Urokinase in rheumatoid arthritis: causal or coincidental?

The past 10 years have witnessed an increased interest in the plasminogen activation system and its potential role(s) in rheumatoid arthritis (RA). Two types of plasminogen activators (PAs), urokinase (uPA) and tissue type PA (tPA), which are structurally, immunologically, and genetically distinct, have been identified in mammals. Both PAs are secreted as a single chain protein, which in the case of uPA is essentially inactive (pro-uPA). Pro-uPA is then converted by a single enzymatic cleavage into two chain active enzyme. uPA and tPA are highly specific serine proteases catalysing the conversion of plasminogen into plasmin.12 Plasminogen, present in plasma and extracellular fluids, can bind to fibrin and also to cell surface plasminogen receptors (PlnR), via its lysine binding sites. The activity of PAs is controlled by natural inhibitors (PAls), which include PAI-1, the main PAI in the circulation, and PAI-2. In addition, uPA activity is blocked by protease nexin 1 (PN-1). Another important step in the regulation of PA activity involves binding of tPA to fibrin, leading to increased catalytic activity of the enzyme, and binding of pro-uPA and uPA through a growth factor domain contained in their amino terminal region, to a specific, high affinity cell surface receptor, uPAR. Binding of uPA to uPAR increases the rate of plasminogen activation and greatly increases extracellular matrix degradation and cell invasion in vitro and in vivo. In addition, upon binding to uPAR, uPA shows effects that are independent of its proteolytic activity.

Non-inflammatory synovial membranes from normal subjects or osteoarthritics patients expressed predominantly tPA, at the activity and antigenic level, which was associated to blood vessels.13 In contrast, in RA synovial tissues, tPA expression was reduced, while uPA activity was increased. Its corresponding antigen and mRNA were also increased, and mainly associated to the synovial lining layer.34 Increased levels of uPAR, PAI-1, and PAI-2 were also found in RA tissues.34 These results from synovial biopsy specimens were in agreement with previous studies on RA synovial fluids, where antigenic levels of uPA, PAI-1, and uPAR were increased compared with corresponding plasma values or with normal knee synovial fluid, while tPA levels were either decreased or unchanged.34 Finally, increased uPA and decreased tPA antigenic levels measured in RA synovial fluids have been associated with the clinical severity of the disease.14 What is the cellular origin of uPA in the RA joint? In vitro studies showed that most of the cells present in the inflamed joint, mononuclear cells, polymorphonuclear cells, synovial fibroblasts, chondrocytes, and bone cells, express uPA together with variable amounts of uPAR and PAls when cultured in vitro (see fig 1). uPA expression can be up regulated by numerous growth factors and cytokines, such as interleukin 1β and tumour necrosis factor α.15 16 By contrast, tPA, mainly produced by endothelial cells, is down regulated by the same cytokines. Thus, increased uPA levels and decreased tPA expression found in RA synovial tissues and fluids are probably accounted for by the opposing effects of cytokines on the two PAs genes.

Effects of uPA overexpression in RA joints can be mediated through both plasmin independent and plasmin dependent pathways (see fig 1). Two types of plasminogen activators (PAs), urokinase (uPA) and tissue type PA (tPA), which are structurally, immunologically, and genetically distinct, have been identified in mammals. Both PAs are secreted as a single chain protein, which in the case of uPA is essentially inactive (pro-uPA). Pro-uPA is then converted by a single enzymatic cleavage into two chain active enzyme. uPA and tPA are highly specific serine proteases catalysing the conversion of plasminogen into plasmin. Plasminogen, present in plasma and extracellular fluids, can bind to fibrin and also to cell surface plasminogen receptors (PlnR), via its lysine binding sites. The activity of PAs is controlled by natural inhibitors (PAls), which include PAI-1, the main PAI in the circulation, and PAI-2. In addition, uPA activity is blocked by protease nexin 1 (PN-1). Another important step in the regulation of PA activity involves binding of tPA to fibrin, leading to increased catalytic activity of the enzyme, and binding of pro-uPA and uPA through a growth factor domain contained in their amino terminal region, to a specific, high affinity cell surface receptor, uPAR. Binding of uPA to uPAR increases the rate of plasminogen activation and greatly increases extracellular matrix degradation and cell invasion in vitro and in vivo. In addition, upon binding to uPAR, uPA shows effects that are independent of its proteolytic activity.

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particular aggregates (the typical ‘rice bodies’) in synovial fluid.25–27 These fibrin deposits are the results of the intra-articular balance between coagulation and fibrinolysis. Increased expression of tissue factor and factor XIII by articular endothelial cells and macrophages have been reported, thus providing the ability to generate and cross link fibrin in RA synovial tissues.28 Degradation of fibrin matrix is performed by plasmin.29 The most tangible evidence of ongoing fibrinolysis is the generation of fibrin degradation products such as cross linked, plasmin modified fibrin (fibrin D-dimer). The presence of fibrin D-dimer was confirmed, using specific antibodies, in RA synovium.28,29 Although the role of uPA in intra-articular fibrin removal has not been assessed in vivo, the concomitant presence of fibrin D-dimer and increased uPA activity in RA synovial tissues, suggests ongoing uPA mediated fibrinolysis in RA joints. Interestingly, in other inflammatory situations, such as septic shock28 and lung injury,29 a correlation between extravascular fibrin deposition and decreased uPA activity has been found, supporting uPA as a crucial enzyme in extravascular fibrin clearance.

Intra-articular fibrin deposits can have potential adverse effects. The presence of fibrin on synovium and cartilage may impede normal nutrition to these tissues, thus amplifying conditions that lead to hypoxia and acidosis in synovial fluid, as suggested by Harris.28 Fibrinogen degradation products may also have direct proinflammatory effects by increasing vascular permeability and inducing chemotaxis at sites of inflammation.22,23 Finally, the extracellular fibrin meshwork serves as a provisional matrix for inflammatory cell adhesion.24 This potential arthrogenic role of fibrin in RA is supported by studies in animals where homologous fibrin injected intra-articularly in previously immunised rabbits induced a chronic inflammatory arthritis.25 The proinflammatory role of fibrinogen in human joints has also been reported: a proportion of patients treated with silicon rubber joint prostheses developed synovial and osseous inflammation, probably mediated by the spontaneous adsorption of fibrinogen on the implanted polymer surfaces.26 uPA mediated proteolytic activity in RA has already been considered as a target for therapeutic intervention in this disease. It has been hypothesised that compounds inhibiting uPA mediated activity could represent new pharmacological tools to reduce joint destruction. Indeed, clinical improvement of arthritic patients was seen after intra-articular injection of urinary trypsin inhibitor, which was shown to inhibit uPA.27 In this context, the finding that glucocorticosteroids suppress uPA activity of human synovial chondrocytes, and mononuclear cells28 could represent one of the mechanisms leading to the beneficial effects of these anti-inflammatory drugs in the treatment of RA. On the other hand, as discussed above, intra-articular fibrin may be highly detrimental in RA. Thus, agents able to decrease extravascular fibrin deposition might ameliorate RA synovitis. Belch et al.29 noted that the anabolic androgenic steroid stanozolol increased plasma fibrinolytic activity and improved inflammation in RA patients. It was postulated that the improvement may be attributable to a reduction in synovial fibrin, mediated by the increased fibrinolytic activity.

Variations in uPA activity levels could occur during the course of the disease and within the joint, favouring one or another feature of RA. The relative importance of the contrasting roles of uPA in RA pathogenesis (deleterious with respect to joint destruction, beneficial in the context of fibrin mediated inflammation) needs to be assessed in vivo. The availability of animals genetically deficient in uPA29 offers the means to clarify the uPA role(s) in arthritis. Ultimately, elucidation of the in vivo role of uPA in arthri-

pathogenesis could lead to the elaboration of new therapeutic strategies for RA.

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