

## Differential expression of the urokinase receptor (CD87) in arthritic and normal synovial tissues

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### Abstract

**Aim**—To determine whether the urokinase plasminogen activator receptor (u-PA; CD87) exhibits a possible pathogenic role in rheumatoid and osteoarthritis.

**Methods**—A semiquantitative, indirect immunoperoxidase histochemical analysis was performed on frozen synovial tissue sections. The recently characterised monoclonal antibody 10G7 recognising transfectants bearing u-PA was used. Synovial tissue was obtained from 10 patients with rheumatoid arthritis, 10 patients with osteoarthritis, and four normal subjects.

**Results**—u-PA was expressed on 70–90% of synovial tissue lining cells and subsynovial, interstitial macrophages from the arthritis patients, but only on a few myeloid cells from the normal subjects. It was also present on more endothelial cells from the rheumatoid and osteoarthritis patients, than from normal synovial tissue.

**Conclusions**—Plasminogen activators are important in joint destruction underlying arthritis. The up-regulated expression of u-PA in diseased versus normal synovial tissue suggests a role for this antigen in the inflammatory and angiogenic mechanisms underlying rheumatoid and osteoarthritis.

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Rheumatoid arthritis is a chronic inflammatory disease characterised by the transendothelial ingress of leucocytes into synovial tissue that is rich in newly formed blood vessels.<sup>1-5</sup> A regulatory network of inflammatory cells and mediators, as well as cellular adhesion molecules (CAMs) play an important role in the pathogenesis of rheumatoid arthritis.<sup>3-4</sup> Angiogenesis is crucial for the perpetuation of leucocyte extravasation into the synovial tissue in rheumatoid arthritis.<sup>5</sup> These elements have also been implicated, although to a lesser extent, in osteoarthritis, an essentially degenerative joint disease with inflammatory features.<sup>4-6</sup>

There is substantial data suggesting that the urokinase plasminogen activator (u-PA), which is highly involved in the proteolytic joint destruction underlying arthritis, interacts with a number of participants in the regulatory network described above. u-PA, a serine protease, activates a cascade of proteolysis, leading to

extracellular matrix (ECM) degradation, and thus it is involved in cell adhesion, migration, and proteolysis underlying tumour invasion, inflammation, angiogenesis, and tissue remodelling.<sup>7-10</sup> The 50 to 65 kD u-PA receptor (u-PA), which binds to the N-terminal end of u-PA and localises u-PA at the cell surface, has recently been clustered as CD87.<sup>11-14</sup> u-PA is a monocyte activation antigen<sup>13-15</sup> that also plays a role during macrophage differentiation.<sup>16</sup> There are some data available on the roles of u-PA and u-PA in inflammatory conditions, including arthritis. Little u-PA is produced by resting monocytes/macrophages, T lymphocytes, endothelial cells, chondrocytes, and synoviocytes. However, the secretion of u-PA by these cells and the expression of u-PA on monocytes and endothelia is highly up-regulated on the activation of these cells by a number of inflammatory and angiogenic mediators.<sup>17-26</sup> u-PA is a glycosyl-phosphatidylinositol (GPI) anchored protein, which forms a complex with the  $\beta 2$  integrins on monocytes.<sup>27-28</sup> It also interacts with other CAMs and ECM components, such as  $\beta 1$  and  $\beta 3$  integrins, and vitronectin on the cell surface.<sup>26-29-31</sup> The co-capping of u-PA with other CAMs on the cell surface, which involves cyclic AMP dependent and protein kinase C dependent signalling, results in the induction of leucocyte adhesion and chemotaxis.<sup>26-27-32-33</sup> The increased production of u-PA by cytokine-stimulated endothelial cells and the up-regulated expression of u-PA in association with integrins on leucocytes plays an important role in leucocyte-endothelial interactions and angiogenesis underlying inflammation.

Some data are available on the abundant production of u-PA and u-PA in rheumatoid arthritis. Increased levels of u-PA and u-PA were detected in synovial tissue extracts from rheumatoid versus osteoarthritis patients.<sup>34</sup> The concentration of u-PA was also increased in synovial fluids from rheumatoid versus osteoarthritis patients.<sup>35-36</sup> In a preliminary study, most myeloid, and some endothelial cells in the synovial tissue from rheumatoid and osteoarthritis patients showed immunoreactivity with the 10G7 monoclonal antibody (MoAb).<sup>37</sup> This MoAb was later found to recognise transfectants bearing u-PA. Descriptive immunohistochemistry performed by others revealed strong expression of u-PA and u-PA in myeloid cells in the rheumatoid arthritis synovial tissue.<sup>34-38</sup> However, semiquantitative immunohistochemical analysis on synovial u-PA has not yet been carried out, nor has the expression of u-PA in normal synovial tissue

been investigated. The present study used detailed semiquantitative immunohistochemistry to determine the distribution of u-PAR synovia from rheumatoid and osteoarthritis patients and normal subjects.

### Methods

Synovial tissue was obtained from 10 rheumatoid and 10 osteoarthritis patients undergoing arthroplasty. The diagnosis of arthritis met the American College of Rheumatology criteria.<sup>39-41</sup> Normal synovial tissue was dissected from fresh pathological specimens or at necropsy. Synovial tissue was snap frozen in OCT compound (Miles, Elkhart, Indiana, USA). All samples were obtained with Institutional Review Board approval.

An MoAb, 10G7, produced in our laboratory, was submitted to the myeloid panel of the Sixth International Workshop and Conference on Leukocyte Differentiation Antigens. Studies carried out for this workshop determined that this antibody reacted with transfectants bearing CD87, u-PAR (Sanna M Goyert, Robert F Todd III, personal communication). Another MoAb, L21, raised against the same antigen was submitted to the previous, fifth international workshop by Dr Boyle (Melbourne, Australia) and this was also used in a pilot study on synovial tissue from three rheumatoid and three osteoarthritis patients for comparison. Anti-factor VIII related antigen (MoAb FVIII; Dakopatts, Carpinteria, California, USA) was used to detect endothelial cells, and MoAb LeuM5 (Becton Dickinson, Mountain View, California, USA) recognising CD11c was used to identify macrophages.<sup>42-43</sup> Purified mouse IgG1 supplied by the fifth workshop was used as an isotype specific negative control. Indirect immunoperoxidase staining was performed as described previously<sup>44</sup> using a Vector ABC assay system (Vector Laboratories, Burlingame, California, USA) and diaminobenzidine (Kirkegaard & Perry, Gaithersburg, Maryland, USA) as a chromogen.

Cell types present in synovial tissue were identified by their morphology after haematoxylin counterstaining, and by using the aforementioned positive control MoAb. Synovial tissue samples were assigned scores for the number of macrophages, degree of inflammation, and the number of blood vessels on a relative scale of 1 to 3, with 3 being the highest score (for example, a macrophage score of 1, 2, and 3 represented < 25, 25-100, and > 100 macrophages per  $\times 400$  microscopic field, respectively). Inflammatory and vessel scores were obtained similarly. The specific cell types evaluated in the synovial tissue were lining cells, subsynovial macrophages, fibroblasts, vascular endothelium, vascular smooth muscle, and lymphocytes. All immunostaining of synovial tissue components was graded by frequency on a scale of 0% to 100%, where 0% indicated no staining and 100% showed that all cells were immunoreactive. Five  $\times 400$  fields were examined per section. Data were pooled and the mean (SEM) was calculated for each data group. In addition, presence ("positive tissues" with > 10% immunoreactive cells) or

absence ("negative tissues" with < 10% immunoreactive cells) of cellular immunoreactivity with MoAb 10G7 was also noted in each tissue. Specimens were analysed by one investigator (GKH) as a blinded observer and, in the case of selected synovial tissue samples, also by the other two investigators. In general, no major differences were found between the analysis of the three observers.

Corresponding data groups were compared using an independent *t* test. In addition, all inflammatory, macrophage, and vessel scores, as well as synovial tissue lining, macrophage, and endothelial immunoreactivity values were pooled, regardless of whether they were obtained from rheumatoid arthritic, osteoarthritic or normal synovial tissue, resulting in a total of 24 values in each data group. A multiway Pearson correlation analysis was performed between any two of these data groups.  $p < 0.05$  was considered significant.

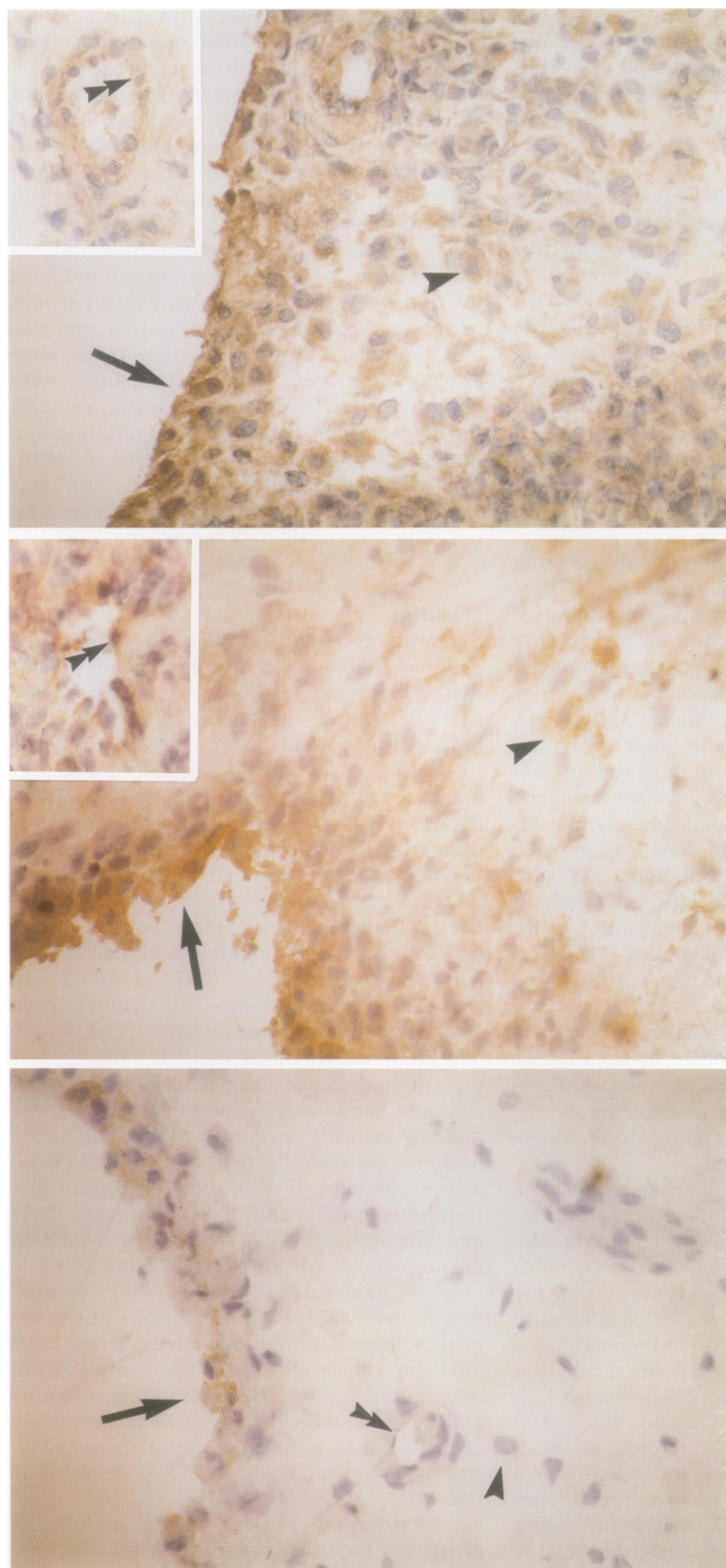
### Results

Synovial tissue from rheumatoid arthritis patients exhibited a significantly higher inflammatory score (2.1 (0.3)), macrophage score (2.3 (0.3)), and vessel score (2.6 (0.2)) than tissue from patients with osteoarthritis (1.3 (0.1), 1.4 (0.2), 1.9 (0.1), respectively) or normal synovial tissue (1 (0), 1 (0), 2 (0), respectively) ( $p < 0.05$ , rheumatoid arthritis versus osteoarthritis or normals). Significant correlations were found between inflammatory and macrophage scores ( $r = 0.76$ ;  $p < 0.05$ ), between inflammatory and vessel scores ( $r = 0.44$ ;  $p < 0.05$ ), and between macrophage and vessel scores ( $r = 0.57$ ;  $p < 0.05$ ) in rheumatoid arthritis, osteoarthritis and normal synovial tissue (data not shown).

u-PAR, recognised by the 10G7 MoAb, was present on the majority of synovial tissue lining cells and subsynovial macrophages from rheumatoid arthritis patients (91% and 78% immunoreactive cells, respectively) and osteoarthritis patients (86% and 68%, respectively). In contrast, significantly fewer lining cells (8%) and macrophages (23%) expressed u-PAR in normal synovia ( $p < 0.05$ ) (figs 1 and 2).

When analysing the number of "positive" tissues, synovial tissue lining cells and macrophages showed immunoreactivity with MoAb 10G7 in almost all (90-100%) samples from rheumatoid and osteoarthritis patients. In normal synovial tissue, lining and macrophages expressed u-PAR in only one of three (33%) and one of four tissues (25%), respectively (table 1).

u-PAR was also present on some endothelial cells from rheumatoid arthritis patients (35% immunoreactive cells), osteoarthritis patients (55%), and normals (25%) (figs 1 and 2). However, while some endothelial staining was observed in a number of synovial tissue sections from rheumatoid arthritis patients (six of 10) and osteoarthritis patients (eight of 10), endothelia from three of four normal synovial tissue sections expressed no u-PAR (an outlier 100% endothelial immunoreactivity was found in one of the normal sections) (table 1).



**Figure 1** Indirect immunoperoxidase staining of frozen synovial tissue sections using MoAb 10G7 recognising the urokinase receptor (original magnification  $\times 1056$ ). (Top) Rheumatoid arthritis, synovial tissue lining layer (arrow), interstitial macrophages (single arrowhead), and endothelial cells (insert, double arrowhead) showing u-PAR immunoreactivity. (Middle) Osteoarthritis, synovial tissue lining layer (arrow), macrophage (arrowhead), and endothelial (insert, double arrowhead) reactivity. (Bottom) Normal synovial tissue showing very low immunostaining of lining layer (arrow). Macrophages (arrowhead) and endothelial cells (double arrowhead) are not reactive.

**Table 1** Immunoreactivity of synovial tissue in patients with rheumatoid or osteoarthritis, and normal subjects with MoAb 10G7 recognising urokinase plasminogen activator receptor

	Lining	Macrophages	Endothelium
Rheumatoid arthritis (n=10)	9*	9	6
Osteoarthritis (n=10)	10	10	8
Normal subjects (n=4)	1*	1	1

Values indicate the number of "positive" tissues. Synovial tissues were considered "positive" if 10% or more cells showed immunoreactivity with MoAb 10G7.

\*No synovial tissue lining was available in one rheumatoid arthritis and one normal sample.

In a pilot experiment, another MoAb (L21) was used to detect u-PAR in synovial tissue from three rheumatoid arthritis and three osteoarthritis patients. This MoAb reacted with 93–98% of lining cells, and 92–98% of subsynovial macrophages in both groups (data not shown).

When using MoAb 10G7, we found a positive correlation between u-PAR reactivity in the synovial tissue lining layer versus subsynovial macrophages in samples from patients with rheumatoid and osteoarthritis, as well as in normal controls ( $r = 0.63$ ,  $p < 0.05$ ; fig 3). u-PAR expression on synovial tissue lining cells also significantly correlated with inflammatory scores ( $r = 0.44$ ;  $p < 0.05$ ). These results suggest that u-PAR is predominantly expressed by cells of myeloid origin, such as lining cells and macrophages from inflamed synovia.

## Discussion

Leucocyte ingress into synovial tissue plays an important role in the pathogenesis of rheumatoid arthritis and, to a lesser extent, of osteoarthritis.<sup>1–3 6</sup> Previous, mainly descriptive, immunohistochemical studies carried out by us and by others suggested that u-PAR is expressed by cells of myeloid origin, such as synovial tissue lining cells and interstitial macrophages in the synovial tissue from rheumatoid arthritis patients.<sup>34 37</sup> Higher levels of u-PAR were detected in the peripheral blood, synovial fluid samples, and synovial tissue extracts of rheumatoid versus osteoarthritis patients.<sup>34–36 45</sup> The expression of u-PAR on peripheral blood monocytes was also increased in rheumatoid versus osteoarthritis and normal subjects.<sup>46</sup> The present, semiquantitative immunohistochemical analysis with MoAb 10G7 found that most synovial tissue lining cells (86–91%) and subsynovial macrophages (68–78%) expressed u-PAR in synovial tissue from both rheumatoid and osteoarthritis patients without major differences between the two diseases. In contrast, u-PAR was present on significantly fewer synovial tissue lining cells (8%) and macrophages (23%) in normal synovia. MoAb L21 also detected u-PAR on more than 90% of myeloid cells in both diseases. Ronday *et al* reported strong u-PAR expression in the synovial tissue lining layer from rheumatoid arthritis patients; however, it was barely detectable in the synovial tissue from osteoarthritis patients.<sup>34</sup> This study did not evaluate the percentage of immunoreactive cells in any synovial

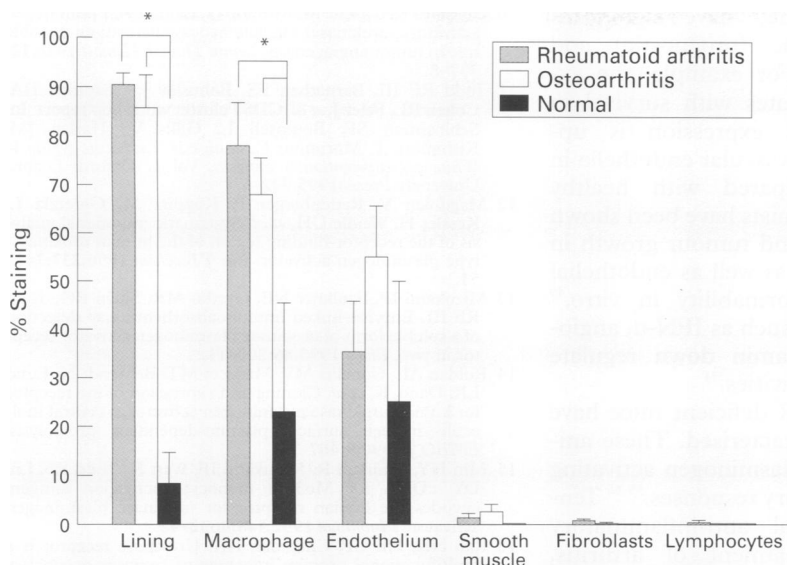


Figure 2 Percentage reactivity of synovial tissue components with MoAb 10G7 raised against u-PAR. Bars represent the mean (SEM). \* $P < 0.05$  between groups. Lining, synovial tissue lining cell layer; macrophage, subsynovial (scattered) macrophages; endothelium, vascular endothelium; Smooth muscle, vascular smooth muscle.

tissue sample.<sup>34</sup> We analysed the number of cells expressing u-PAR, but not the density of u-PAR expression on the cell surface. The number of u-PARs is increased in peripheral blood monocytes from rheumatoid arthritis patients compared with osteoarthritis patients and normal subjects.<sup>46</sup> Therefore, although most myeloid cells express u-PAR in tissues from both rheumatoid and osteoarthritis patients, the number of receptors on these cells may be much lower in synovial tissue from osteoarthritis patients resulting in possible differences in sensitivity between that study and ours. Alternatively, tissue from osteoarthritis patients included in our study may be more inflamed than the ones used by the other group. However, we have also reported that another GPI linked myeloid antigen, CD66, was also up-regulated on synovial tissue lining cells and macrophages in rheumatoid and osteoarthritis versus normal synovia.<sup>47</sup>

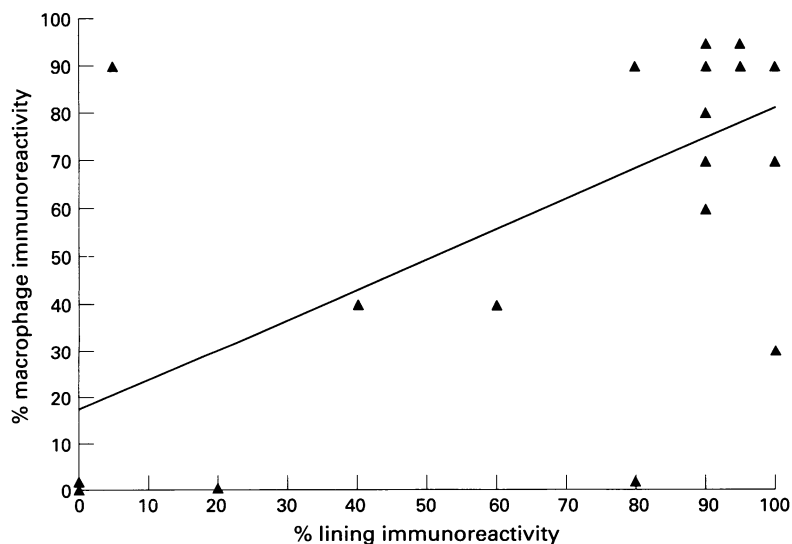


Figure 3 Correlation between u-PAR expression in synovial tissue lining cells versus subsynovial macrophages determined using MoAb 10G7 from 23 patients. ( $r = 0.63$ ;  $P < 0.05$ ).

Plasminogen activators, such as u-PA, may modulate synovial inflammation, angiogenesis, and joint destruction in a number of ways. u-PA may be involved in the recruitment of inflammatory leucocytes into the synovial tissue, as it is chemotactic for neutrophils<sup>48</sup> and it stimulates cytokine dependent monocyte adhesion.<sup>33</sup> u-PAR has been identified as the monocyte activation antigen Mo-3,<sup>15</sup> and its soluble form has been detected in the conditioned media of activated monocytes.<sup>13</sup> u-PAR is involved in macrophage differentiation, monocyte derived pericellular proteolysis, adhesion to the ECM, and the stimulation of metalloproteinase production.<sup>16</sup>

u-PA is also a potent mediator of angiogenesis.<sup>10</sup> Cytokine stimulated endothelia produce high amounts of u-PA that, in turn, enables ECM degradation, endothelial cell migration, and vessel formation.<sup>20 49 50</sup> u-PA acts by inducing plasmin dependent release of the angiogenic basic fibroblast growth factor (bFGF) from the ECM.<sup>18</sup> u-PAR antagonists block neovascularisation in vitro.<sup>49</sup>

u-PAR and its receptor interact with other inflammatory mediators in arthritic synovial tissue. Unstimulated monocytes/macrophages, T cells, endothelial cells, chondrocytes, and synoviocytes produce low amounts of u-PA.<sup>21 23 26</sup> Inflammatory mediators, such as interleukin (IL)-1 $\beta$ , tumour necrosis factor (TNF)- $\alpha$ , transforming growth factor- $\beta$ , and granulocyte-monocyte colony stimulating factor, enhance u-PA secretion by most of these cell types.<sup>17-24</sup> TNF- $\alpha$ , interferon (IFN)- $\gamma$  and bFGF induce u-PAR expression on monocytes.<sup>25</sup> u-PA itself can increase the number of u-PARs on monocytes, resulting in the perpetuation of inflammation, proteolysis and angiogenesis.<sup>25</sup> Most of these cytokines as well as u-PA are present in high quantities, and play an important role in the synovial tissue of rheumatoid and osteoarthritis patients.<sup>4 6 34</sup> In the present study, we also detected strong u-PAR expression on cells of myeloid origin, as well as on some endothelial cells in the majority of the inflamed, but not in normal, synovial tissue.

u-PAR acts in close association with CAMs and ECM components during the adhesion of monocytes and endothelial cells. It forms a complex with the CD11b/CD18  $\beta 2$  integrin, which facilitates integrin dependent adhesive functions.<sup>28</sup> However, co-clustering of the antigen receptor complex with  $\beta 1$  and  $\beta 2$  integrins induce u-PAR expression on T cells.<sup>26</sup> u-PAR serves as a receptor for vitronectin on endothelia, and adhesion to this ECM molecule results in clustering of u-PAR on the endothelial surface, as well as increased u-PA production.<sup>30 31</sup> Blocking the vitronectin receptor  $\alpha V/\beta 3$  integrin results in a decrease in u-PAR mRNA expression suggesting that  $\alpha V/\beta 3$  and u-PAR act in concert during adhesion to vitronectin.<sup>29</sup> The co-capping of u-PAR with other CAMs on the cell surface results in antigen clustering, which regulates focal adhesion, signalling, and pericellular u-PA activities.<sup>9 16 31-33 51</sup>

u-PAR and u-PA may have significant clinical, both diagnostic (prognostic) and therapeutic, relevance. For example, the expression of u-PA correlates with survival in breast cancer.<sup>52</sup> u-PAR expression is up-regulated on brain microvascular endothelia in multiple sclerosis compared with healthy subjects.<sup>53</sup> u-PAR antagonists have been shown to inhibit angiogenesis and tumour growth in syngeneic mice in vivo,<sup>8</sup> as well as endothelial cell migration and deformability in vitro.<sup>49</sup> Anti-angiogenic factors, such as IFN- $\alpha$ , angiostatic steroids, and suramin down regulate u-PA production and activities.<sup>54</sup>

Both u-PA and u-PAR deficient mice have been generated and characterised. These animals showed impaired plasminogen activating potential and inflammatory responses.<sup>55</sup> Tenoxicam, a non-steroidal anti-inflammatory drug used in the treatment of arthritis, down-regulated u-PAR expression on monocytes.<sup>57</sup> Urinary trypsin inhibitor, which blocks u-PA production, also relieved arthritic symptoms in rheumatoid arthritis patients.<sup>36</sup> Thus, the suppression of u-PA production and u-PAR expression using antagonists, antibodies, or antisense oligonucleotides may be useful for the management of inflammatory diseases, including rheumatoid and osteoarthritis.<sup>7 8 29 49 54</sup>

**Addendum:** Additional data obtained from the Sixth International Workshop and Conference on Leukocyte Differentiation Antigens indicated that on the basis of its selective binding to a u-PAR transfectant, MoAb 10G7 may recognise a CD87 epitope, but its reactivity remains to be confirmed by alternate methodology (Todd RF, personal communication).

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