In Vivo Paracrine Interaction between Urokinase and Its Receptor: Effect on Tumor Cell Invasion

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Abstract. Numerous studies have linked the production of increased levels of urokinase type plasminogen activator (uPA) with the malignant phenotype. It has also been shown that a specific cell surface receptor can bind uPA through a domain distinct and distant from the proteolytic domain. In an in vivo model of invasion, consisting of experimentally modified chorioallantoic membrane (CAM) of a chick embryo, only cells that concurrently expressed both uPA and a receptor for uPA, and in which the receptor was saturated with uPA, were efficient in invasion. To test whether uPA produced by one cell can, in a paracrine fashion, affect the invasive capacity of a receptor-expressing cell, we transfected LB6 mouse cells with human uPA (LB6[uPA]), or human uPA-receptor cDNA (LB6[uPAR]). LB6(uPA) cells released into the medium 1-2 Ploug units of human uPA per 10⁶ cells in 24 h. The LB6(uPAR) cells expressed on their surface ~12,000 high affinity (Kd 1.7 × 10⁻¹⁰ M) uPA binding sites per cell. Unlabeled LB6(uPA) and ¹²⁵I-UdR-labeled LB6(uPAR) cells were coinoculated onto experimentally wounded and resealed CAMS and their invasion was compared to that of homologous mixtures of labeled and unlabeled LB6(uPAR) or LB6(uPA) cells. Concurrent presence of both cell types in the CAMS resulted in a 1.8-fold increase of invasion of the uPA-receptor expressing cells. A fourfold stimulation of invasion was observed when cells were cocultured in vitro, prior to in vivo inoculation. Enhancement of invasion was prevented in both sets of experiments by treatment with specific antihuman uPA antibodies, indicating that uPA was the main mediator of the invasion-enhancing, paracrine effect on the receptor-expressing cells.

Numerous studies have shown that proteolytic enzymes produced by tumor cells participate in the degradation of components of the extracellular matrix and basement membrane, thus, facilitating the invasion of surrounding tissues (Dano et al., 1985; Jones and DeClerc, 1980; Mignatti et al., 1986; Mullins and Rohrlich, 1983; Liotta and Stettler-Stevens, 1989; Sas et al., 1986; Sloan et al., 1990; Yagelet al., 1989). Existing evidence suggests that these enzymes are activated and, most likely, utilized sequentially (He et al., 1989; Mignatti et al., 1986; Paranjpe et al., 1980, Werb et al., 1977).

Urokinase type plasminogen activator, a serine protease, has been linked with the malignant phenotype in experimental and naturally occurring tumors (Axelrod et al., 1989; Duffy and O'Grady 1984; Janicke et al., 1990; Markus, 1988; Mira y Lopez and Ossowski, 1987; Ossowski and Reich, 1983; Ossowski, 1988b; Saksela and Rifkin, 1988, Testa and Quigley, 1990; Unkeless et al., 1973; Yu and Schultz, 1990). It is produced and secreted by most cells in the form of a proenzyme (Nielsen et al., 1982; Wun et al., 1982) which is activated by a single proteolytic cleavage. In some tumors, urokinase type plasminogen activator (uPA) is constitutively produced at very high levels, while in others the enzyme level can be substantially increased by growth factors and hormones (Dano et al., 1985). The natural substrate of uPA, plasminogen, is a zymogen present in high concentration in plasma and other body fluids. uPA converts plasminogen into an active serine protease, plasmin (Reich, 1978), which is known to degrade directly several glycoprotein components of the basement membrane and extracellular matrix and to contribute indirectly to the degradation of collagen (He et al., 1989; Mignatti et al., 1986; Paranjpe et al., 1980; Werb et al., 1977).

Although the localization of proteolytic enzymes on the cell surface was suspected for many years (Chapman et al., 1982; Quigley, 1976), uPA was the first enzyme for which a cell surface receptor had been identified (Stoppelli et al., 1985; Vassalli et al., 1985) and cloned (Roldan et al., 1990). We have shown that receptor-bound uPA retains its proteolytic activity and that, under most conditions, the binding of the enzyme does not induce rapid down-regulation of the receptor–uPA complex (Cubellis et al., 1986; Ossowski, 1988).
These properties permit the cell to concentrate a proteolytic activity on its surface. Since invasion requires that extracellular matrix be degraded in the immediate vicinity of an advancing cell, we argued that cells equipped with the uPA receptor, should be more effective in invasion, of an advancing cell, we argued that cellsequipped with that extracellular matrix be degraded in the immediate vicinity. Since invasion requires that artificial air sacs were created to 18 to 24 h before inoculation (wounded and reseeded CAMs, see Ossowski, 1988a). Invasion was quantitated after 24 h of incubation. CAMs were washed with PBS, excised, and both the washes and the CAMs were counted in the gamma counter. The sum of these determinations provided the total number of recovered radioactive cells used for calculation of invasion. The CAMs were then treated with 0.05% trypsin in 1 mM EDTA and again counted in a gamma counter. This method allowed us to distinguish cell types attached to the surface of CAMs, from those that invaded the CAMs and were thus protected from the proteolytic action of trypsin (Ossowski, 1988a). Invasion was expressed as percent of total recovered cells.

Materials and Methods

Materials

COFAL-negative embryonated eggs were obtained from Spafas, Inc. (Norwich, CT); DME and Geneticin from Gibco Laboratories (Grand Island, NY); FBS from Hazleton Research Products Inc. (Lenexa, KS); BSA from Armour Pharmaceutical Co. (Kankakee, IL); [125I]-labeled DNA (specific activity 2,200 Ci/mmol) from New England Nuclear (Boston, MA); Enzymobeads from BioRad Laboratories, Inc. (Richmond, CA); Trypsin from ICN Bio- medicals, Inc. (Costa Mesa, CA); Carnation powdered milk (casein) from Carnation Company, (Los Angeles, CA); Dowex-1, chloride form, Triton X-100, and human urokinase standards from Sigma Chemical Co. (St. Louis, MO); and chromogenic substrate for plasmin (Spectrozyme PL) from American Diagnostica Inc. (Greenwich, CT). Recombinant, single chain human uPA was a generous gift from Abbott Laboratories (North Chicago, IL). Human plasminogen was purified from fresh frozen plasma as previously described (Deutsch and Metz, 1970); anti-human uPA IgG (polyclonal and monoclonal) were as previously described (Ossowski et al., 1991). Mouse uPA was obtained from cultures of MSV-transformed 3T3 cells.

Cell Lines

Mouse LB6 cells transfected with human uPA receptor cDNA under the control of the SV40 promoter have been described and designated clone 19 (Roldan et al., 1990). LB6 cells expressing human uPA cDNA (LB6 clone F) were prepared according to a previously described procedure (Nolli et al., 1989). Both cell types were cotransfected with the pSV-neo plasmid DNA. The cells were treated with 200 µg/ml of Geneticin for 5 days every 4 to 5 wk. For clarity, throughout the manuscript we used the designation of LB6 (uPA) for the uPA receptor-transfected cells and LB6 (uPAR) for uPA receptor-transfected cells.

Methods

Quantitation of Tumor Cell Invasion of Chorioallantoic Membrane Mesenchyme. The methods used were essentially as described previously (Ossowski, 1988a). Briefly, LB6 (uPA) or LB6 (uPAR) cells were labeled in culture for 20 h with 0.2 µCi/ml of [125I]-labeled DNA to a specific activity of 0.05 to 1.0 cpm/cell. Cells were washed extensively with DME with 5% FBS to remove free label, resuspended at 3-4 × 10^8 cells per 50 µl of PBS and inoculated on chorioallantoic membranes (CAMs) of embryos in which artificial air sacs were created 18 to 24 h before inoculation (wounded and reseeded CAMs, see Ossowski, 1988a). Invasion was quantitated after 24 h of incubation. CAMs were washed with PBS, excised, and both the washes and the CAMs were counted in the gamma counter. The sum of these determinations provided the total number of recovered radioactive cells used for calculation of invasion. The CAMs were then treated with 0.05% trypsin in 1 mM EDTA and again counted in a gamma counter. This method allowed us to distinguish cell types attached to the surface of CAMs, from those that invaded the CAMs and were thus protected from the proteolytic action of trypsin (Ossowski, 1988a). Invasion was expressed as percent of total recovered cells.

Plasminogen Activator Determination. We used the chromogenic substrate for plasmin (Spectrozyme PL) to assay PA activity (Mirya Lopez and Ossowski, 1987; Verheijen et al., 1982). Briefly, 10 µl of sample to be tested was mixed with 105 µl of Tris buffer (0.1 M, pH 8.1, with 0.1% Triton X-100) and 10 µl of 0.2 mg/ml of purified human plasminogen in a 96-well, flat bottom tray. The mixtures were incubated for 2 h at 37°C, 15 µl of 1 mg/ml of the chromogenic substrate was added and the generation of color was monitored in a multivariate reader (Dynatech Laboratories, Inc., Chantilly, VA) at 495 nm. A standard curve of human uPA (0.5-10 µM) was included with every assay.

Identification of the Origin of Plasminogen Activator. Two methods were used to determine the origin of uPA produced by mouse cells transfected with the human uPA cDNA. Lysates and conditioned media of these cells (10 µl) were subjected to electrophoresis on SDS-PAGE and simultaneous activation of uPA-producing cells was performed using a zymographic method as described (Granelli-Piperno and Reich, 1978), except the casein from powdered milk, and not fibrinogen, was used in the indicator gels. Bands of lysis produced by the tested cells were compared to those produced by known, standard preparations of uPA. Species origin of uPA was also examined by mixing cell lysates and conditioned media (>1 U of uPA/ml) with 50 µg/ml of purified monoclonal antihuman uPA antibodies, and measuring the residual uPA activity of these mixtures in a chromogenic substrate assay. While the activity produced by the LB6 (uPA) cells was completely inhibited in this assay, a fourfold greater concentration of this antibody did not inhibit a standard preparation of mouse uPA.

Receptor Binding Assay. Recombinant, single chain human uPA (5 µg) was iodinated to specific activity of 4 × 10^6 cpm/µg protein, using Enzymobeads and procedures recommended by the manufacturer. Unbound iodine was removed by passage through a 1-ml washed Dowex-1 column. Cells to be tested were plated in 24-well trays at 1 × 10^5 per well, incubated overnight, washed with DME with 20 mM Hepes and 1 mg/ml of BSA and incubated with dilutions of the radioactive uPA for 45 min at 4°C with, and without, 50-fold excess of unlabeled uPA. After three washes with DME-Hepes-BSA the cells were extracted with 1 N NaOH and the extracts counted in the gamma counter. To determine receptor-bound activity, cells were washed with DME-Hepes-BSA and then with 0.5 ml of Glycine/HCl, pH 3.0, for 3 min (Cubells et al., 1986). The washes were neutralized and tested for uPA.

Paracrine Saturation of Receptors in Mixtures of LB6 (uPA) and LB6 (uPAR) Cells. We plated LB6 (uPA) cells (1 × 10^5) at the bottom of a 60-mm petri dish and the LB6 (uPAR) cells (8 × 10^5) on the inner surface of the Cooper dish cover. (The cover of the Cooper dish is indented so that its distance from the bottom of the dish is only 1 mm.) The bottoms and tops were incubated separately for 20 h. All cultures were then washed with
### Table I. Expression of Human uPA and uPA-receptors in Mouse Cells Transfected with Human cDNAs

| Cell type   | Secreted uPA mU/10^6 cells | Cell associated uPA mU/10^6 cells | uPA-receptor |
|-------------|----------------------------|----------------------------------|--------------|
|             | Mean (SD)                  | Mean (SD)                        | Number/cell | K_d   |
| LB6 (uPA)   | 1,726 (± 476)              | 1,047 (± 326)                    | 0           | N.A.  |
| LB6 (uPAR)  | Below detection            | Below detection                  | 11,700 (± 3,200) | 1.7 (± 0.6) × 10^{-10} M |

Cells transfected with the human uPA-receptor cDNA (clone 19–LB6[uPAR]) or with the human uPA cDNA (clone F–LB6[uPA]), were plated in medium with 10% FBS, grown to semiconfluency, washed once to remove serum, and incubated for 20 h in serum-free medium. Conditioned media and cell lysates were assayed for human and mouse uPA production. (100% of the secreted uPA and 99% of the cell associated activity was inhibited by IgG against human uPA—see Materials and Methods). The binding of uPA to its receptor was determined using radioactive ligand; the number of receptor sites and K_d were calculated from Scatchard plots (see Materials and Methods). The values given are the mean of three experiments. N.A., not applicable. The detection limit of the chromogenic assay is 1 Ploug mU/mL.

### Results

#### Characterization of the Transfected Cell Lines

LB6 cells transfected with human uPA cDNA expressed large amounts of human uPA of which ~60% was found in the medium and the rest was cell associated (Table I). The cell-associated activity was judged to be intracellular since it could neither be eluted by low pH treatment (Cubellis et al., 1986), nor degraded by prolonged exposure to trypsin (results not shown). The released and cell-associated uPAs were identified as being of human origin since their activity was completely blocked by specific monoclonal anti-human uPA IgG (results not shown). Moreover, zymographic analysis (Granelli-Piperno and Reich, 1978) showed that zones of lysis produced by the LB6 (uPA) cells corresponded to those produced by high molecular weight standard human uPA (Fig. 1). The rate of migration of cell-associated uPA was slightly, but consistently, faster than that of the standard. At present, we do not have an explanation for this observation.

The analysis of uPA binding to the receptor-transfected cells revealed the presence of ~12,000 high affinity (K_d of 1.7 (0.6) × 10^{-10} M) specific human uPA binding sites per cell (Table I). The binding was found to be saturable and was completely competed by a 50 M excess of unlabeled human uPA (results not shown).

#### Kinetics of Paracrine Saturation of uPA Receptors in Co-cultures

To determine the length of time required for paracrine loading of uPA receptors, LB6 (uPA) and LB6 (uPAR) cells were cocultivated in Cooper dishes for 3, 7, and 20 h. uPA activity was measured in aliquots of culture media (released activity) and in fractions obtained by brief incubation of each type of cells in low pH (receptor-bound activity—see Materials and Methods). The concentration of free uPA rose with essentially linear kinetics, reaching an estimated concentration of 7 × 10^{-11} M at 20 h. Receptor-bound uPA also rose in a time-dependent manner, reaching at 20 h 3.3 times the level observed at 3 h. Based on the level of released uPA and the

![Figure 1. Analysis of plasminogen activator production by LB6-(uPA) and LB6(uPAR) cells. 10 µl of conditioned media or cell lysates of the two cell types were electrophoresed and assayed by zymography (Granelli-Piperno and Reich, 1978). For preparation of conditioned medium and cell lysates see legend to Table 1. (lanes 1 and 2) LB6(uPAR); (lanes 3 and 4) LB6(uPA); (STD) standards; (lanes 1 and 3) conditioned media; (lanes 2 and 4) cell lysates. (h) Human uPA (55 and 37 kD); (m) mouse uPA (48 kD).](image-url)

### Table II. Kinetics of uPA-receptor Filling in Co-cultures of uPA and Receptor Expressing Cells

| Time of co-culture | Cell type | uPA activity per dish | Medium | Receptor-bound |
|-------------------|-----------|-----------------------|--------|---------------|
|                   |           |                       | mU     | M × 10^{-11}  | mU |
| 3                 | LB6(uPA)  | 324                   | 1.4    | 0             |
|                   | LB6(uPAR) |                       | 90     |               |
| 7                 | LB6(uPA)  | 590                   | 2.6    | 0             |
|                   | LB6(uPAR) |                       | 180    |               |
| 20                | LB6(uPA)  | 1,533                 | 7.0    | 0             |
|                   | LB6(uPAR) |                       | 301    |               |

The uPA-producing LB6 (uPA) and the uPA-receptor-expressing LB6 (uPAR) cells, were plated at 1 × 10^6 or 8 × 10^5 cells, respectively, per Cooper dish (see Materials and Methods) in medium with 10% FBS, incubated for 20 h and, following two washes with serum-free medium, combined so that the two cell types faced each other and were separated only by a 1 mm thick layer of serum-free medium (see Materials and Methods). At the indicated times, conditioned medium uPA, and receptor-bound, acid releasable uPA, were measured (see Materials and Methods). uPA assay duplicates differed by <5%. The experiment was repeated twice with similar results. Specific activity used to convert mU to molar concentration was 100,000 U/mg protein.
The amount of plasmin generated was measured using a chromogenic substrate (see Materials and Methods). The positive control (without antibodies, with plasminogen) generated 1.6 OD₄₉₅ U/h.

![Figure 2. Enhancement of invasion of LB6(uPAR) cells by LB6(uPA) cells; coculture before CAM inoculation.](image)

Receptor expressing cells LB6(uPAR) or human uPA producing cells (LB6-[uPA]) were labeled in culture with 0.2 μCi/ml of [125I]UdR for 24 h. Before inoculation, 1.5 × 10⁶ labeled cells were combined with 3 × 10⁶ unlabeled receptor or uPA expressing cells and inoculated on CAMs in 50 μl of PBS. Invasion of CAMs was measured 24 h postinoculation (for details see Materials and Methods and Ossowski, 1988a). The data shown are a summary of two identical experiments; each group included 12 embryos. Analysis of variance (Systat Statistics) gave a p value of 0.063. The p value for uPA + uPAR vs. uPAR was 0.032, for uPA + uPAR vs. uPA was 0.046, and for uPA vs. uPAR was 0.698.

established Kd (Table I), ~33% of the receptors would be expected to be filled with uPA after 20 h of co-cultivation. The measured level of receptor-bound uPA (Table II) indicated that a somewhat greater actual receptor occupancy may have been achieved.

**Interaction between uPA and uPA Receptor–producing Cells In Vivo**

To test whether the presence in the CAM of uPA secreting cells would affect the efficiency of invasion we used a quantitative in vivo model of tumor invasion (Ossowski, 1988a). In this model 9-d-old embryos with artificially wounded CAMs were incubated until the wounds were sealed (usually 24–30 h) by a layer of fibroblasts resulting in structures partially resistant to tumor cell invasion. The model was adapted to allow a measurable, but low level, invasion by the receptor-transfected cells by reducing the time between wounding and inoculation. LB6 (uPAR) cells were labeled in culture with [125I]UdR, and coinoculated with unlabeled LB6 (uPA) cells on the sealed CAMs. Labeled uPAR or uPA cells, mixed with homologous, unlabeled cells were used as controls. Results in Table III indicate that the coinoculation of uPA-producing cells leads to an enhancement of invasion by the receptor-expressing cells. Qualitatively similar results, showing an enhancement of invasion from 1.5- to 2.1-fold, were observed in eight additional experiments (results not shown).

Since the level of receptor saturation is dependent on the ligand concentration, and since under conditions of continuous uPA production, ligand concentration rises with time (see Table II), we tested whether receptor cells cocultured with uPA-producing cells for 20 h before inoculation on the CAMs would invade more efficiently. In this experiment the receptor cells were labeled with [125I]UdR before coculture. Control mixtures included labeled and unlabeled receptor cells and labeled and unlabeled uPA-producing cells. This approach produced a fourfold (p = 0.006) enhancement of receptor-expressing cell invasion by the uPA-producing cells (Fig. 2).

To test whether the enhancement of invasion was mediated solely via a transfer of uPA from an enzyme producing cell to a receptor expressing cell (paracrine mechanism) we car-

**Table III. Stimulation of Receptor-expressing Cells Invasion by uPA-producing Cells**

| Cells on CAM | Labeled (uPAR) | Unlabeled (uPAR) | Radioactivity (% of total recovered) | Mean (SD) | Median | Range |
|--------------|----------------|-----------------|------------------------------------|----------|--------|-------|
| LB6(uPAR)    | 5.4 (±3.3)     | 4.4 (2.3-12.3)  | -                                  |          |        |       |
| LB6(uPA)     | 5.6 (±3.7)     | 4.4 (1.9-13.9)  | -                                  |          |        |       |
| OB6(uPAR)    | 10.0 (±3.8)    | 11.5 (4.6-13.6) | -                                  |          |        |       |

Table IV. Inhibition of Receptor-bound uPA by Specific Antibodies

| Antibody treatment | Plasminogen in uPA assay | uPA (%) control |
|--------------------|--------------------------|----------------|
| −                  | +                        | 100.0          |
| −                  | +                        | 0.7            |
| +                  | +                        | 1.4            |
| +                  | −                        | 1.7            |

Mixtures (5 × 10⁶ cells each) of receptor-expressing and uPA-producing cells were co-cultured overnight in 24-well trays in DME with 10% FBS, detached, washed extensively, and incubated in suspension (1 × 10⁶ cells/ml) with 50 μg/ml of anti-human-uPA IgG for 1 h at 37°C. The cells were washed three times and incubated for 30 min at 37°C with 100 μg/ml of human plasminogen.

**Table V. The Effect of Anti-uPA Antibodies on Invasion**

| Experiment | Mean (SD) | P    |
|------------|-----------|------|
| 1          | 69.8 (±26.0) | 0.013 |
| 2          | 85.8 (±12.0)  | 0.008 |

LB6(uPAR) cells were labeled in culture with [125I]UdR for 20 h, detached, mixed with an equal number of LB6(uPA) cells, and further incubated in 60-mm dishes for 24 h. The following preimmune or anti-human uPA IgGs were used: in experiment 1, in which 18 embryos were used, 300 μg of monoclonals and 1 mg of rabbit polyclonals added to cells immediately before inoculation and also added to CAMs 1 h before inoculation; in experiment 2, in which 16 embryos were used, mAbs (50 μg/ml) were added to cells for the 20 h of in vitro coculture and also added to the inoculum. Invasion of cells treated with similar concentrations of normal IgG (mouse or rabbit) was considered 100% and used to calculate percent inhibition. The significance of the difference between the control and antibody treated groups (p values) was tested using the t test.
ried out the invasion assays in the presence of specific inhibitory antihuman uPA antibodies. This antibody was capable of inhibiting receptor-bound uPA activity (see Table IV), indicating that the epitope it recognized was not hindered by binding to the receptor. Inclusion of the inhibitory antibodies in both experimental designs (Table III and Fig. 2) resulted in a significant inhibition of invasion (Table V).

Discussion

Using a functional complementation analysis we showed that uPA produced by one cell can, by a paracrine in vivo interaction, modulate the invasive potential of a uPA-receptor-expressing tumor cell. This result is of particular interest for two reasons. First, it confirmed our previous observations which implicated uPA receptor in the enhancement of uPA-dependent invasion of CAMs (Ossowski, 1988a). Second, to the best of our knowledge, it represents the few documented cases in which a known mediator affected the malignant potential of tumor cells in vivo via a paracrine interaction.

Although the mechanism of enhancement of invasion by receptor-bound uPA is not yet understood, it may function by focusing the proteolytic activity on the cell surface. This notion is supported by the findings that plasmin generation on the surface of cells is more effective than in solution (Plow et al., 1986). One possible explanation for this effect may be found in the observation (Quax et al., 1991) that receptor-bound pro-uPA (the form commonly released by most cells) may be more susceptible to activation by plasmin. Other mechanisms responsible for the enhanced proteolysis by the receptor-bound uPA are currently being investigated.

The analysis of paracrine interactions between tumor cells and their role in the enhancement of malignancy was made possible by the availability of the molecular clones of uPA and its receptor and an in vivo model of invasion, which could be adapted to accommodate cells with greatly differing invasive potentials (Ossowski, 1988a). We have previously shown that under the most stringent conditions of the CAM model only highly malignant cells yielded measurable invasion (Ossowski, 1988a). These cells were found to produce, in addition to uPA, at least one other proteolytic enzyme belonging to the metalloproteinase family (Ossowski, L., manuscript in preparation). However, it was possible, by reducing the stringency of resistance of the CAM to invasion, to assess quantitatively even cells with marginal invasive potential such as the LB6 (uPAR) cells.

We found that inoculated individually, the transfected cells, even under conditions of low stringency, produced very low levels of CAM invasion (Table III). Coinoculation of the two cell types enhanced the invasion of the receptor-expressing cells 1.8-fold. The relatively small enhancement was not unexpected since, although the LB6 (uPAR) bind uPA with high affinity (Kd 1.7 × 10^-10 M; Table I), the number of their plasma membrane binding sites (11,700) is lower (Table I) than that reported for highly malignant tumor cells ranging from 40,000 to 100,000 (Ossowski, 1988a; Schlechte et al., 1989). Also, Schlechte et al. (1989) have shown that the ability to invade in an in vitro model was correlated with the number of surface uPA receptors. Moreover, it should be kept in mind that coinoculation of the two cell types on the CAM, in which relatively small numbers of cells in suspension (3-4 × 10^5) encounter relatively large areas of tissue, may not accurately represent the topography that exists in a primary tumor. This may reduce the effective concentration of uPA in the vicinity of receptor-expressing cells and thus reduce the level of receptor saturation by the enzyme. In spite of this relative inefficiency of the system, the results clearly show that uPA produced by one cell can be utilized, in a paracrine fashion, to potentiate the invasive ability of another cell. The fact that in experiments in which the two cell types were cocultured before inoculation the potentiation of invasion was much greater (Fig. 2), gives additional support to the notion that during the brief period (24 h) in the CAM, cell interactions may be less than optimal and that in a naturally developing tumor the paracrine effects may be of much greater magnitude.

We used inhibitory anti–human uPA IgGs to positively identify uPA as the mediator of the observed enhancing effect on invasion. This treatment, which specifically interrupted the paracrine interaction, greatly reduced the potentiation by LB6 (uPA) cells (Table V), implicating uPA as the main mediator of the effect. The magnitude of the inhibition indicated that no other paracrine signals, important for invasion of connective tissue, were exchanged between the two cell types.

Overall, using a functional complementation analysis of genetically engineered cells, we proved that specific paracrine interactions, involving uPA and its receptor, lead to increased tumor invasion in vivo. The significance of this finding is underscored by the recent observation showing that, in colon carcinomas in man, uPA and its receptor are produced by distinct populations of cells (Pyke et al., 1991), suggesting that interactions of the kind described here may be operative and necessary for the expression of the full malignant potential of tumors.

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