A urokinase-sensitive region of the human urokinase receptor is responsible for its chemotactic activity

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The role of urokinase-type plasminogen activator (uPA) and its receptor (uPAR/CD87) in cell migration and invasion is well substantiated. Recently, uPA has been shown to be essential in cell migration, since uPA−/− mice are greatly impaired in inflammatory cell recruitment. We have shown previously that the uPA-induced chemotaxis requires interaction with and modification of uPAR/CD87, which is the true chemotactically active molecule acting through an unidentified cell surface component which mediates this cell surface chemotaxis activity. By expressing and testing several uPAR/CD87 variants, we have located and functionally characterized a potent uPAR/CD87 epitope that mimics the effects of the uPA–uPAR interaction. The chemotactic activity lies in the region linking domains 1 and 2, the only protease-sensitive region of uPAR/CD87, efficiently cleaved by uPA at physiological concentrations. Synthetic peptides carrying this epitope promote chemotaxis and activate p56/p59hck tyrosine kinase. Both chemotaxis and kinase activation are pertussis toxin sensitive, involving a Gαo protein in the pathway.

Keywords: chemotaxis/G protein/pertussis toxin/receptor/urokinase-type plasminogen activator

Introduction

Cell recruitment is an essential component in inflammation, neo-angiogenesis and cancer invasiveness. Urokinase-type plasminogen activator (uPA), its receptor (uPAR/CD87) and inhibitor (PAI-1) are directly involved in these mechanisms (Danø et al., 1994; Blasi, 1997). An important proof of this statement is the profound impairment of inflammatory cell recruitment in uPA−/− mice which do not respond properly but succumb to infection by Cryptococcus neoformans, due to deficient T lymphocyte and monocyte–macrophage recruitment (Gyetko et al., 1996). Moreover, uPA-deficient mice fail to support the growth and development of experimental melanomas, while inhibition of the uPA–uPAR/CD87 interaction in vivo blocks angiogenesis, growth and spreading of syngeneic tumors (Min et al., 1996; Shapiro et al., 1996). A chemotactic activity of uPA has long been recognized in vitro on different cell types in culture (Gudewicz and Bilbo, 1987; Fibbi et al., 1988; Busso et al., 1994; Gyetko et al., 1994; Resnati et al., 1996). The chemotactic activity of uPA strictly depends on binding to its receptor uPAR (CD87): it does not occur in murine cells lacking uPAR/CD87, or containing uPAR/CD87 but not recognizing human uPA; can be restored by transfection of the uPAR/CD87 cDNA; and is inhibited by antibodies that prevent uPA–uPAR interaction (Resnati et al., 1996). Occupancy of uPAR/CD87 transduces a signal that results in the movement of cells; indeed, a rapid and transient activation of intracellular serine and tyrosine kinases (PTK) has been observed in different cells (Busso et al., 1994; Resnati et al., 1996). That tyrosine kinase activation is required for the chemotactic activity of uPA is shown by the ability of 3T3 fibroblasts from wild-type but not from Src−/− mice to respond to uPAR/CD87-mediated chemotaxis (our unpublished data), or by the block of ligand-induced chemotaxis by tyrosine kinase inhibitors in THP-1 cells (Resnati et al., 1996).

uPAR/CD87 is a glycosylphosphatidylinositol (GPI)-anchored protein made up of three cysteine-rich CD59-like domains connected by short linker regions (Ploug and Ellis, 1994), expressed by a variety of cells including neutrophils, T lymphocytes, monocytes–macrophages and fibroblasts. In addition, most cancer cells express uPAR/CD87, often at rather high levels (Blasi, 1988). In T lymphocytes and monocytes, uPAR/CD87 is an activation antigen and its expression appears to be relevant to the function of these cells, at least in culture (Picone et al., 1989; Nykjaer et al., 1994; Bianchi et al., 1996).

The membrane attachment of uPAR/CD87 via a GPI anchor (Ploug et al., 1991), i.e. the lack of an intracytoplasmic domain capable of connecting with the cytoplasmic signal transducers, suggests that transmembrane adaptor(s) must mediate the activation of intracellular transducers. Indeed, uPAR/CD87 cells can respond to a chemotactic stimulus induced by exogenous, soluble uPAR (suPAR), indicating that the binding of suPAR to uPAR/CD87 transforms the latter into a ligand for another cell surface component. However, this activity of suPAR is apparent only after chymotrypsin cleavage at Tyr87 (Resnati et al., 1996), a modification causing a major conformational change, similar to uPA binding (Ploug et al., 1993; Ploug and Ellis, 1994). The chemotactic properties of uPA and chymotrypsin-cleaved suPAR are identical, i.e. they both induce p56/p59hck tyrosine kinase activation with the same time course (Resnati et al., 1996), indicating that the same mechanism operates in both cases.

The above data show, therefore, that uPAR/CD87 is a cell surface chemokine-like molecule, the activity of which is regulated by the binding of its ligand uPA which in an as yet undefined way unveils the chemotactic epitope. Here we demonstrate that the uPAR/CD87 chemotactic
epitope is located in the sequence linking domains 1 and 2, a region highly sensitive to cleavage by uPA and other proteases (Høyer-Hansen et al., 1997). We also show that a synthetic uPAR/CD87 chemotactic epitope activates p56\(^{hck}\) and that both chemotaxis and activation of p56\(^{hck}\) are pertussis toxin (PTX) sensitive, implying a connection between G proteins and intracellular protein tyrosine kinases. Here, we indicate with uPAR/CD87 the ‘normal’ membrane-attached uPA receptor, and with suPAR the exogenous, recombinant receptor.

**Results**

**Identification and mapping of the uPAR/CD87 chemotactic epitope**

We employed suPAR truncated mutants to identify the uPAR/CD87 chemotactically active region (see Figure 1 for a scheme, and Materials and methods for terminology). We had shown previously that chymotryptic cleavage of exogenous suPAR induced a very efficient chemotactic activity at picomolar concentrations in cells which did not express this receptor (Resnati et al., 1996). To identify the region of suPAR responsible for the activity, we first purified the FLAG-tagged, full size three-domain suPAR, digested it with chymotrypsin and separated the resulting fragments D\(_{11-87}\) and D\(_{2388-274}\) on FLAG™ antibody affinity chromatography columns. The two fragments were tested individually in chemotaxis assays on human THP-1 cells. We chose THP-1 cells, even though they are poor responders to chemotactic signals, because their basal level of tyrosine phosphorylation is low (Resnati et al., 1996) and hence make the subsequent biochemical analysis faster. However, the same results were obtained with several other cells (mouse uPAR/CD87–/– fibroblasts, NIH-3T3 fibroblasts, rat smooth muscle cells and mouse LB6 tumor cells), which respond ~2-fold better to the chemotactic stimuli of suPAR fragments and other suPAR derivatives (see below). The C-terminal fragment, D\(_{2388-274}\), but not the N-terminal D\(_{11-87}\), elicited a dose-dependent chemotactic response (Figure 2E and F). We then tested individual recombinant suPAR mutants. Of these, only D\(_{11-92}\) induced a dose-dependent chemotactic effect (Figure 2A), while D\(_{2393-274}\), D\(_{2393-191}\) and D\(_{3192-274}\) failed to induce chemotaxis through the entire concentration range analyzed (Figure 2B–D). These findings show that only fragments containing the sequence 88–92 of uPAR/CD87, SRSRY, had a chemotactic effect. Since the presence of this motif correlates with enhanced migration of THP-1 cells, it may represent the chemotactically active epitope. Interestingly, the 88–92 sequence appears to be active both when present at the N-, as in D\(_{2388-274}\), as well as at the C-terminus, as in D\(_{11-92}\).

To confirm the above interpretation, we tested synthetic uPAR/CD87 peptides for their chemotactic activity on THP-1 cells. Peptide 1 [AVTYSRSRYLEC, which corresponds to the amino acid sequence 84–95 of human uPAR/CD87 (Roldan et al., 1990)] and its shorter version, peptide 3 (SRSRYLEC, residues 88–95), exhibited chemotactic activity with a maximal response at concentrations as low as 0.1 pM (Figure 3A and C). On the other hand, peptide 4 (TLVEYYSRASCR), a scrambled version of peptide 1, and peptide 2 (YTARLWGTLTT), which covers residues 301–313, and thus is absent from processed mature uPAR/CD87 (Ploug et al., 1991), had no effect on cell migration over the entire concentration range analyzed (Figure 3B and D). These results support the conclusion that the sequence SRSRY of human uPAR/CD87 has a potent chemotactic activity. It is important to note that this region is cleaved efficiently by uPAR-bound uPA at chemotactically active concentrations (Høyer-Hansen et al., 1997; see Discussion).

Since THP-1 cells are not very sensitive to chemotactic stimuli, we tested peptide 1 on other cells. Peptide 1 was found to be active also on several other cells, like murine uPAR/CD87–/– fibroblasts, NIH-3T3 and LB6 cells, or on rat aortic smooth muscle cells (data not shown). On some of these cells, the effect is much more pronounced (~4-fold...
over basal migration), but has an identical concentration dependence as in THP-1 cells.

The chemotactic effect of suPAR fragments and synthetic peptides was not a general effect on migration but was dependent on the establishment of a concentration gradient. Also, these agents did not display a chemokinetic effect on THP-1 cells, as addition in the upper well (containing cells) had no effect on migration (data not shown).

**Activation of p56/p59^hck by chemotactic suPAR/CD87 peptides**

Treatment of THP-1 cells with the uPA fragment ATF at chemotactic concentrations induces the activation of p56/p59^hck tyrosine kinase. Treatment with exogenous chymotryptsin-cleaved suPAR, that substitutes for uPA in inducing chemotaxis in uPAR/CD87-lacking cells, also activates p56/p59^hck (Resnati et al., 1996; unpublished data). We therefore tested the effect of chemotactic concentrations of peptide 1 on the activity of p56/p59^hck kinase. Treatment of THP-1 cells at 37°C with 1 pM peptide 1 induced increased autophosphorylation as well as phosphorylation of the exogenous substrate enolase (Figure 5A). The effect was already apparent after 2 min, reached a maximum after 10 min and returned to the basal level after 30 min. This time course is very similar to that of ATF or chymotryptsin-cleaved suPAR (Resnati et al., 1996). No effect was observed when 1 pM of the corresponding scrambled peptide (peptide 4) was used in the assay (data not shown). Thus, the effect of peptide 1, containing the SRSRY sequence of uPAR/CD87, resembles that of uPA and chymotryptsin-cleaved suPAR not only in stimulating chemotaxis but in the activation of the same tyrosine kinase. This supports the view that peptide 1 is the, or one of the, chemotactic epitope(s) of uPAR/CD87.

**Involvement of heterotrimeric G proteins**

The activity of many chemokines is mediated through specific receptors by signal–effector coupling GTP-binding proteins (G proteins). PTX inhibits the activity of some chemokines through the αi subset of G proteins; the inhibitory effect of PTX is therefore an indication of the involvement of heterotrimeric Gαi proteins in the signaling pathway (Baggiolini et al., 1994; Neer, 1995). To test the involvement of G proteins in the uPA/suPAR/synthetic peptides-induced chemotaxis, we tested the effect of PTX and one of its inactive mutants. Chemotaxis of THP-1 cells induced by the N-terminal fragment of uPA (ATF, Stoppelli et al., 1985) also proved to be PTX sensitive (data not shown). As shown in Figure 4, pre-incubation of THP-1 cells with PTX also inhibited chymotryptsin-cleaved suPAR and peptide 1-induced chemotaxis, while
Fig. 3. Chemotactic response of THP-1 cells to different concentrations of the indicated peptides. THP-1 cells migrated towards a solution containing different concentrations of the chemoattractants. Random cell migration of untreated THP-1 cells is referred to as 100% migration.

an inactive mutant had no effect. An identical result was obtained on peptide 1-induced chemotaxis of rat smooth muscle cells. This result indicates that the uPAR/CD87 epitope exerts its chemotactic activity through a G protein-coupled cell surface protein.

Since uPA as well as the chemotactic uPAR/CD87 peptides also activate p56/p59hck, in a separate experiment we also tested the effect of the toxin on the activation of p56/p59hck by peptide 1. As shown in Figure 5B and C, the activation of p56/p59hck activity by peptide 1 was also PTX sensitive. Inhibition of p56/p59hck activation by peptide 1 was observed in the presence of 100 ng/ml wild-type, but not mutant, PTX. This result confirms the G protein dependence of uPAR/CD87 chemotaxis and suggests that the activation of tyrosine kinases is a downstream step from the heterotrimeric G\textsubscript{i/o} protein.

Discussion

GPI-anchored proteins are potent signaling molecules. However, the lack of a transmembrane domain requires that they interact with another transmembrane 'adaptor', i.e. with the real signaling molecule. This has in fact been shown to be true for several GPI-anchored proteins, including, among others, CD14 (Uhlevitch and Tobias, 1995). In the case of uPAR, the adaptor (Resnati et al., 1996) is not yet known; however, in the present study, by identifying the chemotactic epitope of uPAR, we in fact suggest the nature of the actual ligand for such an adaptor.

Fig. 4. Effect of PTX on chemotaxis of THP-1 cells in response to peptide 1 or chymotrypsin-cleaved suPAR. THP-1 cells were pre-incubated for 4 h with (100 ng/ml) PTX or its inactive mutant (Pizza et al., 1989), washed and their chemotactic response measured. Medium alone (black bars), 0.1 pM peptide 1 (dotted bars), 1 pM peptide 1 (white bars), 10 pM chymotrypsin-cleaved suPAR (striped bars). Random cell migration of untreated THP-1 cells is referred to as 100% migration.

We have shown that peptide 1 can reproduce, with the same time course, the chemotactic effect of uPA and chymotrypsin-cleaved suPAR, causing cell migration and rapid and transient activation of p56/p59hck in THP-1 and other cells, through a PTX-sensitive signaling system. The concentration of peptide 1 required to elicit the signaling
uPA receptor region responsible for chemotaxis

Fig. 5. Peptide 1 activation of p56/p59$^{hck}$ kinase activity and its inhibition by PTX. Metabolically $^{35}$S-labeled THP-1 cells were acid washed and treated with 1 pM peptide 1 for the indicated times at 37°C, then lysed and immunoprecipitated with anti-p56/p59$^{hck}$ antibody. $^{35}$S: direct analysis by SDS–PAGE of the amount of p56/p59$^{hck}$ employed. $^{32}$P: in vitro kinase assay in the presence of [y-$^{32}$P]ATP and 5 µg of rabbit muscle enolase. (A) Effect of peptide 1; (B) Effect of peptide 1 on cells pre-treated with mutated PTX (100 ng/ml); (C) Same as in (B) in the presence of 100 ng/ml wild-type PTX. The data in (A) and in (B) and (C) are from two different experiments.

Fig. 6. The uPAR/CD87 linker sequence between domains 1 (D1) and 2 (D2) and the protease-sensitive sites.

effects (~0.1 pM) is far lower than the concentration of uPA or ATF required to generate the same biological response (0.5–5 nM, Resnati et al., 1996), but is of the same order of magnitude as the effect obtained with chymotrypsin-cleaved suPAR. This represents an enormous functional amplification which follows or bypasses the uPA–uPAR/CD87 interaction step. The shape of the dose–response curve for the various peptides closely resembles that for the chymotrypsin-cleaved uPAR (Resnati et al., 1996), and appears to be typical of the chemotactic response.

Our data show that the chemotactic effect of peptide 1 is not species specific, as murine and rat cells can respond as well. This differs from the binding of uPA to uPAR/CD87 which is species specific (Appella et al., 1987; Estreicher et al., 1989).

The chemotactic epitope is located within the linker region connecting domains 1 and 2 (see Figure 6). This is the only region that is in fact susceptible to proteases (Behrendt et al., 1991). The question arising from the identification of the chemotactic epitope is whether exposure of this epitope occurs physiologically through proteolytic cleavage. While we cannot exclude that a ligand-induced conformational change of uPAR/CD87 is sufficient to transduce migratory signals, we must take note that a relevant proteolytic event does indeed occur in vivo. (i) In different cell lines and human cancer tissues, uPAR/CD87 fragments missing domain D1 have been demonstrated (Høyer-Hansen et al., 1992; Sølberg et al., 1994). (ii) In purified suPAR, the linker region between domains 1 and 2 (Figure 6) is susceptible to cleavage by uPA and other proteases, like chymotrypsin (Høyer-Hansen et al., 1992; Ploug and Ellis, 1994). (iii) Importantly, at chemotactically active concentrations, endogenous or exogenous receptor-bound uPA cleaves 50% of surface uPAR/CD87 at residue 83 or 89 (Høyer-Hansen et al., 1997). The former site would generate a chemotactically active uPAR/CD87, starting with an N-terminal AVTYSR-SRY sequence, identical to that of peptide 1 (see Figure 6). (iv) Binding of uPA to uPAR/CD87 activates cell surface plasminogen to plasmin which also might cleave uPAR/CD87 in the same region (Høyer-Hansen et al., 1992; Ploug and Ellis, 1994).

While these data suggest that proteolysis activates the chemotactic activity of uPAR/CD87, in cultured cells the uPA protease domain is not necessarily required, since chemotaxis can be stimulated by uPA derivatives lacking protease activity (i.e. ATF or pro-uPA) (Fibbi et al., 1988; Resnati et al., 1996). It is known that ligand binding induces a conformational change (Ploug et al., 1994). Possibly, this may allow cleavage of uPAR/CD87 by a different protease substituting for uPA. Since the sequence SRSRY is active when positioned both N- and C-terminally, different types of proteases may be involved. Again, we cannot exclude that a conformational change may be sufficient to expose the SRSRY epitope, and that the observed proteolytic event may be unrelated.

A proteolytic event activating the cell surface uPAR chemokine may explain the paradox that the high level of expression of uPAR and uPA in human tumors is a strong negative prognostic factor, independently of whether uPAR is expressed by cancer or stromal cells. While in colon cancer, for example, uPAR/CD87 is expressed by cancer cells, in breast cancer it is confined mostly to stromal
cells. In both cases, however, high tumor levels of uPAR are predictive of cancer relapse (Pyke et al., 1991, 1993; Bianchi et al., 1994; Grøndahl-Hansen et al., 1995). Depending on the protease involved, cleavage of uPAR might generate either a soluble chemotactoacting domain D1 acting on distant cells, or a membrane-attached chemotactic domain D23, which would then act on the very cell which is expressing it. In other words, a cell surface chemokine or a soluble chemokine can be generated, depending on the protease involved.

It is also interesting to note that uPA has two potential cleavage sites in the sequence of uPAR linking domain D1 to domain D2. The second site, at position 89/90 (Figure 6), might destroy the chemotactactic activity, as it would cleave within the conserved region. It is interesting to note that the efficiency of cleavage by uPA at this second site is lower (Høyer-Hansen et al., 1997), suggesting that the cleavage might actually occur only at high concentrations of uPA.

Whatever the mechanism, the data reported here show that the uPA–uPAR system is a cell surface-regulated chemokine. The exposed chemotactic epitope must interact with a cell surface (adaptor) protein which can signal through a heterotrimeric G protein. The nature of the adaptor is still unknown: among already known proteins, possible candidates are the chemokine receptors, integrins and caveolin. Chemokine receptors, as members of the seven transmembrane-spanning protein family, may interact with heterotrimeric G proteins (Bagnoli et al., 1994). Integrins and caveolin, on the other hand, have been shown to co-immunoprecipitate with uPAR (Bohuslav et al., 1994). Synthetic peptides had the following sequences: peptide 1, AVTYSRSRYLEC; peptide 2, YTARLWGGTLLT; peptide 3, LLQVYTYCSSLE; peptide 4 (scrambled version of peptide 1), TLVEYYSRSRYLEC. Depending on the protease involved, these agents would be acting downstream of the primary uPA–uPAR/CD87 interaction step, and might have properties different from those of uPAR/CD87 antagonists, expanding the application of agents inhibiting this functions.

**Materials and methods**

**Materials and cells**

Human AT1F was a generous gift of Dr Jack Henkin (Abbott Laboratories, IL). Soluble uPAR/CD87 was prepared and chymotrypsin-cleaved as described before (Resnati et al., 1996). Recombinant PTX and its inactive mutant (Pisza et al., 1989) were generous gifts of Dr Maria Grazia Pizza (IRIS, Siena, Italy). Synthetic peptides had the following sequences: D11–92, domain 1, from residue 1 to 92; D29–191, domain 2, from residue 9 to 191; D31–274, domain 3, from residue 9 to 274. Mutant cDNAs encoding soluble molecules since they employed the basic cDNA modification described previously (Roldan et al., 1991; Ploug and Ellis, 1994). All uPAR/CD87 constructs expressed soluble molecules since they employed the basic cDNA modification described previously (Roldan et al., 1991; Masucci et al., 1999). Pharmaceuticals of uPAR/CD87 is designated D123. Truncated molecules are identified by the number of the domains (i.e. D1, D2, D3) and by the number of amino acids they express (i.e. D1, D2, D3 domains, 1, from residue 1 to 92). Mutant cDNAs encoding soluble uPAR/CD87 were generated by PCR with the following primers (see Table I for sequences): 1, ATGSRSKLYCE; peptide 2, YTARLWGGTLLT; peptide 3, RSRRYCE; peptide 4 (scrambled version of peptide 1), TLVEYYSRASCR.

**Construction of plasmids expressing soluble, truncated uPAR/CD87 molecules**

uPAR/CD87 has three domains, an N-terminal (D1), an intermediate (D2) and a C-terminal domain (D3), which is connected to the GPI anchor (Ploug et al., 1991; Ploug and Ellis, 1994). All uPAR/CD87 constructs expressed soluble molecules since they employed the basic cDNA modification described previously (Roldan et al., 1991; Macucci et al., 1999). The full size soluble uPAR/CD87 is designated D123. Truncated molecules are identified by the number of the domains (i.e. D1, D2, D3) and by the number of amino acids they express (i.e. D1, D2, D3 domains, 1, from residue 1 to 92). Mutant cDNAs encoding soluble uPAR/CD87 were generated by PCR with the following primers (see Table I for sequences): 1, ATGSRSKLYCE; peptide 2, YTARLWGGTLLT; peptide 3, RSRRYCE; peptide 4 (scrambled version of peptide 1), TLVEYYSRASCR.

**Table I. Primers used in PCR**

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<tr>
<th>Primer</th>
<th>Sequence (5′–3′)</th>
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<tbody>
<tr>
<td>FRA18</td>
<td>ATATACCTGGAGAAGAGTGGACCCCGGCA</td>
</tr>
<tr>
<td>D1.3/T</td>
<td>TTATCGATGGTACAGCGCCCTGGGAATA</td>
</tr>
<tr>
<td>D2.3/T</td>
<td>TTATCGATGGGCTACTGGCGGAGATT</td>
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<tr>
<td>D2.5/T</td>
<td>AAATCGACTGGGCCCCAACAGGGCTGGA</td>
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<tr>
<td>D3.5/T</td>
<td>TCTATCGATGCTTTCCTCTGACGTGTTAA</td>
</tr>
<tr>
<td>K/FOcs</td>
<td>GCAGAGGACCTCGTCCGACTATAAGGTGACGAGACAAAGTA</td>
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<td>GCTTTACCTGCTCGTCCATCACCCCA</td>
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<tr>
<td>NSI</td>
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</tr>
<tr>
<td>4D</td>
<td>CAAGCTTACCTGCTCGTCCATCC</td>
</tr>
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and succumb to bacterial infections (Gyetko et al., 1996). The mechanism outlined in this study is likely to contribute to the role of uPA in cell recruitment. The inability to induce a conformational change and/or to cleave uPAR/CD87 proteolytically may explain the immunodeficiency of uPAR− mice. If this hypothesis is correct, the uPAR−/− mice will have to be immunodeficient as well. In man, uPAR/CD87 is an activation antigen in T lymphocytes and monocytes, and is required for T cell migration, at least in vitro (Nykjær et al., 1994; Bianchi et al., 1996).

Finally, the results described here may have a practical application, as it is conceivable that agents acting on uPAR/CD87 chemotaxis, for example by blocking the function of the chemotactic epitope, may be employed to modify inflammatory reactions or cancer invasiveness. These agents would be acting downstream of the primary uPA–uPAR/CD87 interaction step, and might have properties different from those of uPAR/CD87 antagonists, expanding the application of agents inhibiting this functions.

F.Fazioli et al.
D23+, D23+24, the D1 region in the D12 and D123 constructs was deleted by substituting the Nrl–NcoI fragment containing the D1 coding region, with a fragment generated by amplifying the uPAR/CD87 cDNA with the primers FRA18 and D3.5'T and digesting with the same enzymes. To generate D3+24, the D1 region in D123 was deleted by substituting the Nrl–NcoI fragment containing the D12 coding region, with a fragment generated from uPAR/CD87 cDNA with the primers FRA18 and D3.5'T and digesting with the same enzymes. All mutant receptors were tagged at the C-terminus with the peptide sequence HRRASVDYKDDDDK, which includes a protein kinase substrate and the FLAG™ epitope, by inserting in the C-terminal Cid site a linker made by annealing the two oligonucleotides K/FoCs and K/FoAs. All the recombinant coding regions were amplified with the primers NS1 and 46D, digested with NcoI and transferred to the eukaryotic expression vector pBSEN (Pallisgaard et al., 1994) digested with NcoI and Klenow-treated EcoRI.

**Purification of recombinant soluble uPAR/CD87 molecules**

Semifluidic COS7 cells were harvested in phosphate-buffered saline (PBS)–1 mM EDTA, washed with RPMI medium and the suspension (0.8 ml, 1–2×10⁶ cells/ml in RPMI) electroporated in 0.4 cm Bio-Rad cuvettes containing plasmid DNA (30 µg, 1 µg/ml in water) at 960 µF, 240 V (GenePulser, Bio-Rad). After recovery overnight, cells were collected and fresh medium added. Recombinant proteins were purified 240 V (GenePulser, Bio-Rad). After recovery overnight, cells were collected and fresh medium added. Recombinant proteins were purified over the FLAG™ affinity column. D1 1–87 was recovered in the flow-through while the D23 88–274 containing the FLAG™ epitope was retained through while the D23 88–274 containing the FLAG™ epitope was retained.

**Chemotaxis assays**

Chemotaxis analysis were performed in modified Boyden chambers, using polyvinylpyrrolidone-free polycarbonate filters (13 mm diameter, 5 µm pore size) coated with type I collagen (100 µg/ml in PBS pH 7.4). After an acid wash (Stoppelli et al., 1986), 2×10³ THP-1 cells in serum-free medium were added in the upper chamber. Attractants were diluted in serum-free medium at the indicated concentrations and added in the lower chamber. The assays were incubated at 37°C in 5% CO₂ in air for 90 min. The upper surface of filters was scraped free of cells, the filters fixed in methanol, stained with crystal violet and cells counted.

**Tyrosine kinase assay**

The kinase assay was performed as previously described (Resnati et al., 1995) using THP-1 cells metabolically labeled with [γ-³²P]ATP, -3000 Ci/ml (Amersham, 5 µg rabbit muscle enolase substrate, 5 min, room temperature) and resolved by SDS-PAGE.

**Acknowledgements**

We gratefully thank R.Pardi and P.C.Marchisio who provided helpful suggestions and critical comments. We thank Dr Maria Grazia Pizza (I.R.I.S., Siena, Italy) for providing the inactive mutant of pertussis toxin and Dr Jack Henkin (Abbott Laboratories, IL, USA) for the human ATF. N.S. is the recipient of a fellowship from the Danish Research Academy. This work was supported in part by grants from the Italian Association for Cancer Research (AIRC), the Research (A.I.R.C.), the EU Biomed Program (contract no. CT96-0017) and the AIDS fund of the Italian Ministry of Health.

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Received on August 9, 1997; revised on September 24, 1997