Urokinase-type plasminogen activator enhances invasion of human T cells (Jurkat) into a fibrin matrix

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Abstract: The receptor for urokinase-type plasminogen activator (uPA-R) localizes uPA to the cell surface. The receptor-bound uPA converts plasminogen to the trypsin-like endopeptidase plasmin. Thus uPA is involved in the initiation of pericellular proteolysis. Pericellular proteolysis is assumed to facilitate the cellular infiltration into surrounding tissue. The uPA-R has recently been identified as a surface antigen of activated human T lymphocytes. We have characterized the uPA-R of the human CD4⁺ T cell line Jurkat by immunological (flow cytometry), biochemical (ligand blotting), and physico-chemical (Scatchard blotting) methods. The collective data suggest that the human CD4⁺ T cell line Jurkat expresses a cell surface receptor for uPA similar to that of myelo/monocytes and normal T cells with regard to size, affinity, ligand specificity, and antigenicity. Binding studies using exogenous uPA and subsequent functional assays revealed that receptor-bound uPA retains its enzymatic activity. Saturation of the Jurkat cell uPA-R with exogenous uPA facilitated cellular invasion into fibrin matrices in vitro. uPA-dependent invasion was inhibited in the presence of an anti-catalytic monoclonal anti-uPA antibody. We propose that uPA-R-bound uPA may facilitate the invasiveness of uPA-R-positive T lymphocytes. *J. Leukoc. Biol.* 56: 110–116; 1994.

Key Words: *pericellular proteolysis · tumor cell infiltration · T cell activation · inflammation*

INTRODUCTION

Urokinase-type plasminogen activator (uPA) is one of the two serine proteinases involved in extracellular proteolysis by converting plasminogen into plasmin [1–3]. A cell surface receptor (uPA-R) with high affinity ($K_d \approx$ 0.1–1 nM) for uPA was isolated previously from the myelo/monocytic cell line U 937 [4, 5]. The uPA-R is a cysteine-rich, highly glycosylated protein with a molecular mass ranging between 55 and 60 kDa. It is attached to the plasma membrane by a glycosylphosphatidyl inositol (GPI) anchor [6]. pro-uPA as well as functionally active high molecular weight (HMW) uPA bind to the uPA-R via their growth factor domain [7].

The specific binding of uPA to the cell surface may provide cells with the mechanism to focus plasmin formation in the pericellular space [8, 9]. Anti-uPA antibodies prevent invasiveness of human melanoma cells in vivo [10, 11] and in vitro [12, 13]. Moreover, uPA-R expression is oriented to the leading edge of monocytes infiltrating into protein gels in vitro [14]. The findings indicate that uPA-R expression and uPA-mediated plasmin formation may favor the invasive behavior of migrating cells via the breakdown of pericellular matrix barriers. One major component of the matrix environment in inflamed tissue is fibrin [15], which is a favorable substrate for plasmin [16].

The uPA-R is expressed by activated human T cells [17]; however, its functional role in T cells remained elusive. Here we report that a human CD4⁺ T lymphocyte line (Jurkat) binds enzymatically active uPA via the uPA-R, and that this process facilitates invasion of the cells into a fibrin matrix in vitro.

MATERIALS AND METHODS

Reagents and cells

HMW-uPA, isolated from human urine, was from Serono (Ukidan; Freiburg, Germany). Recombinant human pro-uPA was kindly provided by the Grünenthal GmbH (Aachen, Germany). The anticatalytic mAb against uPA (HD-UK 1) [18] and the uPA-R mAb [19, 20] have recently been described. Normal murine IgG was purchased from Dianova (Hamburg). The Jurkat cell line was kindly provided by Dr. D. Kabelitz (Paul-Ehrlich Institut, Langen, Germany). The U937 cell line, as a positive control for uPA-R expression [4, 6], was kindly provided by Dr. G.M. Hänsch, Heidelberg. By flow cytometry, the Jurkat line was found to have the same phenotype (CD2⁺, CD4⁺, CD8⁻, CD25(IL-2R)⁻) as described for the original cell line [21, 22].

Flow cytofluorometry

Cells were washed in glycine/HCl buffer (0.1 M, pH 3; 1 min, rt), immediately neutralized in 0.5 M hepes, 0.1 M NaCl, pH 7.5 and suspended in phosphate-buffered saline (PBS, pH 7.2) containing BSA (1% w/v) and sodium azide (0.05% w/v) (PBS/BSA/NaN_3), and washed twice in the same buffer. For staining of the uPA-R, Jurkat cells were either incubated with FITC-labeled pro-uPA (40 μg/ml) or mAb 3B10 (a 1:100 dilution of ascites fluid; [19, 20]) in PBS/BSA/NaN_3 for 1 h on ice. After extensive washing, the FITC-pro-uPA-labeled cells were analyzed by flow cytometry. The antibody-stained cells were incubated with 2 μg/ml of phycoerythrin (PE) -labeled species-specific goat anti-

Abbreviations: DSS, disuccinimidyl suberate; GPI, glycosylphosphatidyl inositol; uPA, urokinase-type plasminogen activator; HMW-uPA, high molecular weight uPA; PBS, phosphate-buffered saline; PE, phycoerythrin; PI-PLC, phosphatidyl-inositol phospholipase C; pro-uPA, enzymatically inactive proenzyme of uPA.

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mouse IgG (Fab')$_2$ (Dianova, Hamburg) in PBS/BSA/NaN_3 buffer (30 min, on ice). After extensive washing, the cells were resuspended and fixed in PBS containing 1% paraformaldehyde and then analyzed by flow cytometry (FACScan, Becton Dickinson, Heidelberg).

Treatment of cells with glycine/HCl buffer was originally included into the protocol to remove endogenous ligand from the uPA-R of uPA-producing cells, e.g., the uPA-R-expressing U 937 cell line, which is included as a positive control in our staining reactions for the uPA-R. The Jurkat cells do not contain uPA, as indicated by the following findings (data not shown): *1)* Jurkat cells do not express uPA-specific mRNA, as shown by sensitive PCR analyses, *2)* lysates of the Jurkat cells do not contain measurable uPA activity, *3)* we cannot demonstrate surface-bound uPA by flow cytometry, even if Jurkat cells that have not been pretreated with glycine/HCl buffer are analyzed, *4)* neither in Scatchard-type analyses nor in FITC-uPA binding do we observe a difference in uPA binding when glycine/HCl-treated and mock-treated Jurkat cells are compared. However, to assure comparability of the staining results between the Jurkat cells and the positive control (i.e., the uPA-producing U937 cell line), we have also included glycine/HCl treatment in the protocol for staining of the Jurkat cells (compare **Fig. 1**).

Detergent phase separation of Jurkat cell membranes and ligand blotting

Membrane proteins were prepared by detergent phase separation as described previously [23]. In brief, the Jurkat cells were incubated in a glycine/HCl buffer (0.1 M, pH 3; 1 min, rt), then neutralized with hepes/NaOH buffer (0.5 M Hepes, 100 mM NaCl, pH 7.4), immediately suspended in PBS, and washed twice in PBS. Cells (2×10^8) were lysed in 4 ml of 0.1 M Tris/HCl, 1% Triton X-114 (v/v), 10 mM EDTA, 1 mM PMSF, 10 μg/ml aprotinin for 4 min on ice. The lysate was clarified by centrifugation (16,000 $\times$ *g*; 4°C). The clear supernatant was then incubated for 10 min at 37°C and centrifuged again at 1800 $\times$ *g* at 25°C. The lower phase was collected and washed by addition of ice-cold 0.1 M Tris/HCl, pH 8.0. Temperature-induced phase separation was repeated and the lower phase was mixed with 2 ml 0.1 M Tris/HCl containing 0.5% CHAPS (v/v) pH 8.1 to avoid further temperature-induced phase separation. 20 μl of the solubilized membrane proteins was separated electrophoretically by using a 10% SDS polyacrylamide gel under nonreducing conditions and then blotted onto an Immobilon-P membrane. After blocking (50 mM Tris/HCl, 150 mM NaCl, 2 mM $CaCl_2$, 3% milk powder, 0.05% Tween 20 (v/v), pH 8.1 for 2 h at 25°C), the membrane was incubated with [^{125}I]pro-uPA (50 pM) with or without an excess of unlabeled pro-uPA (100 nM) in blocking buffer overnight at 4°C. After washing extensively in blocking buffer, the membrane was analyzed by autoradiography.

[^{125}I]pro-uPA binding to Jurkat cells and release of receptor-bound [^{125}I]pro-uPA by phospholipase C treatment

Radiolabeled human recombinant pro-uPA was prepared by using the chloramine T method [Immundiagnostik GmbH, Bensheim, Germany]. The concentration-dependent binding of [^{125}I]pro-uPA was tested as follows: the cells were washed with glycine/HCl buffer (0.1 M, pH 3; 1 min, 25°C) and were then neutralized with hepes/NaOH buffer (0.5 M Hepes, 100 mM NaCl, pH 7.4) and immediately suspended in binding buffer (124 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 2.5 mM NaH_2PO_4, 25 mM Hepes, 1% BSA, 5 μg/ml aprotinin, pH 7.4). After washing twice in binding buffer, the cells (6×10^6/600 μl) were incubated with increasing concentrations of [^{125}I]pro-uPA with or without a 200-fold excess of pro-uPA for 16 h at 4°C. Bound [^{125}I]pro-uPA was separated from unbound ligand by the addition of 200 μl of the cell suspension to microfuge tubes containing 200 μl silicone oil (having a density of 1.025), followed by centrifugation. The bottom of the tube containing the pelleted cells freed from unbound ligand was cut and measured for radioactivity in a gamma counter [17].

The glycosyl phosphatidyl-inositol (GPI) -anchored uPA-R of the Jurkat cells was solubilized by treatment of the cells with a bacterial phosphatidyl-inositol phospholipase C (PI-PLC) as previously described [6]. In brief, the cells were treated with glycine/HCl buffer (see above). After washing with incubation buffer (RPMI 1640, 2% Ultroser, 25 mM

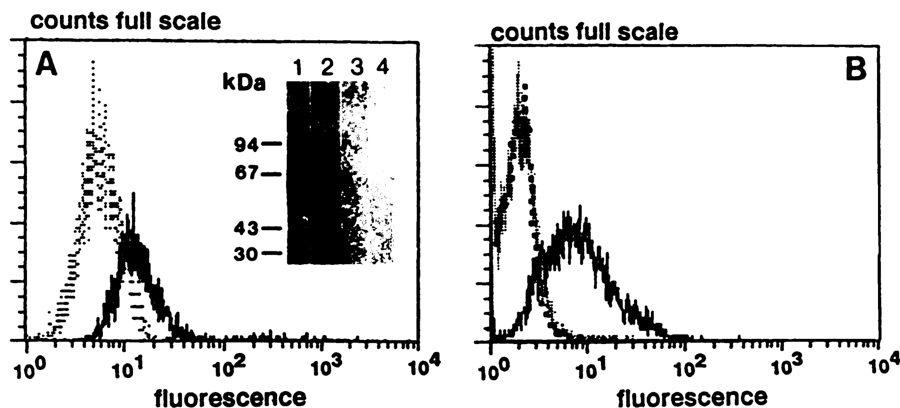

Fig. 1. The human T cell line Jurkat expresses a cell surface receptor for (pro-)uPA. (A) FITC-pro-uPA binding to Jurkat cells was analyzed by flow cytofluorometry. Jurkat cells were incubated with FITC-labeled pro-uPA in the absence (solid line) or presence (broken line) of an excess (5×) of unlabeled pro-uPA. FITC-pro-uPA binding, expressed as fluorescence intensity (abscissa), was assessed by flow cytofluorometry; a total of 5000 cells was counted. Inset: [^{125}I]pro-uPA binding to plasma membranes isolated from U 937 cells (lanes 1 and 3) or Jurkat cells (lanes 2 and 4). Plasma membranes were isolated by the detergent phase separation method. Afterward, the membrane proteins were electrophoretically separated by SDS-PAGE and transferred onto an immobilon membrane by electroblotting. After blocking, the membrane was incubated with [^{125}I]pro-uPA (50 pM) in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of an excess of unlabeled pro-uPA (100 nM). After extensive washing of the filters, [^{125}I]pro-uPA binding was visualized by autoradiography. In plasma membrane preparations of U937 and Jurkat cells, a pro-uPA binding protein of ≈ 55 kDa was disclosed. (B) Staining of Jurkat cells with the uPA-R-specific mAb 3B10. Labeled cells were analyzed by flow cytofluorometry. Dotted lines: only second antibody added; broken lines: isotype-matched control antibody (LA-1 [29]) and PE-labeled second antibody added; solid lines: mAb 3B10 and PE-labeled second antibody added.

Hepes/NaOH, pH 7.4), the cells were incubated in the same buffer with [^{125}I]pro-uPA (1 nM) for 2 h at 4°C. After washing, the cells (1 × 10^7) were incubated in incubation buffer with or without PI-PLC (1 U/ml [= 1.6 μg/ml], 1 h 37°C). The cell-free supernatants were collected and the proteins contained within the supernatant were covalently cross-linked by treatment with disuccinimidyl suberate (DSS; 5 mM final concentration; incubation for 15 min at 25°C). Control supernatants were not treated with DSS. The reaction was stopped by the addition of ammonium acetate (100 mM final concentration, 15 min, 25°C). The proteins were precipitated by acetone, and the precipitate was electrophoretically separated by using a 10% SDS polyacrylamide gel under nonreducing conditions followed by autoradiographic analysis.

Saturation of Jurkat cells with HMW-uPA and activity determination of bound uPA

1 × 10^6 cells/ml were washed in PBS, treated with glycine/HCl buffer (0.1 M, pH 3; 1 min, 25°C), and then neutralized with hepes/NaOH buffer (0.5 M Hepes, 100 mM NaCl, pH 7.4). Afterward, the cells were immediately suspended in PBS/1% BSA and washed twice in PBS. Cells were either treated with PI-PLC (1 U/ml in PBS, 30 min, 37°C) or with PBS. The cells were then washed twice in PBS containing 1% (w/v) BSA (PBS/BSA), saturated with HMW-uPA (100 μM in PBS/BSA; 30 min 37°C), washed twice in PBS/BSA, and resuspended in 60 μl of assay buffer (30 mM Tris/HCl, 60 mM NaCl, pH 7.4). Then 20 μl of plasminogen solution in assay buffer was added (final concentration 0.5 U/ml in the presence or absence of control or anticatalytic anti-uPA antibody) to yield a final concentration of 100 μg/ml. Finally, 20 μl of the chromogenic plasmin substrate S-2251 (HD-valyl-leucyl-lysyl-para-nitroanilide) diluted in assay buffer was added to start the color reaction (final substrate concentration: 0.4 mg/ml). The color reaction caused by liberation of free nitroaniline was monitored at 405 nm using a microplate reader.

In vitro invasion of Jurkat cells

The in vitro invasion of the Jurkat cell line was studied in a double-filter assay as described previously [12, 13, 24]. In brief, fibrin gels were located between two filters: a lower nitrocellulose filter and an upper polycarbonate filter. The fibrin matrix used in the current studies was made in PBS containing the following additions: 12.5 mg/ml fibrinogen, 2 mg/ml casein, 10% (v/v) acid-treated fetal calf serum, 1 U/ml thrombin, 10% (v/v) RPMI medium (containing 2 mM glutamine). The method has been described in great detail elsewhere [24]. Cells to be studied for invasion were suspended in RPMI 1640 (supplemented with 10% (v/v) human serum and 2 mM L-glutamine) at a concentration of 5 × 10^5/ml, and placed on top of the double filter arrangement. To reach the protein gel between the upper and lower filter, cells had to migrate through the pores of the upper filter, which were filled with gel [24]. After a 20 h incubation period at 37°C in a humidified atmosphere of 7% CO_2, the whole setup was fixed with glutaraldehyde and the filters were then separated and stained. Polycarbonate filters were stained with Giemsa's solution (Merck, Darmstadt, Germany) and nitrocellulose filters were stained with hematoxylin. The protein gel (containing the cells that have invaded) remains attached to the lower filter allowing separation and quantitation of adherent cells (upper filter) and cells that have invaded (lower filter). Percent of invasion was calculated from the number of cells that have invaded (= cells present

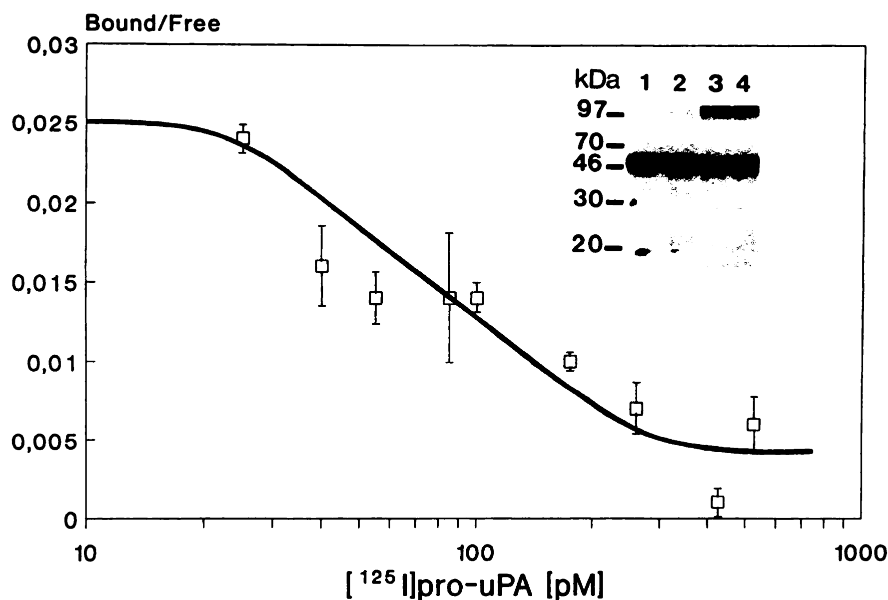

Fig. 2. Concentration dependence of [^{125}I]pro-uPA binding to the uPA-R of Jurkat cells uPA-R and release of pro-uPA/uPA-R complexes by PI-PLC treatment. Jurkat cells were incubated with increasing concentrations of [^{125}I]pro-uPA (abscissa) in the presence of an excess of unlabeled pro-uPA. Cell-bound and free radioactivity were separated by centrifugation as described in Materials and Methods. Data are depicted as bound radioactivity/free radioactivity (ordinate). Inset: Release of receptor-bound pro-uPA by phosphoinositol-phospholipase C (PI-PLC) treatment. The Jurkat cells (lanes 1 and 3) or the U937 cells (lanes 2 and 4) were incubated with [^{125}I]pro-uPA (1 nM) and washed extensively. Afterward the following was analyzed: lanes 1 and 2: [^{125}I]pro-uPA spontaneously released from the cells into the culture supernatant by PI-PLC treatment (1 U/ml, 30 min, 37°C); lanes 3 and 4: [^{125}I]pro-uPA released from the cells into the culture supernatant by PI-PLC treatment (1 U/ml, 30 min, 37°C) followed by DSS-mediated chemical cross-linking of the solubilized proteins. The proteins contained in the samples were precipitated using acetone and separated by SDS-PAGE under nonreducing conditions using a 10% gel. Radioactive labeled proteins were visualized by autoradiography. PI-PLC treatment resulted in the release of [^{125}I]pro-uPA as a [^{125}I]pro-uPA/uPA-R complex, having a molecular weight of ≈ 100 kDa.

at the lower filter) divided by the total number of cells (= cells present in both filters) according to the following formula:

$$\% \text{ invasion} = \frac{\text{no. of cells in lower filter}}{\text{total no. of cells (in both filters)}} \times 100$$

Alternatively, the lower filter supporting the gel with the invaded cells was analyzed by confocal fluorescence microscopy using a Zeiss LSM 3 laser scanning microscope (Carl Zeiss, Oberkochen, Germany). An argon ion laser with a wavelength of 488 nm was used to excite the fluorescence of the eosin in the stained cells. Consecutive horizontal optical sections with appropriate spacings were scanned through the gel and the resulting images were photographed with a focus imagecorder (Focus Graphics, Farchant, Germany).

RESULTS

uPA-R on Jurkat cells

(pro-)uPA binding by Jurkat cells was studied by flow cytofluorometry using FITC-labeled pro-uPA as well as a uPA-R-specific mAb [19, 20] (Fig. 1). Jurkat cells bound FITC-pro-uPA (40 μg/ml) and binding was counteracted by a fivefold excess of unlabeled HMW-uPA (Fig. 1A). To characterize the uPA binding site (or sites) at a cellular level, Jurkat cells or U 937 cells, the latter as a positive control for uPA-R expression, were lysed in Triton X-114 and the membrane proteins were partially purified by temperature-induced detergent phase separation [23]. Enriched membrane proteins were separated by gel electrophoresis, electroblotted onto nitrocellulose, and incubated with [^{125}I]pro-uPA. Autoradiography revealed a ≈ 55-kDa pro-uPA-binding protein in membrane preparations of both cell types (Inset Fig. 1A; lanes 1 and 2). Binding of radiolabeled pro-uPA to the 55-kDa binding protein was prevented by an excess of unlabeled pro-uPA (Inset Fig. 1A; lanes 3 and 4). Surface expression of the uPA-R by Jurkat cells was further proven by staining with the uPA-R-specific mAb "3B10" (Fig. 1B).

Binding experiments with [^{125}I]pro-uPA showed a dose-dependent binding of the radiolabelled ligand to the Jurkat cells (a representative experiment is shown in **Fig. 2**). Scatchard transformation of the data (not shown) revealed an apparent K_d of ≈ 1.2 × 10^{-10} M at 4°C and a density of ≈ 2000 uPA-R per cell: affinity and receptor density thus were in the same range as for nonactivated myelo/monocytes [4, 5, 8, 9] or activated T cells [17].

In all cell lines tested thus far, the uPA-R is attached to the surface membrane by a GPI anchor, and is therefore—at least in part—susceptible to cleavage by a bacterial PI-PLC. To test whether this was also true for the uPA-R of the Jurkat T cell, their uPA-R were saturated with [^{125}I]pro-uPA and the cells were subsequently treated with PI-PLC. Chemical cross-linking of the solubilized material with disuccinimidyl suberate led to the formation of a stable complex (≈ 100 kDa; inset, Fig. 2) consisting of [^{125}I]pro-uPA (≈ 50 kDa) and the putative uPA-R of ≈ 55 kDa.

Flow cytofluorometric analysis of the Jurkat cells with anti-uPA mAb [18] did not reveal uPA at the cell surface (data not shown), indicating that the uPA-R was not saturated to a significant degree with its natural ligand under the conditions used for Jurkat cell culture. Exogenous uPA bound to the uPA-R (compare also Fig. 1A) and the bound enzyme retained its functional activity: activation of exogenous plasminogen was observed when uPA-saturated Jurkat cells were tested (**Fig. 3A**; open rectangles). Plasminogen activation was inhibited in the presence of an anticatalytic anti-uPA mAb (Fig. 3A, closed rectangles), but not by isotype-matched control antibodies (Fig. 3A, open circles) or by normal mouse IgG (not shown). Furthermore, plasminogen activation by uPA-saturated Jurkat cells was reduced by ≈ 50% when the saturated cells were treated with PI-PLC before the plasminogen activator assay (Fig. 3B).

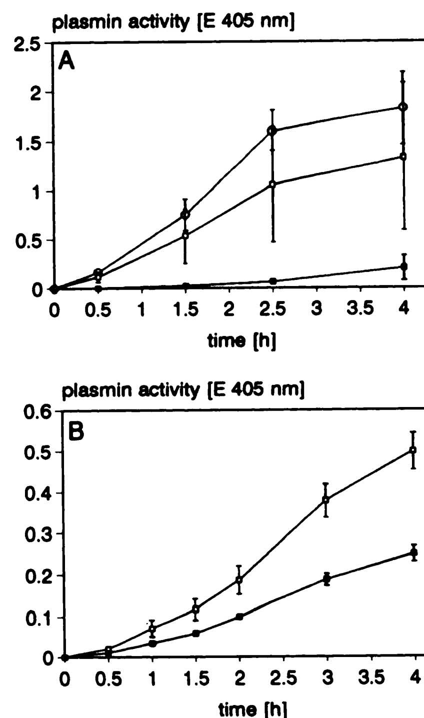

Fig. 3. Activity of cell surface-bound uPA. (A) The Jurkat cells were saturated with HMW-uPA. After extensive washing the uPA activity of the saturated Jurkat cells was assessed in an indirect chromogenic substrate assay using exogenous plasminogen and a plasmin-specific chromogenic peptide substrate. Plasminogen activator activity in saturated cells (open squares) was inhibited in the presence of an anticatalytic anti-uPA antibody (HD-UK 1 [18]) (filled squares) but not in the presence of an isotype-matched control antibody (HD-a2M 2 [30]) (open circles). (B) Jurkat cells were treated with PI-PLC (filled squares) or the PI-PLC incubation buffer only (open squares) and later saturated with exogenous HMW-uPA. The cell-associated uPA activity was lower in the cells pretreated with PI-PLC, indicating partial removal of the uPA binding structure (or structures) by PI-PLC treatment. E_{405} nm = extinction at 405 nm, as determined by an automated ELISA reading apparatus.

In vitro invasiveness of the human T cell line Jurkat

To measure the in vitro invasiveness of the Jurkat cells, we used a double-filter cell invasion assay as described previously [24]. Cellular invasion into fibrin gels was either quantified by microscopical enumeration of infiltrating cells (**Fig. 4**) or analyzed morphologically by confocal laser scanning microscopy (**Fig. 5**). The role of surface-bound uPA during the process of infiltration was analyzed by comparing uPA-saturated and nonsaturated Jurkat cells and by testing the influence of an anticatalytic anti-uPA antibody. Nonsaturated Jurkat cells displayed low invasion (Fig. 4, column A), which was reflected by low cell numbers in all sections of the fibrin gel (Fig. 5A). Jurkat cells saturated with uPA before the invasion assay displayed increased invasion, which was ≈ 10-fold above that of nonsaturated Jurkat cells (Fig. 4, col. B). Increased invasion of saturated cells was also proven by the presence of numerous cells in all levels of the fibrin gel

as revealed by confocal laser scan microscopy (Fig. 5B). Invasion of saturated cells was inhibited in the presence of an anticatalytic anti-uPA antibody (Fig. 4, col. C, and 5C) but not by noninhibitory control antibodies (normal mouse IgG (Fig. 4, col. D) or isotype-matched mAb (not shown)).

DISCUSSION

We have identified a specific uPA-binding protein, termed uPA-R, in the CD4⁺ human T cell line Jurkat. By ligand binding analyses, the uPA-R was found to be indistinguishable from the uPA-R of myelo-/monocytes [5, 6, 19, 20] and activated T cells [17]. Identity was further proven by using the uPA-R-specific monoclonal antibody 3B10 that originally was generated against the uPA-R of human macrophages [19, 20]. uPA bound to the surface-associated uPA-R retains functional activity (Fig. 3) and receptor saturation with active uPA-enhanced Jurkat cell invasion into a fibrin matrix by a factor of ≈ 10 (Fig. 4). The uPA-dependent invasiveness was counteracted by a monoclonal anti-uPA antibody with specific inhibitory activity, but not by control antibodies (Figs. 4, 5).

Our data (Fig. 2; inset; Fig. 3B) indicate that only part of the uPA-R can be removed from the Jurkat cell surface by treatment with PI-PLC. This is reminiscent of previous findings with myelo-/monocytes [6, 20] and does not necessarily indicate the existence of an additional subpopulation of transmembrane-anchored uPA-R molecules or an alternative binding mechanism for surface-associated uPA. The incomplete release of uPA-R observed after PI-PLC treatment may merely reflect a partial acylation of the 2-hydroxyl group of the inositol residue in the glycolipid anchor of the uPA-R, a modification found in some GPI-anchored proteins that hampers the catalytic mechanism of PI-PLC [6]. It remains open whether the part of the cell-bound uPA that is not releasable by PI-PLC treatment may be bound by alternative mechanisms, which could encompass binding to plasma membrane gangliosides [25].

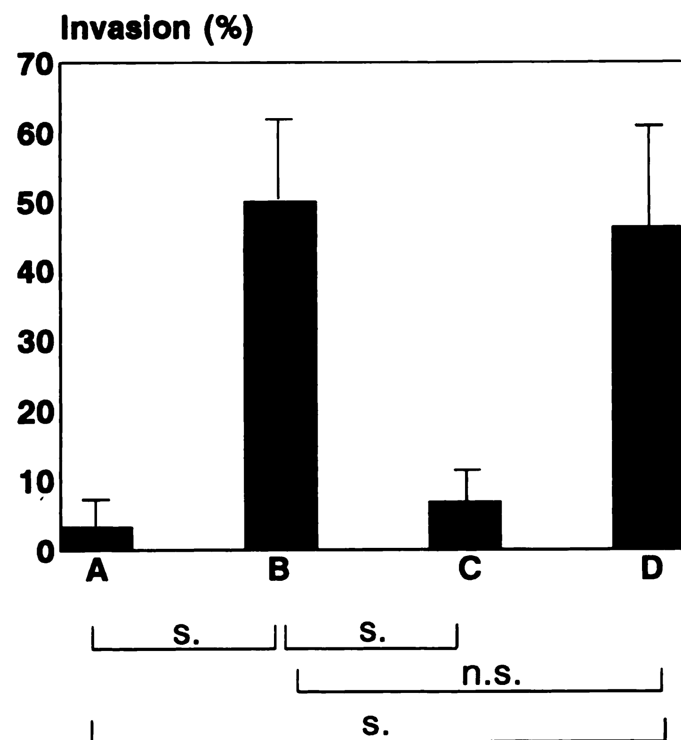

Fig. 4. Quantitative analysis of Jurkat cell invasion into fibrin gels. Jurkat cell invasion was studied by using a double-filter in vitro invasion assay described previously [24]. The filters were analyzed by light microscopical counting and the data (mean value plus standard deviation obtained from 5 replicate assays) was processed as detailed under Materials and Methods to obtain "% invasion" (ordinate). Column A: Jurkat cells not saturated with HMW-uPA; col. B: Jurkat cells saturated with HMW-uPA. Col. C: Jurkat cells saturated with HMW-uPA and cellular invasion studied in the presence of the anticatalytic monoclonal anti-uPA antibody HD-UK 1 (100 μg/ml); col. D: Jurkat cells saturated with HMW-uPA and invasion studied in the presence of normal murine IgG (100 μg/ml). Statistics were calculated by using the Wilcoxon paired rank sum test. s: difference significant ($P < 0.001$). n.s.: difference not significant.

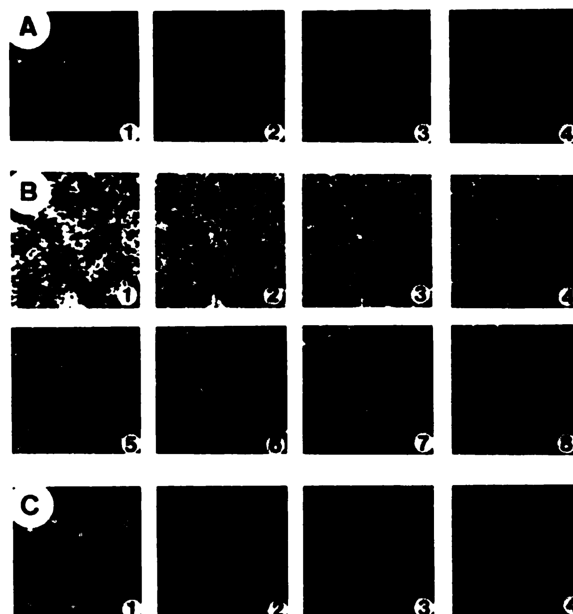

Fig. 5. Morphological analysis of Jurkat cell invasion into fibrin gels in vitro. Cellular invasion was studied by confocal laser scanning microscopy. Consecutive cross sections of 8 μm (B) or 20 μm (A, C) thickness of the lower filter that supports the protein gel were documented; the numbers indicate consecutive cross sections of the respective thickness. (A) Invasion of Jurkat cells not saturated with HMW-uPA: only a few scattered cells were found in the upper level of the fibrin gel. (B) Invasion of Jurkat cells saturated with HMW-uPA: many cells were found in all levels of the entire fibrin gel. (C) Invasion of Jurkat cells saturated with uPA and incubated in the presence of the anticatalytic anti-uPA antibody HD-UK 1: only a few scattered cells were found in the upper level of the fibrin gel.

So far, we are unable to detect pro-uPA-specific mRNA in Jurkat cells even by using the highly sensitive polymerase chain reaction [U. Vettel et al., unpublished data]. This is similar to the findings with activated T cells [17]. uPA-R expressing T cells may therefore rely on binding of exogenous (pro-)uPA. The blood concentration of (pro-)uPA is about 15–25 pM, implying a receptor occupancy of ≈ 20%. Higher concentrations of (pro-)uPA are expected in the vicinity of (pro-)uPA-producing cells, such as endothelial cells, macrophages, or fibroblasts [for review, see refs 1–3]. Activation of such cell types, in particular under conditions of inflammatory reactions, may even enhance uPA synthesis and secretion [1–3]. Inflammatory foci may therefore provide a microenvironment where uPA-R occupancy will be greater than 20%.

uPA is synthesized and secreted as an inactive precursor, i.e., pro-uPA. Previously we have found that a T cell-associated serine proteinase, termed HuTSP-1 or, synonymously, granzyme A, can activate pro-uPA [26, 27]. HuTSP-1 is induced by mitogen- or antigen-induced activa-

tion of T cells stored within intracytoplasmic granules [28], and can be released into the pericellular space by receptor-triggered exocytosis [26]. It has therefore been suggested that after its release into the extracellular space, HuTSP-1 may not only directly cleave extracellular matrix substrates, but may also induce the plasminogen activator system via activation of pro-uPA. The relevance of this pathway is currently being explored.

The natural substrate for uPA is plasminogen, which is converted by limited proteolysis into the heterodimeric endopeptidase plasmin. Plasmin has trypsin-like activity and can degrade a wide range of extracellular matrix proteins [for review, see refs 1-3]. We have demonstrated that uPA bound to the Jurkat cell uPA-R can activate plasminogen (Fig. 3). As the invasion assay has been performed in the presence of plasminogen-containing serum and fibrin is a favorable substrate for plasmin [16], it is to be expected that the proinvasive effect of surface-bound uPA may be mediated via activation of plasminogen. The possible involvement of plasmin(ogen) in cellular invasion is presently being examined.

Fibrin is a major constituent of the stroma in inflammatory foci [15]. The biological relevance of uPA-R occupancy was therefore addressed by the quantitative and morphological analysis of Jurkat cell invasiveness into fibrin gels in vitro. Plasmin, however, can degrade not only fibrin, but also a wide variety of glycoprotein constituents of the extracellular matrix [1-3]. Accordingly, it had been demonstrated that plasmin generated in a uPA-dependent manner can facilitate the invasion of uPA- or tPA-expressing melanoma cells into more complex matrices [12, 13]. Therefore, in our future studies we will determine whether T cells can invade into more complex substrata.

Our experiments were inspired by previous results that had suggested a role for plasminogen activators in the invasiveness of nonlymphoid cells [10-14]. Our data provide evidence that uPA-mediated plasminogen activation may also play a role in the invasiveness of T lymphocytes. This assumption is even further corroborated by the fact that activated normal human T lymphocytes as well as T cell clones can express the uPA-R [17; and own observations], and that the invasion of cloned human T cell lines into fibrin gels can also be increased by uPA binding [M.D. Kramer et al., unpublished data].

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