**Communication**

Purified \( \alpha_2 \)-Macroglobulin Receptor/LDL Receptor-related Protein Binds Urokinase·Plasminogen Activator Inhibitor Type-1 Complex

EVIDENCE THAT THE \( \alpha_2 \)-MACROGLOBULIN RECEPTOR MEDIATES CELLULAR DEGRADATION OF UROKINASE RECEPTOR-BOUND COMPLEXES

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Complexes between \( ^{125} \)I-labeled urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) bound to purified \( \alpha_2 \)-macroglobulin (\( \alpha_2 \)M) receptor (\( \alpha_2 \)MR)/low density lipoprotein receptor-related protein (LRP). No binding was observed when using uPA. The magnitude of uPA·PAI-1 binding was comparable with that of the \( \alpha_2 \)M-associated protein (\( \alpha_2 \)MRAP). Binding of uPA·PAI-1 was blocked by natural and recombinant \( \alpha_2 \)MRAP, and about 80% inhibited by complexes between tissue-type plasminogen activator (tPA) and PAI-1, and by a monoclonal anti-PAI-1 antibody.

In human monocytes, uPA·PAI-1, like uPA and its amino-terminal fragment, bound to the urokinase receptor (uPAR). Degradation of uPAR-bound \( ^{125} \)I-uPA·PAI-1 was 3–4-fold enhanced as compared with uncomplexed uPAR-bound uPA. The inhibitor-enhanced uPA degradation was blocked by \( \alpha_2 \)MRAP and inhibited by polyclonal anti-\( \alpha_2 \)M/LRP antibodies. This is taken as evidence for mediation of internalization and degradation of uPAR-bound uPA·PAI-1 by \( \alpha_2 \)MR/LRP.

The \( \alpha_2 \)-macroglobulin receptor, \( \alpha_2 \)MR (1–3), identical with the low density lipoprotein receptor-related protein, LRP (4, 5), consists of an 85-kDa membrane-spanning \( \beta \)-chain and a 500-kDa ligand-binding \( \alpha \)-chain (4–6). \( \alpha_2 \)MR/LRP binds several apparently unrelated ligands: \( \alpha_2 \)M-proteinase complex and methyamine-treated \( \alpha_2 \)M (7), \( \beta \)-migrating very low density lipoprotein (\( \beta \)-VLDL) activated by enrichment with apolipoprotein E (apoE) (8), and a 40–25, 5, 9) or 39-kDa (3, 4, 10) copurifying protein designated \( \alpha_2 \)MR-associated protein (\( \alpha_2 \)MRAP). Moreover, the receptor is reported to bind lipoprotein lipase (11), \( \alpha_2 \)MR/ LR P is a monocyte differentiation antigen (12) not expressed in lymphocytes (12, 14).

The receptor (uPAR) for urokinase (uPA) is a 55-kDa, 313-residue (15) glycoprotein (16) anchored to the cell membrane by a glycosyl-phosphatidylinositol (GPI) moiety (17). It binds single-chain pro-uPA, active two-chain uPA, and complexes between uPA and the plasminogen activator inhibitors (PAI-1 and PAI-2). Binding of uPA does not involve the serine proteinase domain (18) and the 10-kDa amino-terminal fragment (ATF) binds as well as pro-uPA and uPA (19). uPA is expressed on numerous cell types including monocytes (20, 21), T-lymphocyte-derived interleukin-2-activated killer (LAK) cells (21), and several established cell lines (22).

As expected for a GPI-anchored receptor (23), uPAR-bound uPA and ATF are poorly internalized and degraded. Surprisingly, uPA·PAI-1 complex, when added in solution or formed on the cell surface by the addition of PAI-1 to uPAR-bound uPA, is readily internalized and degraded in lysosomes. These results were obtained with the following \( \alpha_2 \)MR/ LR P-bearing cells: JAR cells (13), monocyte U-937 cells (24), and blood-derived monocytes (21). Analogous results were obtained in monocytes with uPA-plasminogen activator inhibitor type-2 (25). On the other hand, LAK cells, not expressing \( \alpha_2 \)MR/ LR P, degrade uPA·PAI-1 complex poorly (21). These observations led us to investigate whether \( \alpha_2 \)MR/ LR P might be involved in binding and endocytosis of uPA·PAI-1 complex.

**Materials and Methods**

\( \alpha_2 \)MR/ LR P and Ligands—Human \( \alpha_2 \)MR preparation, two-chain \( \alpha_2 \)MR, and \( \alpha_2 \)MRAP were produced as described (2, 7, 9). Human two-chain uPA (50 kDa) was purchased from Serono (Switzerland). Low M<sub>c</sub> uPA was from Green Cross Corporation, Osaka, Japan. ATF was a gift from Dr. J. Krause, The
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1 The abbreviations used are: \( \alpha_2 \)MR, \( \alpha_2 \)-macroglobulin receptor; \( \alpha_2 \)M, human \( \alpha_2 \)-macroglobulin; LRP, LDL receptor-related protein; \( \alpha_2 \)MRAP, \( \alpha_2 \)MR-associated protein; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor; ATF, amino-terminal uPA fragment; PAI-1, plasminogen activator inhibitor-type 1; FX<sub>c</sub>, coagulation factor X; apoE, apolipoprotein E; \( \beta \)-VLDL, \( \beta \)-migrating very low density lipoprotein; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Heps, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GPI, glycosyl-phosphatidylinositol; BSA, bovine serum albumin.

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RESULTS

Fig. 1 (lane 1) shows the SDS-PAGE-resolved components of the human placental α2MR/LRP preparation. Lane 2 shows binding of the established ligand α2MRAP to the α-chain.

Binding of labeled natural and rα2MRAP were not distinguishable (not shown). Labeled uPA-PAI-1 (lane 3), but not ATF (lane 4), bound to the α-chain. Lanes 5 and 6 show that α2MRAP and uPA-PAI-1 bound at the position of the α2MR/LRP α-chain in SDS-PAGE resolved placental membranes, but not at other positions.

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These results raised the question whether the established ligands compete for binding. Fig. 3 shows that 100 nM natural α2MRAP or rα2MRAP blocked the binding of 125I-uPA-PAI-1. Control experiments (not shown) with immobilized α2MRAP demonstrated absence of 125I-uPA-PAI-1 binding to this protein. The binding of uPA-PAI-1 to α2MR/LRP was sensitive to EDTA and heparin. α2-M-methylamine (100 nM) did not inhibit, ATP and low M, uPA had no effect, whereas 100 nM uPA inhibited slightly. Fig. 3 also shows that tPA-PAI-1 complex, but not tPA, inhibited 76%, and reactive center-cleaved PAI-1 inhibited 55%, suggesting that the PAI-1 moiety might contribute to the binding. This hypothesis was supported by the finding that one anti-PAI-1 monoclonal antibody inhibited 85%. The monoclonal anti-uPA antibody against the COOH-terminal serine protease domain inhibited 77%, whereas no inhibition (not shown) was observed with the anti-uPA antibody, clone 6, inhibiting binding of uPA to uPAR (21).

The functional consequence of uPA-PAI-1 binding to α2MR/LRP was explored in monocytes. Table I shows that binding of labeled uPA-PAI-1 at 4 °C was blocked by unlabeled ATP, but not by rα2MRAP, confirming binding to uPAR (21, 24). Under these conditions 125I-ATF was 14% bound (not shown). Conversely, binding of labeled rα2MRAP was blocked by unlabeled rα2MRAP, but not by ATP. Acid treat-
JAR cells (13) and U-937 cells. The radioactivity remaining associated to monocytes
clones 2, directed against the serine proteinase domain. The radioactivity remaining associated to monocytes
beled ATF inhibited the cell association of both tracers. presumably reflecting the low turnover of this tracer. Unlabeled
uPA was degraded 6-7% (not shown). Similar results, includ-
ing the inhibition by the polyclonal anti-α2MR/LRP antibody (Ab) was corrected for the effect of non-
monocytoid cells
purified α2MR/LRP, while uPA alone is unable to do so. This
membrane caused rapid dissociation of both tracers indicating
binding to the cell surface as previously shown for uPA-PAI-
1 in U-937 monocyteoid cells (24).

Fig. 4 shows the results of incubation experiments with 125I-
-uPA-PAI-1 and 125I-ATF for 5 h at 37 °C. The upper panel
shows a higher cell-associated radioactivity when using ATF,
preumably reflecting the low turnover of this tracer. Unlabeled
uPA-PAI-1 and 125I-α2MRAP present in monocyte suspensions by 5 h,
minor binding to α2MR/LRP. The following experiment sup-
ports this hypothesis. 125I-uPA and 125I-uPA-PAI-1
were prebound to monocytes at 4 °C and washed. α2MRAP
was present during the incubation at 4 °C to prevent any
minor binding to α2MR/LRP. Wash was in the presence of
EDTA since uPA-PAI-1 binding to α2MR/LRP (Fig. 3), but
not to uPAR, is Ca2+-dependent. The cells were then trans-
ferred to 37 °C. Fig. 5 shows that complexing with PAI-1
caused a 3.2-fold increase in degradation of uPAR-bound 125I-
-uPA by 4 h in agreement with the previously described time
courses (21). This inhibitor-enhanced degradation was essen-
tially blocked by α2MRAP (Fig. 5).

**FIG. 3.** Inhibition of 125I-uPA-PAI-1 binding to immobilized
two-chain α2MR/LRP. The incubations were with 20 pm tracer at
4 °C for 16 h. The unlabeled ligands were present at a concentra-
tion of 100 nM, EDTA at 10 mM, and heparin at 100 units/ml. The filled
columns show the effects of the monoclonal antibodies (100 μg of
IgG/ml): anti-PAI-1, clone 7; anti-PAI-1, clone 3; and anti-uPA,
clone 2, directed against the serine proteinase domain.

**Table 1**

| Additions (100 nM) or acid treatment | Monocyte-bound tracer |
|-------------------------------------|-----------------------|
|                                     | 125I-uPA-PAI-1 | 125I-α2MRAP |
| None                               | 11.1 ± 0.4 | 10.3 ± 0.6 |
| ATF                                | 0.3 ± 0.2 | 9.8 ± 0.3 |
| α2MRAP                             | 10.7 ± 0.2 | 0.3 ± 0.2 |
| pH 3.0                             | 1.7 ± 0.0 | 2.2 ± 0.3 |

**FIG. 4.** Binding and degradation of 125I-labeled ligands in
monocyte suspensions at 37 °C. Monocytes (2 × 10⁷/ml) were
incubated for 5 h with 20 pm labeled ligand as indicated. The
unlabeled ligands were present at a concentration of 100 nM. The
ordinate shows the percentage of the radioactivity added to the
suspension. Upper panel, the bars show cell-associated (bound) tracer. The
inset shows the presence of α2MR/LRP and uPAR in Triton X-
114 detergent phase extract of 7 days cultured monocytes; lane 1,
ligand blot showing binding of 125I-α2MRAP; lane 2, binding of 125I-
ATF. Lower panel, the bars show the percentage of degraded tracers in
the monocyte suspension after 5 h. The inhibition by the polyclonal
anti-α2MR/LRP antibody (Ab) was corrected for the effect of non-
imune IgG (8% inhibition). The trichloroacetic acid-precipitable
radioactivity in the medium (not shown) accounted for the part of
the added radioactivity which was not cell-associated or degraded.

**DISCUSSION**

The data show that uPA-PAI-1 complex can bind to the
purified α2MR/LRP, while uPA alone is unable to do so. This

![Image](image_url)
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binding seems to involve the PAI-1 moiety, as judged by the
inhibitions with monoclonal anti-PAI-1 antibody, reactive
center-cleaved PAI-1, and tPA-PAI-1 complex, but does not
involve the NH2-terminal receptor-binding domain of uPA.
It is remarkable that αM-methyleneimine did not inhibit the
binding of uPA-PAI-1 to the purified receptor. Previous experi-
ments have shown that receptor-active αM is a poor
inhibitor of αM-PAI/LRP (9). In fibroblasts, αM inhibits binding of apoE-activated β-VLDL,
but only at high concentration, suggesting that competition
might result from steric hindrance (35). On the other hand,
proteinase-related complexes constitute only one out
of several ligand families transported over the plasma
membrane by αM-PAI/LRP-mediated endocytosis. The present
names of this receptor do not reflect its function as a clearance
receptor for multiple ligands. We propose the name Charon
Receptor (CharR) after the ferryman who, according to Greek
mythology, sailed the souls across the river Styx to Hades.

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