do these gene products of MPC contribute to the pathogenesis of RA?

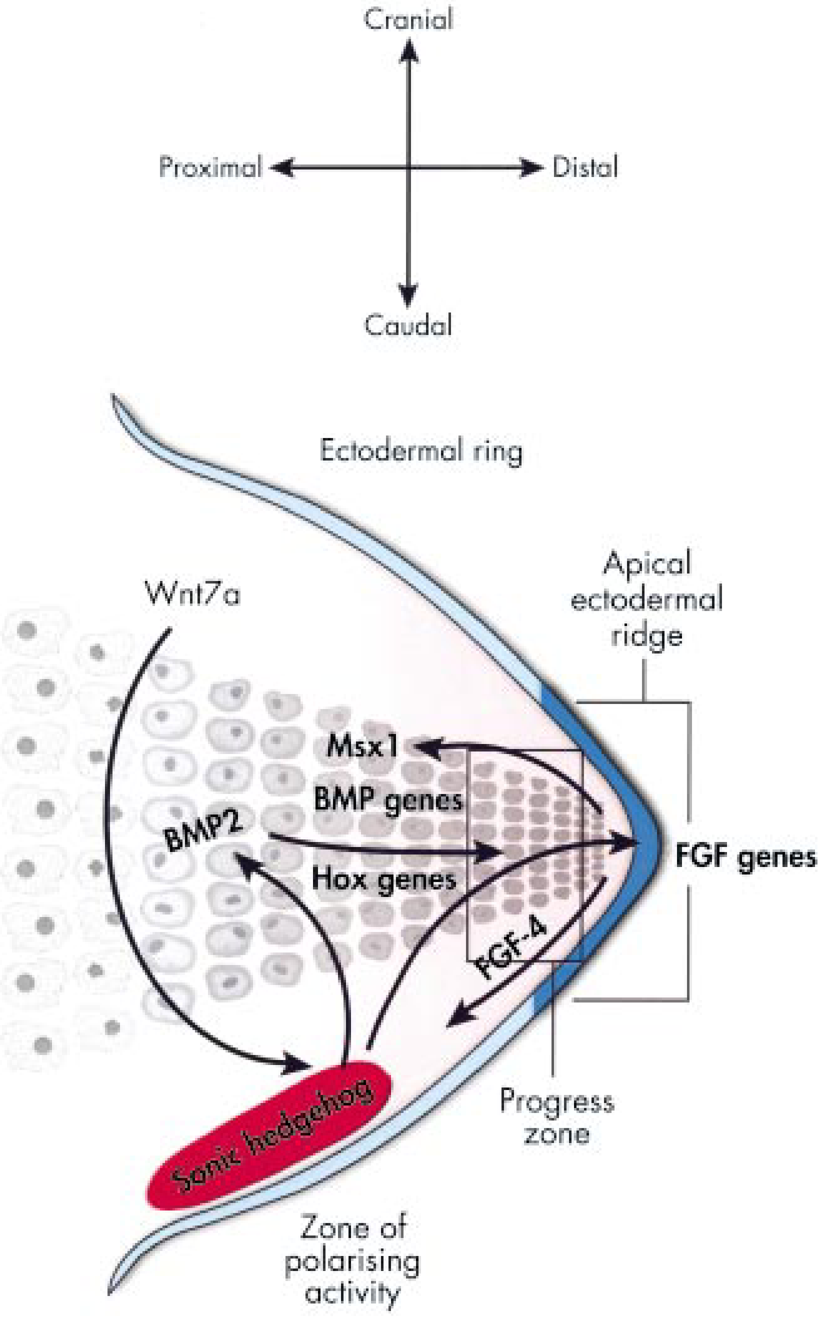

Figure 1 A schematic representation of the early events in limb development depicted in a proximal-distal and cranial-caudal orientation. Shown in colour are the locations of the relevant signalling regions: apical ectodermal ridge (AER), zone of polarising activity (ZPA), and the progress zone, where the most immature mesenchymal stem cells reside. Also displayed are the expression patterns of several relevant genes—namely, fibroblast growth factor (FGF), bone morphogenic protein (BMP), and homeobox (Hox).

RA—REGENERATION GONE AWRY?

The differential expression of proteins usually associated with embryonic limb patterning in the rheumatoid synovium might represent a physiological attempt to heal and restore inflamed or damaged tissue. However, a disruption of the regulated postnatal expression of these proteins can result in an abnormal phenotype, as suggested by certain hereditary syndromes. Pseudorheumatoid dysplasia is an autosomal recessive disorder associated with mutations in Wnt inducible protein 3.[26] This genetic deficiency is manifest by cartilage loss and destructive bone changes in children as they age, at times necessitating joint replacement surgery by the third decade of life. This syndrome suggests that limb patterning molecules function in normal homoeostasis of bone and joint structure and integrity. Perturbations of these molecules that maintain bone and cartilage could potentially lead to structural loss.

> "Mesenchymal precursor cells may be attempting to restore the damaged joint in RA"

A recruitment or influx of MPC could gradually replace the fibroblasts of the normal synovial lining. Rheumatoid synovial fibroblasts express embryonic morphogens that have roles in both limb bud mesenchyme and bone marrow stem cell development. The aggressive phenotype of invasive pannus, stimulated by intra-articular inflammatory cytokines, might be further accentuated by embryonic growth factors. The Wnt/frizzled signalling pathway is associated with transcriptional control of cell cycle proteins, adhesion molecules, and MMP-7 through β-catenin.[27–29] Moreover, Wnt5a has been reported to activate protein kinase C, thereby enhancing nuclear translocation of NFκB.[30] Transfection of synovial fibroblasts with a Wnt5a encoding construct results in enhanced interleukin (IL)6, IL8, and IL15 production.[3,4] Rheumatoid synoviocytes support osteoclast formation in vitro[31] and IL15 can stimulate the differentiation of osteoclast precursors.[32] In addition, frizzled signalling pathway might also influence the production of the osteoclast differentiation factor receptor activator of NFκB ligand (RANKL) by synovial fibroblasts.[4] Certainly, osteoclastic activity has a key role in the formation of erosions in RA.

In summary, the presence of an expanded number of MPC in the inflamed synovium in conjunction with the expression of morphogenic genes indicates previously unrecognised components in the pathogenesis of RA. The association between inflammation and wound repair suggests that these cells may be attempting to restore the damaged joint by a process akin to recapitulating the embryonic programme. Inflammatory messengers may be driving this process and enlarging the channels connecting the bone marrow with the synovial cavity. Growth of the pannus may result from a repopulation of the synovium with MPC that are stimulated to undergo differentiation. Cascading effects could then influence cell adhesion, cytokine secretion, osteoclast differentiation, and bone homoeostasis.

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