Regulation of Plasminogen Receptor Expression on Monocytoid Cells by \( \beta_1 \)-Integrin-dependent Cellular Adherence to Extracellular Matrix Proteins

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Plasminogen binding sites function to arm cell surfaces with the proteolytic activity of plasmin, critical for degradation of extracellular matrices. We have assessed the effects of adhesion of the representative monocytoid cell lines, THP-1 and U937, to purified extracellular matrix proteins on their expression of plasminogen receptors. After adhesion to immobilized fibronectin, adherent and nonadherent subpopulations of cells were separated. Plasminogen binding to the nonadherent population of cells increased 3-fold, whereas binding to the adherent population decreased by 60%. These changes were due to differences in the plasminogen binding capacities of the cells, while the affinities of the cells for plasminogen were unchanged. The up-regulation of receptor expression in the nonadherent cell population was: 1) induced rapidly and reversibly, 2) independent of new protein synthesis, 3) required an interaction between adherent and nonadherent cell populations, and 4) associated with an enhanced ability of the cells to promote plasminogen activation and to degrade fibronectin. Other immobilized adhesive proteins, laminin and vitronectin, also supported up-regulation of plasminogen receptors in the nonadherent cells. Carboxypeptidase B treatment eliminated the increment in the plasminogen binding capacity of the nonadherent cells, suggesting that the increase in binding was due to exposure of new carboxyl-terminal lysyl residues on the cell surfaces. Furthermore, both the adherence of the cells and up-regulation of plasminogen binding sites was abolished by \( \beta_1 \)-integrin monoclonal antibodies. These results suggest that proteins found in extracellular matrices have the capacity to modulate the expression of plasminogen binding sites, thus regulating local proteolysis and cell migration.

The expression of cell surface binding sites for plasminogen is widespread among cells of both prokaryotic (reviewed in Refs. 1 and 2) and eukaryotic organisms (reviewed in Ref. 3). Among eukaryotes, plasminogen receptors are expressed on cells within the vasculature including platelets (4), monocytes (5), lymphocytes (5), and endothelial cells (6–8), as well as on a variety of cells found within solid tissues including hepatocytes (9), fibroblasts (10), epidermal cells (11), and keratinocytes (12). By enhancing plasminogen activation (4, 6) and protecting cell-bound plasmin from inactivation by \( \alpha_2 \)-antiplasmin (10, 13, 14), plasminogen receptors function to arm cell surfaces with the broad spectrum proteolytic activity of plasmin. Such cell surface proteolytic activity facilitates processes involving cell migration through extracellular matrices.

The ability to modulate plasminogen binding capacity provides a mechanism for regulation of cell surface proteolytic activity. Expression of plasminogen receptors on U937 monocytoid cells is up-regulated by interferon-\( \gamma \) and vitamin D\(_3\) (15, 16). Down-regulation of plasminogen receptors is elicited by glucocorticoid treatment of HT-1080 fibrosarcoma cells (17) or thrombin treatment of endothelial cells (7). In the presence of differentiation inducing agents (phorbol 12-myristate 13-acetate and vitamin D\(_3\)), monocytoid cells (THP-1 and U937) change their adhesive properties and up-regulate plasminogen binding site expression (18). The importance of cell adhesion in modulating the cell-surface association of components of the plasminogen system has been emphasized by studies showing that adhesion modulates the expression and distribution of urokinase and its receptor (19) and the identification of the matrix-associated protein, vitronectin, as a ligand for the urokinase receptor (20).

It has now been established in many reports that cells respond to the presence and composition of the extracellular matrix by adhering, spreading, migrating, differentiating, and altering gene transcription and cellular phenotype (reviewed in Ref. 21). These matrix-dependent effects are exemplified in studies demonstrating that extracellular matrix attachment influences mammary epithelial cell differentiation (reviewed in Ref. 22) and expression of milk proteins (23). Adhesion of cells to the extracellular matrix also induces metalloproteinase expression (24). Many of these responses require attachment to the extracellular matrix via \( \beta_1 \)-integrins (reviewed in Ref. 25). In other cell types, integrin-mediated attachment to the extracellular matrix suppresses apoptosis in both two-dimensional (26) and three-dimensional cultures (27).

In view of the close interrelationship between cell adhesion and proteolysis, we have examined the effect of adherence of monocytoid cells to purified extracellular matrix proteins (in the absence of exogenously added agonists) on plasminogen receptor expression. Adhesion is shown to directly influence the
plasminogen binding capacity of cells, and a role for β1-integrins in receptor expression is demonstrated. Data are developed to indicate that a specific subset of plasminogen receptors is altered by cell adhesion, and this subset influences plasmin generation. The ability of adhesion to modulate plasminogen receptor expression has important implications in several diverse physiological and pathophysiological processes.

EXPERIMENTAL PROCEDURES

Proteins—Glu-plasminogen1 was purified from fresh human blood collected into 3 ml benzamidin, 3 ml EDTA, 100 units/ml Trasylol (FBA Pharmaceuticals, New York, NY), 100 μg/ml soybean trypsin inhibitor (Sigma). The plasma was subjected to affinity chromatography on lysine-Sepharose (28) in 0.01 M sodium phosphate, pH 7.3, 0.15 M NaCl (PBS) with 1 mM benzamidin, 0.02% NaN3, and 3 mM EDTA, followed by molecular exclusion chromatography on Ultrigel AAc44. The plasminogen concentration was determined spectrophotometrically at 280 nm using an extinction coefficient of 16.8 (29). Fibronectin (Fn) was purified from fresh plasma by affinity chromatography on gelatin-Sepharose as described previously (30). Vitronectin (Vn) was purified as described previously (31), and its concentration was determined using an extinction coefficient of 13.8 (31). Laminin (Lm) was purchased from Boehringer Mannheim. Single chain urokinase type plasminogen activator (u-PA) was kindly provided by Dr. Dick Henkin, Abbott Laboratories, Abbott Park, IL. Urokinase (u-Pa) was from Calbiochem (La Jolla, CA). Sc-PA, gl-plasminogen, and Fn were radiiodinated using a modified chloramine T procedure as described elsewhere (4).

β1-Integrin Monoclonal Antibodies—SG19 (32) was provided by Dr. K. Miyake, 4B3 (33) by Dr. C. Morimoto (Dana-Farber Cancer Institute, Boston, MA), and SA2 (34) by Dr. Dres. N. Kovach and J. Harlan (University of Washington, Seattle).

Cells—The human monocytoid cell line, THP-1 (35) was cultured in RPMI 1640 (Whittaker/MA Bioproducts, Walkersville, MD) containing penicillin G (100 units/ml penicillin G, 100 μg/ml streptomycin, 1 mM sodium pyruvate, 0.05 μM HEPES, pH 7.35, 5 × 10−6 β-mercaptoethanol, and 5% fetal calf serum. The cells were incubated with 25 μCi/ml [35S]methionine (ICN Biochemicals, Inc., Costa Mesa, CA) for 2 h at 37°C in an atmosphere with 95% humidity and 5% CO2. Incorporation of [35S]methionine into protein was determined as counts precipitable by 30% trichloroacetic acid from cells lysed in 20 mM Tris-HCl, 150 mM NaCl, 0.1% Triton X-100, 0.05% Tween 80, 50 mM octylglucoside, 2 mM phenylmethylsulfonyl fluoride, 0.5% NaN3, 10 mM EDTA, 5 units/ml Trypsin, 10 mM benzamidin, and 10 μg/ml soybean trypsin inhibitor.

Statistics—Data are given as mean ± S.D.

Reagents—Carboxypeptidase B was from Sigma. The plasmin substrate, D-Val-Leu-Lys-pNA (S-2251) was from Kabi Vitrum, Malmo, Sweden. Goat anti-mouse IgG and normal mouse IgG were from Calbiochem. Transwell dishes were from Costar, Cambridge, MA. Asciates containing mAb W6/32 was kindly provided by Dr. Martin Schwartz, The Scripps Research Institute.

RESULTS

Role of Cell Adhesion in Modulation of Plasminogen Receptor Expression.—We tested whether adhesion of THP-1 monocytoid cells to isolated extracellular matrix proteins could modulate plasminogen receptor expression. THP-1 cells were allowed to adhere to Petri dishes coated with either Fn, Vn, or Lm or to uncoated control wells. The cells adhered to the three matrix proteins, but not to the uncoated dishes. By producing a limited surface, a nonadherent and an adherent cell population could be recovered separately from each substrate and the extent of adhesion and receptor expression were measured. Plasminogen binding to the nonadherent cell population was greatly enhanced compared to the control cells incubated in uncoated dishes. A typical experiment is shown in Fig. 1. With the different matrix proteins, the percent plasminogen binding (relative to control cells at 100%) was 310 ± 55 (Fn) (n = 22), 167 ± 20 (Vn) (n = 3), and 140 ± 35 (Lm) (n = 3). In contrast, plasminogen binding to the adherent population decreased with each matrix protein compared to the control cells: the percent 125I-plasminogen binding was 61 ± 16 (Fn, n = 22), 45 ± 9 (Vn, n = 3), and 64 ± 35 (Lm, n = 3).

The relationship between extent of adhesion and receptor up-regulation was then explored. Since the greatest degree of adherence and receptor up-regulation was attained with Fn (Fig. 1), this substrate was used in subsequent experiments. Petri dishes were coated with varying quantities of Fn to attain different degree(s) of cellular adhesion. Maximal adhesion of the cells (85% adherence) was attained when the wells were coated with 50 μg of Fn (Fig. 2, panel A). Plasminogen receptor expression in the nonadherent cells increased with increasing adhesion (Fig. 2, panel B). In contrast, plasminogen binding to the adherent cells decreased to 60 ± 16% (n = 6) relative to control cells (data not shown) without any apparent relation to the extent of adhesion. As specific examples, with 85, 64, and 38% adhesion, the decrease in plasminogen binding of the adherent cells was 31, 18, and 40%. The role of adhesion was

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1 The abbreviations used are: Glu-plasminogen, the native form of plasminogen; NH-terminal Glu-plasminogen; bovine serum albumin (BSA); CBP, carboxypeptidase B; Fn, fibronectin; HBSS, Hanks' balanced salt solution; Lm, laminin; PBS, phosphate-buffered saline; scu-PA, single chain urokinase type plasminogen activator; S-2251, D-Val-Leu-Lys-pNA; pNA, p-nitroalnine; u-PA, urokinase-type plasminogen activator; Vn, vitronectin; mAb, monoclonal antibody.
Regulation of Plasminogen Receptor Expression

Separate and incubate at 1 °C, respectively, under "Experimental Procedures." The adherent (cross-hatched bars) and nonadherent (filled bars) cells were recovered separately and incubated at 1 × 10⁶ cells/ml with 125I-plasminogen (100 nM). Specific binding of 125I-plasminogen is shown. The data are from one representative experiment from at least three separate experiments. The percent adherence of the cells to each extracellular matrix protein was 83, 37, and 31% for Fn, Vn, and Lm, respectively, in the experiment shown.

![Figure 1](http://www.jbc.org/)

**Figure 1.** Effect of adherence to extracellular matrix proteins on plasminogen receptor expression. THP-1 cells (1.5 × 10⁶ cells/10 ml) were incubated in Petri dishes coated with either Fn, Vn, Lm, or uncoated, as described under "Experimental Procedures." The adherent (cross-hatched bars) and nonadherent (filled bars) cells were recovered separately and incubated at 1 × 10⁶ cells/ml with 125I-plasminogen (100 nM). Specific binding of 125I-plasminogen is shown. The data are from one representative experiment from at least three separate experiments. The percent adherence of the cells to each extracellular matrix protein was 83, 37, and 31% for Fn, Vn, and Lm, respectively, in the experiment shown.

![Figure 2](http://www.jbc.org/)

**Figure 2.** Relationship between adhesion and plasminogen receptor expression. Petri dishes were coated with varying quantities of Fn. The coating efficiency was 19 ± 4% (n = 3), as determined by adding 125I-Fn as a tracer to dishes coated with 100 µg of Fn. THP-1 cells (1.5 × 10⁶ cells) were incubated with either the Fn-coated dishes or uncoated dishes (control cells). Nonadherent and adherent cells were recovered separately and percent adhesion was determined (panel A). Plasminogen receptor up-regulation (expressed as the quotient of plasminogen binding to the control cells) was plotted against percent adhesion (panel B). In this experiment, the control cells bound 1.04 ± 0.2 × 10⁶ molecules/cell of 125I-plasminogen.

Also examined by comparing up-regulation of plasminogen binding sites in an additional cell type. In parallel experiments, using dishes coated with 100 µg of Fn, THP-1 cells were 94% adherent and U937 monocytoid cells were 76% adherent. Plasminogen binding to the nonadherent THP-1 cells increased 2-fold over controls, while plasminogen binding to the nonadherent U937 cells increased 1.6-fold. Moreover, the up-regulation of plasminogen receptors did not reflect a general effect on cell surface receptors. When 125I-scu-PA binding to the cells was examined in parallel with a 1 nM input concentration of 125I-scu-PA, 1.19 ± 0.26 × 10⁶ (n = 3) and 1.38 ± 0.29 × 10⁶ (n = 3) molecules were bound per cell in the nonadherent and adherent THP-1 cell populations, respectively.

To further explore the requirement for adherence to substrate, we tested whether soluble Fn could induce plasminogen receptor up-regulation in the nonadherent cell population. Cells (1.5 × 10⁶) were incubated in polypropylene tubes in the presence of 100 µg of soluble Fn. Under this condition, no up-regulation of plasminogen binding sites was observed. In parallel experiments in which the cells were incubated with immobilized Fn, 70% adhesion was achieved, resulting in a 4.5-fold up-regulation of plasminogen receptor expression in the nonadherent population.

Next, the time dependence of plasminogen receptor up-regulation in the nonadherent cell population was investigated (Fig. 3). THP-1 cells were incubated with Fn-coated dishes for varying times. The nonadherent population was recovered and plasminogen binding and percent adhesion were determined. Plasminogen receptor up-regulation was induced rapidly, and could be detected by 10 min, reaching a maximum between 30 and 120 min. The receptor up-regulation decayed rapidly after 120 min. Interestingly, the extent of adhesion paralleled the receptor up-regulation until 120 min, but decayed more slowly. We tested also whether the up-regulation of plasminogen binding sites was reversible. Cells were incubated with Fn-coated dishes, and 69% of the cells adhered. The nonadherent population was recovered and then allowed to adhere to a second Fn-coated dish. Sixty-eight percent adherence was obtained. Therefore, the distinction between adherent and nonadherent populations was not due to an unique subpopulation of cells, but, apparently, to the availability of surface for adherence. As discussed above, plasminogen receptors were up-regulated 2-fold in the nonadherent cell population during the first adhesion step (Table I). Following the second adhesion of the nonadherent population of cells, plasminogen receptor expression was further up-regulated by 2-fold in the newly nonadherent population and was downregulated 2.5-fold in the newly adherent population (Table I). Thus, both up-regulation and down-regulation of plasminogen binding site exposure was reversible.

Mechanisms of Plasminogen Receptor Up-regulation—To determine whether the increase in plasminogen binding was due to a change in affinity and/or capacity of the nonadherent cells for plasminogen, binding isotherms were constructed for the cells that did not adhere to Fn and for control cells incubated in uncoated dishes. Under equilibrium binding conditions, the isotherms gave evidence of saturation and, therefore, the data were plotted in Scatchard plots (Fig. 4). Straight lines were obtained and provided good fits for both sets of data points (r = 0.98 for control cells and r = 0.99 for the nonadherent cells), suggesting a single class of plasminogen binding sites with respect to affinity. The affinities of both cell populations for plasminogen were not statistically different: Kᵢ values of 447 ± 141 nM and 393 ± 25 nM were calculated for nonadherent and control cell populations, respectively (n = 3). However, the number of binding sites was 4-fold higher in the nonadherent population: β_max = 28 ± 5 × 10⁶ molecules/nonadherent cell versus 7 ± 1 × 10⁶ molecules/control cell. Thus, the increase in plasminogen binding to the nonadherent cells was due to a change in capacity but not in affinity for plasminogen.

In order to determine whether de novo protein synthesis was required for plasminogen receptor up-regulation, THP-1 cells were treated with cycloheximide prior to exposure to the immobilized Fn. To insure that cycloheximide was exerting its anticipated effects, we verified that [⁵¹]Smethionine incorporation into total cellular protein was inhibited by greater than 90% (Table II). Under this condition, plasminogen receptor up-regulation was not affected (Table II). In controls, cycloheximide treatment altered neither cell viability nor the ability of the cells to adhere to Fn.

The possibility that soluble factors released during the adhesion step could be responsible for the up-regulation was explored. Conditioned medium was collected after adhesion of the cells to Fn for 1 h and was then added to fresh THP-1 cells in uncoated dishes for 1 h. No plasminogen receptor up-regulation was observed in the cells exposed to the conditioned medium (106 ± 3% (n = 3) relative to control cells exposed to...
and percent adhesion were measured. Were removed, and plasminogen binding point, nonadherent and adherent cells incubated for varying times. At each time point, nonadherent and adherent cells were removed, and plasminogen binding and percent adhesion were measured. 125I-Plasminogen binding to the control cells was 0.33 × 10^6 molecules/cell in this experiment (representative of two experiments).

Experimental format was used. Cells were cultured in transwell mouse 3-

The role of β3-integrins in up-regulation of plasminogen binding sites following adherence to fibronectin was explored. Cells were preincubated with two anti-β3-integrin monoclonal antibodies that block adhesion to fibronectin, SG19 (32) and 4B4 (33). Under these conditions, no adherent cells were recovered (Fig. 5B), and plasminogen binding sites were not increased in the nonadherent population (Fig. 5A). Using the activating mAb, 8A2 (34), adherence was not blocked and plasminogen binding sites were up-regulated 3.7-fold compared to control cells incubated in the absence of Fn. Cells incubated with Fn in the presence of an irrelevant mAb exhibited a 2.5-fold up-regulation in plasminogen binding ability compared to the control cells. We found no up-regulation of plasminogen binding sites when cells were incubated with the activating

Under these conditions, no up-regulation of plasminogen receptors was observed in cells cultured in the upper chamber, although in the bottom chamber, a 2.3 ± 0.2-fold up-regulation (n = 3) was induced in the nonadherent population. Thus, no evidence was obtained for up-regulation of plasminogen receptors by soluble factors.

Regulation of Plasminogen Receptor Expression

**TABLE I**

Reversibility of plasminogen receptor up-regulation

| Cell population | 125I-Plasminogen bound |  |
|-----------------|------------------------|---|
|                 | First adhesion         | Second adhesion (nonadherent) |
| Control cells   | 1.0 ± 0.09             | 0.99 ± 0.29                     |
| Adherent cells  | 0.5 ± 0.12             | 0.8 ± 0.24                      |
| Nonadherent cells | 2.0 ± 0.28         | 4.2 ± 1.25                     |

**FIG. 3.** Time course of plasminogen receptor up-regulation. THP-1 cells (1.5 × 10^7 cells/ml) were either treated with cycloheximide (10 μg/ml) at 22 °C for 30 min or untreated. Then the treated and untreated cells were added to the Fn-coated dishes or uncoated dishes and incubated at 37 °C for 1 h. Plasminogen binding to the separated cell populations was measured.

**FIG. 4.** Scatchard plots of plasminogen receptor expression. Nonadherent cells (squares) obtained by incubation with plates coated with 100 μg of fibronectin or control cells (triangles) (10^6 cells/ml) were incubated with varying concentration of 125I-plasminogen (50 nM to 4 μM). The data obtained in these binding isotherms were subjected to Scatchard analysis.

Nonadherent cells (squares) obtained by incubation with plates coated with 100 μg of fibronectin or control cells (triangles) (10^6 cells/ml) were incubated with varying concentration of 125I-plasminogen (50 nM to 4 μM). The data obtained in these binding isotherms were subjected to Scatchard analysis.

nonconditioned medium). To test whether a transiently active soluble factor, released from the adherent cells during the adhesion step, might induce receptor up-regulation, a second experimental format was used. Cells were cultured in transwell dishes with 3-μm pore size nucleopore polycarbonate membranes. Fn was coated onto the surface of the lower chamber, and no Fn was present in the upper chamber. Thus, in the lower chamber, both adherent cells and nonadherent cells were present; in the upper chamber, all cells were nonadherent.

**TABLE II**

Effect of cycloheximide on plasminogen receptor up-regulation

| Treatment   | Plasminogen binding up-regulation | \[^{35}S\]Methionine incorporation |
|-------------|----------------------------------|----------------------------------|
| Untreated   | 2.84                             | 100                              |
| Cycloheximide | 2.75                         | 8                                |

^a Fold up-regulation was determined as plasminogen binding to the nonadherent cell population divided by binding to the control cells. To determine the effects of cycloheximide on total cellular protein synthesis, THP-1 cells (1.5 × 10^7) in methionine-free Dulbecco’s modified Eagle’s medium were treated either with or without cycloheximide. Then, \[^{35}S\]Methionine (25 μCi/ml) was added and the cells were further incubated at 37 °C for 2 h.

\[^{35}S\]Methionine incorporation into protein was determined as counts precipitable in 10% trichloroacetic acid from cell lysates as described under “Experimental Procedures.” Percent incorporation into protein was determined by dividing trichloroacetic acid precipitable counts in the presence of cycloheximide by trichloroacetic acid precipitable counts in the absence of cycloheximide. Binding of 125I-plasminogen to cells cultured in uncoated plates was 1.1 × 10^6 molecules/cell for both untreated and cycloheximide-treated cells.

Under these conditions, no up-regulation of plasminogen receptors was observed in cells cultured in the upper chamber, although in the bottom chamber, a 2.3 ± 0.2-fold up-regulation (n = 3) was induced in the nonadherent population. Thus, no evidence was obtained for up-regulation of plasminogen receptors by soluble factors.
antibody, 8A2, in uncoated dishes (data not shown), excluding the possibility that 8A2 could stimulate the cells to increase plasminogen binding sites in the absence of a β1-integrin ligand.

To examine whether the up-regulation observed in the non-adherent population might require attachment followed by release of cells, we tested whether up-regulation could occur when adherent cells were prevented from detaching. Petri dishes were incubated with goat-anti-mouse IgG to capture either mAb W632, directed against the major histocompatibility complex, or normal mouse IgG as described (37). The THP-1 cells adhered to each type of coating, presumably via an interaction with Fc receptors and/or the major histocompatibility complex (Fig. 6B). Nonadherent and adherent cell populations were recovered from these dishes and plasminogen binding compared with nonadherent and adherent cells recovered from fibronectin-coated dishes. Under all conditions that induced adherence, plasminogen binding was increased to a similar extent in the nonadherent cell populations and decreased in the adherent populations, compared to control cells incubated in uncoated dishes (Fig. 6A). Therefore, the up-regulation in receptor expression in the nonadherent population cannot be attributed to changes occurring when cells detach from a surface.

**Nature of the Up-regulated Plasminogen Receptor Population**—Proteins with carboxyl-terminal lysyl residues are candidate plasminogen receptors (38). We sought to determine the extent to which plasminogen receptor up-regulation depended upon exposure of carboxyl-terminal lysyl residues. Adherent and nonadherent populations were generated by incubating THP-1 cells in Fn-coated dishes, and control cells (squares) incubated in uncoated dishes, were incubated with increasing concentration of CPB (diisopropyl fluorophosphate-treated) at 37 °C for 30 min. The cells were washed three times with HBSS-BSA, and 125I-plasminogen binding was measured.

**Effect of carboxypeptidase B treatment on plasminogen receptor up-regulation**. Nonadherent (triangles) and adherent (circles) cell populations, generated by incubating THP-1 cells in Fncoupled dishes, and control cells (squares), incubated in uncoated dishes, were incubated with increasing concentration of CPB (diisopropyl fluorophosphate-treated) at 37 °C for 30 min. The cells were washed three times with HBSS-BSA, and 125I-plasminogen binding was measured.

**Functional Consequences of Plasminogen Receptor Up-regulation**—We sought to determine whether up-regulation of plasminogen binding in a dose-dependent manner, reaching a plateau at approximately 60% inhibition (Fig. 7), consistent with previously published data (38–40). With the nonadherent cell population, CPB treatment decreased plasminogen binding to the level of the CPB-treated control cells (Fig. 7). These results suggested that the up-regulated plasminogen binding sites are composed predominantly of proteins exposing carboxyl-terminal lysyl (or arginyl) residues on the cell surface.
minogen receptors was associated with an increased ability of the cells to promote plasminogen activation. Adherent and nonadherent cell populations were produced after incubation with Fn-coated dishes. These cells and control cells were incubated with 15 μM Glu-plasminogen. The unbound plasminogen was removed by centrifugation and u-PA and the plasmin substrate, S-2251, were added. Plasmin activity was measured as cleavage of the S-2251. After 30 min, the plasmin amidolytic activity of the nonadherent cells was 3.5 times higher than that of the adherent cells and 2.5 times higher than the control cells (Fig. 8). The extent of promotion of plasminogen activation correlated with the plasminogen binding capacities of the cells in this experiment. Plasminogen binding to the nonadherent cells was approximately 2.5- and 2-fold higher than to either the adherent or control cells, respectively.

The relationship between plasminogen receptor expression and the ability of the cells to degrade a representative extracellular matrix component, Fn, was explored. Nonadherent cell populations were generated by adherence of THP-1 cells to Fn-coated plates and control cells were incubated in uncoated plates. These cells were incubated with 10 μM Glu-plasminogen and washed to remove unbound ligand. Then u-PA and soluble 125I-Fn were added and incubated with the cells. After incubation for 2 h, 125I-Fn was degraded to a major product with a Mr of 190,000 (Fig. 9, lanes 2 and 3), while the integrity of either Fn alone or Fn plus u-PA was not changed between 0 and 2 h (data not shown). The extent of degradation in the nonadherent cells was ~2-fold greater than that of the control cells, as determined by laser densitometric scanning of autoradiograms of the gels. After 20 h of incubation, intact 125I-Fn was no longer detectable after incubation with the cells (Fig. 8, lanes 4 and 5). At this time point, the 190,000 band was further degraded into smaller fragments. The decrease in intensity of the 190,000 band was more extensive (~4-fold) in the nonadherent cell population compared to control cells. Greater than 95% of the 125I-Fn remained intact following incubation with buffer or with u-PA for 20 h (data not shown). Thus, the ability of the cells to degrade Fn was enhanced in the nonadherent cell population coinciding with plasminogen receptor up-regulation.

**DISCUSSION**

In this study, we provide the first evidence that adhesion of monocytoïd cells to extracellular matrix proteins, via β₁-integrins, induces increased expression of plasminogen binding sites. The up-regulation of these sites occurred in the subpopulation of cells that was nonadherent, although the presence of adherent cells was required. Three different adhesive proteins, fibronectin, vitronectin, and laminin, supported this up-regulation. The following conclusions can be drawn from our analyses. 1) The presence of a population of cells adhering to the extracellular matrix in an integrin-dependent manner is sufficient to induce up-regulation of plasminogen binding sites in the nonadherent population of cells. In contrast, plasminogen binding to the adherent cells is decreased compared to cells maintained in suspension. 2) The up-regulation of plasminogen binding sites is a reversible process; when nonadherent cells are allowed to adhere to substratum, exposure of plasminogen binding sites is down-regulated. This reversibility suggests that the nonadherent cells did not represent a distinct subpopulation within the cell line. Furthermore, this implies that proteolysis does not play a role in the up-regulation. 3) The up-regulation is rapid and independent of new protein synthesis. 4) The newly available plasminogen receptors are proteins exposing carboxyl-terminal lysines or arginines. These receptors exhibit the same affinity for plasminogen as the receptors on resting cells. 5) Up-regulation of plasminogen binding sites increases the ability of the cells to promote plasminogen activation and to degrade Fn.

The ability of cells to promote plasminogen activation is absolutely dependent upon binding of plasminogen to the cell surface (41, 42). Both protein (38) and nonprotein (e.g., gangliosides) (43) components of the cell membrane bind plasminogen. In the present study, the increase in plasminogen binding capacity of the nonadherent cells was entirely sensitive to CPB. Thus, these new binding sites are predominantly proteins exposing carboxyl-terminal lysines. Furthermore, the nonadherent cells exhibiting enhanced plasminogen binding were 3.5-fold more effective than control cells in promoting plasminogen activation. These observations indicate a role of newly exposed carboxyl-terminal lysines in enhancing plasminogen activation and support the dependence of cell surface plasminogen activation on plasminogen receptor occupancy.

The presence of a population of cells that adhered to extracellular matrix proteins was necessary and sufficient to induce up-regulation of plasminogen receptors in the nonadherent cell population. The up-regulation of plasminogen binding sites did not require the addition of exogenous agonists. The adherence of the THP-1 cells to Fn was dependent upon β₁-integrin(s).
β1-Integrins are known to transmit external signals from the extracellular matrix to stimulate intracellular signaling pathways (reviewed in Ref. 44). However, activation of β1-integrins on the monocytoid cells using an activating antibody in the absence of β1 ligands, was not sufficient to up-regulate plasminogen binding site expression. The ability to induce plasminogen receptor up-regulation was not limited to extracellular matrix proteins: Adherence of cells to immobilized antibodies also induced plasminogen receptor up-regulation in the nonadherent cell population, suggesting that the predominant determinant of up-regulation is the presence of adherent cells and may not be dependent upon intracellular signaling.

In analyzing plasminogen binding site up-regulation, we were unable to detect the presence of a soluble factor which could induce receptor up-regulation in the nonadherent cell population. Furthermore, plasminogen binding site up-regulation was not diminished by pretreatment with cycloheximide, suggesting that new protein synthesis was not required to achieve up-regulation. Another potential mechanism which could explain up-regulation is proteolytic modification of cell surfaces to increase the number of plasminogen binding sites (39, 45). However, plasminogen binding site expression was down-regulated when nonadherent cells were allowed to adhere to a new surface. This observation argues that proteolytic digestion of membrane proteins is not responsible for the up-regulation induced by adherence.

With elimination of the foregoing potential mechanisms to explain up-regulation of plasminogen receptor expression in the nonadherent cells, the following model is most compatible with our results. Direct contact between the adherent cell population and nonadherent cells induces reversible reorganization of the cell membrane which increases surface exposure of proteins with carboxyl-terminal lysines. The adhesion step induced down-regulation of receptor expression in the adherent cells. Furthermore, when the nonadherent cells (that exhibited receptor up-regulation) were allowed to adhere, adhesion induced receptor down-regulation. Down-regulation, likewise, may be induced by reorganization of the cell membrane during adherence.

The ability of cells to regulate their expression of plasminogen receptors is critical for local regulation of proteolysis. When cells encounter an adhesive substrate, receptor expression is up-regulated in the nonadherent cell population. When this increase is combined with clustering of urokinase receptors at the leading edge of the cell (46), focal proteolytic activity is generated on the cell surface to promote matrix degradation. After the cells adhere, plasminogen receptors and, consequently, cell surface proteolytic activity are down-regulated, to aid in the establishment of stable cell-substratum contacts.

This regulation of proteolytic activity at local environments should have important consequences for biological processes in which cells must degrade extracellular matrices in order to migrate, such as in wound healing, inflammation, metastasis, and angiogenesis.

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