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Expression and functional role of urokinase-type plasminogen activator receptor in normal and acute leukaemic cells

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Summary. Urokinase-type plasminogen activator receptor (UPA-R-CD87) is a GPI-anchored membrane protein which promotes the generation of plasmin on the surface of many cell types, probably facilitating cellular extravasation and tissue invasion. A flow cytometric quantitative analysis of expression levels for UPA-R was performed on fresh blast cells from patients with acute myeloid leukaemia (AML, $n = 74$), acute lymphoblastic leukaemia (ALL, $n = 24$), and biphenotypic leukaemia (BAL, $n = 3$) using two CD87 monoclonal antibodies (McAbs) (3B10 and VIM5). Peripheral blood and bone marrow (BM) cells from 15 healthy adults served as controls. Using 3B10 McAb, UPA-R was expressed (>99%) by blood monocytes, neutrophils, and BM myelomonocytic precursors in controls, whereas resting T and B lymphocytes, and CD34^{+} cells were UPA-R negative. We also attempted to clarify whether UPA-R has a role in mediating neutrophil functions. Oriented locomotion induced by different chemotaxins and lysozyme release by granules stimulated with fMLP or PMA were significantly decreased when UPA-R was neutralized by CD87 McAb. In contrast, the anti-UPA-R McAb had no effect on superoxide anion generation of normal neutrophils. Blasts from AML showed a heterogenous pattern of expression for the UPA-R McAbs, with reactivity strictly dependent on FAB subtype. The highest UPA-R expression was seen in the M5 group: all patients tested ($n = 20$) showed strong positivity for the UPA-R McAb whereas only 12% (3/24) of ALL patients were CD87 positive, and 2/3 of BAL patients showed a dim expression for CD87. The number of receptors expressed by blast cells in 6/74 (8·1%) AML patients was higher than those of normal samples; in addition, since co-expression of UPA-R and CD34 was not found in normal haemopoietic cells, it may be postulated that CD87 can be used alone (when over-expressed) or in combination with CD34 for the detection of minimal residual disease. Results also indicated that patients with UPA-receptors $>12 \times 10^{3}$ ABC/cell, irrespective of FAB subtype, had a greater tendency for cutaneous and tissue infiltration and a higher frequency of chromosome abnormalities, thus suggesting the concept that cellular UPA-R content positively correlates with the invasive potential of AML cells. The combination of higher UPA-R positivity, abnormalities of chromosome 11, and M5 FAB morphology may identify a peculiar subset of AML, characterized by a more aggressive clinical course.

Keywords: UPA-R, acute myeloid leukaemia, AML M5 morphology, tissue infiltration, chromosome abnormalities.

Urokinase is a specific serine protease which catalyses plasminogen conversion to plasmin principally within the extravascular compartments. Plasmin is a broad-spectrum protease which is able to degrade protein constituents of the extracellular matrix and adhesion molecules in the pericellular space. As a consequence, these enzymes are thought to contribute to the invasive properties and metastasis formation of tumour cells (Bu *et al*, 1994; Stephens *et al*, 1996; Lijnen, 1996). Urokinase plasminogen activator (UPA)-mediated cell surface plasminogen activation occurs on a specific membrane receptor that can be found in many cell types including monocytes, granulocytes, a subset of activated natural killer cells, endothelial cells, fibroblasts, smooth muscle cells, keratocytes, hepatocytes and placental trophoblasts, as well as in several tumour cells such as fibrosarcoma, rhabdomyosarcoma, melanoma, breast, prostate and colon carcinoma, and the U937 leukaemia cell line (Pyke *et al*, 1991; Todd *et al*, 1995; Lanza *et al*, 1994; Sitrin *et al*, 1994). The UPA-receptor (UPA-R) is a specific and

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saturable receptor, composed of a single-chain, highly glycosylated protein with a molecular weight of 55–60 kD. CD87 serves as a receptor for pro-UPA and UPA with high affinity (K_d 10^{-9}–10^{-10} molar) (Todd *et al*, 1995). The protein is linked to the plasma membrane via a glycosyl-phosphatidylinositol (GPI) anchor and thus has no intra-cytoplasmic domains; as a consequence, signal transduction by UPA-R may require an adaptor protein connected to the cell surface. Since UPA-R is spatially and temporally associated with cellular structures that regulate cell adhesion and migration, it has been hypothesized that formation of complexes between UPA-R and integrins such as CD11b (Mac-1) might provide an integrin mediated link between UPA-R and the cytoskeleton, thus promoting adhesion (Gyetko *et al*, 1994, 1995, 1996; Xue *et al*, 1994; Plesner *et al*, 1994; Cao *et al*, 1995; Kindezelskii *et al*, 1996; Simon *et al*, 1996). When UPA is complexed to its specific inhibitors PAI-1 (plasminogen activation inhibitor-1) and PN-1, it is internalized and degraded through a mechanism requiring both UPA-R and the $\alpha 2$ macroglobulin receptor. CD87 may also form a functional linkage with $\beta 1$, $\beta 2$, $\beta 3$ integrins or with other proteins that have tyrosine kinase activity (Wei *et al*, 1996).

Recently, UPA-R cDNA has been cloned and the primary structure of the protein defined; it consists of 313 amino acid residues organized in three repeating sequences of approximately 90 amino acids (Todd *et al*, 1995). The human CD87 gene has been mapped to chromosome 19q1.3.

At the Fifth International Workshop on Leukocyte Differentiation Antigens, held in Boston, U.S.A., on 3–7 November 1993, six monoclonal antibodies (McAbs) were verified as recognizing the UPA-R, and were referred to as CD87 (CD stands for cluster of differentiation) (Todd *et al*, 1995; Lanza *et al*, 1994). Four further CD87 McAbs were assigned in 1996 at the Sixth International Workshop on Leukocyte Differentiation Antigens.

The aim of this study was to investigate the cellular expression and functional role of UPA-R in haemopoietic cells obtained from healthy adult subjects; in addition, UPA-R expression was evaluated by flow cytometry in acute leukaemic blasts, and the results were tentatively correlated with clinical and biological features of the patients examined.

MATERIALS AND METHODS

Subjects. Fresh bone marrow (BM) cells obtained from patients with acute myeloid leukaemia (AML, $n = 74$), acute lymphoblastic leukaemia of B-cell phenotype ($n = 24$), AML with minimal phenotypic deviation ($n = 3$), and biphenotypic acute leukaemia ($n = 3$) were investigated within 4 h of collection = Peripheral blood and bone marrow cells from 15 healthy adult subjects were also included in this study. Written informed consent was obtained from all study participants as approved by the institutional review board of the Hospital.

Diagnosis of leukaemia was based on morphological, cytochemical, cytogenetics, molecular genetics and immunological criteria. According to the FAB (French–American–British) classification, AML patients were distributed as follows: M0: 7; M1: 12; M2: 20; M3: 4; M4 11; M5: 20. ALL patients were subdivided into the following immunological subgroups: pro-B (B-I) ($CD19^+$, $cyCD22^+$, $CD79\alpha^+$, $CD10^-$), two cases; common B (B-II): $CD10^+$, $CD19^+$, $CD22^+$, $CD79^+$, 14 cases; pre-B (B-III): $c\mu^+$, $CD10^+$, $CD19^+$, $CD22^+$, $CD79\alpha^+$, four cases; mature B (B-IV): $SigM^+$, $CD19^+$, $CD22^+$, $CD79\alpha^+$, $CD10^-$, four cases (Bené *et al*, 1995).

Patients with acute leukaemia had >50% blasts in the specimen analysed (mean 69%). The diagnosis of biphenotypic leukaemias or acute leukaemia with minimal phenotypic deviation was made according to the proposals of the EGIL group (Bené *et al*, 1995).

AML patients were treated with a DAT-based induction regimen, followed by consolidation chemotherapy. Patients with B-lineage ALL were treated according to a modified VPD based regimen (vincristine, prednisone, daunorubicin) combined with cytosine-arabinoside, cyclophosphamide and asparaginase.

Flow cytometry analysis. Whole blood samples and purified $CD34^+$ cells were analysed with a Facscan flow cytometer (Becton Dickinson, San José, U.S.A.) equipped with a 15 mW argon-ion laser. The instrument was calibrated with FITC (isothyocyanate of fluorescein) and PE (R-phycoerythrin) beads provided by Becton Dickinson. Data were analysed with appropriate negative (isotypic) and positive (blood cells from healthy adult subjects) controls using CellQuest and Paint-a-Gate research software (Lanza *et al*, 1993). 20 000 cells were analysed for each sample at a flow rate of approximately 300 particles per second. The blast cell population was identified using a combined approach based on a multicolour analysis and evaluation of light scattering properties of the cells (forward scatter and side scatter) (Lanza *et al*, 1996).

In normal $CD34^+$ bone marrow cells the analysis of UPA-R expression has been performed using a multiparametric live-gate approach. In two cases the cytofluorimetric analysis was also performed on $CD34^+$ cells which were purified using a positive selection technique (MiniMacs system, Miltenyi Biotec, Milan, Italy) (Lanza *et al*, 1997a). $CD34^+$ progenitor cells mobilized with G-CSF were also analysed by flow cytometry in 10 healthy subjects.

Flow cytometry data were expressed as percentage of positivity (using the standard marker approach), as molecules of equivalent soluble fluorochrome (MESF), and as antibody binding capacity (ABC) (Poncelet *et al*, 1996). Based on the analysis of the minimum detection threshold of our flow cytometry, and on variation in MESF and ABC values for UPA-R in control and pathological samples (evaluated within and between different specimens), we estimated that the lower limit of quantitation was 590 MESF/cell. Below this limit the enumeration of antigenic determinants per cell was considered unreliable and unreproducible. However, as stated earlier, the lower limit of detection was variable and ranged from 0·47 to $0{\cdot}59 \times 10^3$ MESF/cell over a period of 4 years (Papa & Lanza, 1996; Poncelet *et al*, 1996; Lanza *et al*, 1997a). Using the QIFIKIT assay, the lower limit of detection was $0{\cdot}9 \times 10^3$ ABC/cell.

Immunophenotypic analysis. The immunophenotypic profile of leukaemic cells was assessed with a Facscan flow cytometer using a large panel of McAbs, including CD13, CD33, CD15, anti-MPO (MPO-7, CLB-MPO1), anti-lysozyme (LZ-1), CD34 (HPCA-2), CD11b, CD11c, CD65, CD41, CD61, glycophorin A, CD35, CD117, CD56, Thy-1 (CD90), HLA-DR, CD38, CD14, CD10, CD19, CD22, CD3, CD5, CD7, cytoplasmic (cy) CD3, cyCD22, CD79α, TdT (Becton Dickinson; Dakopatts, Glostrup, Denmark; Immunotech, Marseille, France; Serotec, U.K.; Caltag, U.S.).

The concentrations of McAbs were titrated towards a saturating titre, as assessed by flow cytometry analyses performed on normal haemopoietic cells and cell lines. Experiments of single, double and triple staining were employed in all cases.

MPO and lysozyme measurement was performed using Fix and Perm fixation- permeabilizing agent (Caltag) (Lanza *et al*, 1997b). McAbs were used at saturating concentration. In brief, whole blood (1×10^6 cells) was incubated for 15 min at room temperature with 100 μl of reagent A (fixation medium), then washed once with PBS before adding 50 μl of reagent B (permeabilization reagent) plus anti-MPO or lysozyme McAb. For the detection of lysozyme, whole blood cells were washed three times before their treatment with the permeabilization agents, in order to remove serum lysozyme. Detection of MPO and lysozyme was performed using a direct immunofluorescence staining method. To study MPO, we used a FITC-conjugated anti-MPO McAb (clone: MPO-7, IgG1 isotype, from DAKO), while reactivity for lysozyme was evaluated by using a FITC-conjugated anti-lysozyme McAb (clone: LZ-1, IgG1 isotype, purchased from Caltag).

Monoclonal antibodies to UPA-R. The expression of UPA-R on normal and leukaemic cells was assessed by using two different unconjugated McAbs: 3B10 (Mizukami *et al*, 1990) and VIM5 (Gadd *et al*, 1990), the latter was developed in the laboratory of Professor Walter Knapp (Institute of Immunology, Vienna, Austria).

Negative controls with isotype-matched non-relevant McAbs (mouse IgG1, IgG2a, IgM; Dakopatts, Silenus) were done in all experiments. All the McAbs were utilized using an indirect immunofluorescence technique. As second step we employed FITC-conjugated rabbit F(ab′) Ig fragments specific for mouse Ig (fluorochrome/protein ratio 2·3; Dakopatts). Human AB serum was added prior to antibody incubation to avoid non-specific binding of McAbs to Fc receptors. All samples were further incubated for 10 min with a lysing buffer (Facslysing solution, Becton Dickinson) to eliminate red blood cells. Two- and three-colour fluorescence staining experiments were performed using the PE-conjugated VIM-5 McAb (CD87, Pharmingen, U.S.A.), in combination with McAbs directed against various surface markers.

Detection of cyUPA-R. For the detection of cytoplasmic (cy) UPA-R, whole blood cells were permeabilized with Fix and Perm permeabilization-fixation kit (Caltag), as described above, and then analysed by flow cytometry. For each sample, the MESF values of the isotypic control was subtracted from the MESF values of the positive cell population. The intracellular content of UPA-R was calculated by subtracting the fluorescence intensity (expressed either in the form of mean fluorescence intensity, MFI or MESF) of surface UPA-R evaluated on unfixed cells from the total amount of UPA-R detected by flow cytometry after fixation and permeabilization (Lanza *et al*, 1997b). CyUPA-R was analysed on neutrophils and monocytes obtained from healthy subjects, as well as in acute leukaemic blasts from AML. All experiments were performed in duplicate.

MESF and ABC calculation. To evaluate the number of antigenic determinants per cell for the various UPA-R McAbs, we used FITC quantitative microbead calibration standards (Quantum 26p beads, Flow Cytometry Standard Corporation, FCSC, purchased from Walter Occhiena srl, Torino, Italy) in conjuction with the Quantum Simply Cellular beads (FCSC, Walter Occhiena) (Poncelet *et al*, 1996). The use of calibrated microbeads made it possible to express the flow cytometry data in the form of molecules of equivalent soluble fluorochrome (MESF) values. The calculation of MESF was based on the evaluation of the peak channel expressed by cells and different populations of microbeads. For each sample the MESF value of the isotypic control was subtracted from the MESF value of the positive cell population.

Data were also expressed as Antibody Binding Capacity (ABC) by using the QIFIKIT test (Dakopatts). This kit contains a series of beads coated with different, but well-defined, quantities of mouse monoclonal antibody molecules. These beads mimic cells with different antigen densities which have been labelled with a primary mouse monoclonal antibody, isotype IgG, and they serve as a set of standards to calibrate the fluorescence scale of the flow cytometer in units of ABC (Poncelet *et al*, 1996).

For the PE-conjugated VIM5 McAb, Quantum Simply Cellular beads were used to calculate the number of antigens per cell (Poncelet *et al*, 1996).

For each sample the ABC value of the isotypic control was subtracted from the ABC value of the positive cell population. The range of ABC values for the various normal haemopoietic cells and for leukaemic cells was calculated using the QIFIKIT assay.

DNA analysis by flow cytometry. The assessment of cellular DNA content was made with a Facscan flow cytometer (Becton Dickinson), according to a previously published method (Lanza *et al*, 1995). Bromodeoxyuridine incorporation and a monoclonal antibody against bromodeoxyuridine (Becton Dickinson) were used to calculate the size of population of cells in S phase of the cell cycle (Lanza *et al*, 1995).

Purification of the cells. Cells were obtained from the blood of healthy subjects and neutrophils were purified employing the standard techniques of dextran sedimentation (Pharmacia, Uppsala, Sweden), centrifugation on Ficoll-Paque (Pharmacia) and hypotonic lysis of contaminating red cells. The cells were washed twice, resuspended in Krebs-Ringer-phosphate containing 0·1% w/v glucose (KRPG), pH 7·4, at a final concentration of 50×10^6 cells/ml and kept at room temperature until used. The percentage of neutrophils was 98–100% pure and >99% viable as determined by Trypan blue exclusion test.

Random locomotion. Random locomotion was evaluated

using 48-well microchemotaxis chamber (BioProbe, Milan, Italy) (Spisani *et al*, 1996), by estimating the distance (μm) which the leading-front of the cell migrated, using the method of Zigmond & Hirsch (1973), after 90 min incubation at 37°C. A 3 μm pore-size filter (Millipore, Roma, Italy) separated upper and lower compartments.

Chemotaxis. Chemotaxis was studied by adding the chemoattractant to the lower compartment. The chemotactic factors used were: 2 mg/ml casein and 10^{-8} M N-formyl-methionyl-leucyl-phenylalanine (fMLP). Casein ('Hammarsten' Merck, Darmstadt, Germany) stock solution 10 mg/ml in KRPG was diluted before use in KRPG containing 1 mg/ml bovine serum albumin (KRPG-A); fMLP (Sigma Chemical Co., St Louis, Mo., U.S.A.) stock solution 10^{-2} M in dimethylsulphoxide (DMSO) was diluted before use in KRPG-A. The DMSO did not interfere with any of the biological assays performed.

Superoxide anion (O_2^-) production. The superoxide anion production was measured by the superoxide dismutase-inhibitable reduction of ferricytochrome c (Sigma) (Spisani *et al*, 1996) modified for microplate-based assays. The tests were carried out in a final volume of 200 μl containing 4×10^5 neutrophils, 100 nmol cytochrome c and KRPG. At zero time the stimulant was added and the plates (Falcon Microtest III, Becton Dickinson Labware, Milano, Italy) were incubated into a microplate reader (Ceres 900, Bio-TeK instruments Inc.) with the compartment T set at 37°C. Absorbance was recorded at wavelengths of 550 and 468 nm. Differences in absorbance at the two wavelengths were used to calculate nmoles of O_2^- produced, using a millimolar extinction coefficient for cytochrome c of 15·5.

The stimulants employed were phorbol 12-myristate 13-acetate (PMA, Sigma) 100 ng/ml and fMLP 10^{-6} M in KRPG. Neutrophils were preincubated with 5 μg/ml cytochalasin B (Sigma) for 5 min prior to activation by fMLP. Results were expressed as net nmoles of $O_2^-/2\times10^6$ cells/5 min. Assays were currently done in triplicate for each experimental condition. PMA stock solution of 1 mg/ml in DMSO was diluted before use in KRPG.

Granule enzyme assay. Release of neutrophil granule enzymes was evaluated by determining lysozyme activity (Spisani *et al*, 1997) modified for microplate-based assays. 3×10^6 cells were incubated in microplates wells in the presence of the stimulus for 15 min at 37°C. The plates were then centrifuged for 5 min at 400 ***g*** and the lysozyme was quantified nephelometrically by the rate of lysis of cell wall suspension of *Micrococcus lysodeikticus* (Sigma). Reaction rate was measured with a microplate reader at 465 nm. Enzyme was expressed as a net percentage of total enzyme content released by 0·1% Triton X-100. Total enzyme activity was 85 ± 1 μg/1×10^7 cells/min. The degranulating agents used were PMA 0·1 μg/ml and fMLP 10^{-6} M, in KRPG. Cells were preincubated with cytochalasin B 5 μg/ml for 15 min prior to activation by fMLP. Assays were performed in triplicate for each experimental condition.

McAb treatment. When appropriate, cells were incubated at 20°C for 30 min with 3B10 or VIM5 McAbs, washed twice with KRPG and then evaluated for cell functionality. The controls were without McAbs, with an irrelevant mouse IgG1 or IgG2a.

Chromosome analysis. Cytogenetic studies of leukaemic cells were performed at diagnosis to detect the presence of structural or numerical abnormalities. It was based on both a direct technique or a short-term culture. In some cases synchronization with methotrexate and thymidine was carried out. In all cases G-binding was applied, and karyotypes were expressed according to the International System for Human Cytogenetic Nomenclature. The presence of three or more events of translocation or non-disjunction in the same clone was defined as complex karyotype (Castoldi & Cuneo, 1996).

Statistical analysis. UPA-R expression and clinical and biological characteristics were compared using parametric and non-parametric statistics (Student's *t* test, Wilcoxon test, linear regression model).

RESULTS

Quantification of UPA-receptor in normal haemopoietic cells

The reactivity with UPA-R in haemopoietic cells from 15 healthy donors are shown in Table I. Briefly, 3B10 and VIM5

Table I. Flow cytometry quantification of UPA-R expression in normal haemopoietic cells using two different CD87 McAbs.

Cell population	3B10 McAb (% positive cells;* MESF/cell$\times10^3$; ABC/cell$\times10^3$; mean (range)†)	VIM5 McAb (% positive cells;* MESF/cell$\times10^3$; ABC/cell$\times10^3$; mean (range)†)
PB neutrophil granulocytes	99·3%; 32·8 (24·8–37·5); 3·5 (2·9–4·2)	99·1%; 31·2 (24·2–35·7); 3·6 (2·5–4·0)
PB monocytes	99·4%; 31 (19·8–37·1); 3·3 (2·5–3·7)	99·2%; 29·7 (7·4–36·2); 2·9 (2·3–3·6)
BM myeloid precursors	96%; 34·9 (11·9–52·6); 5·5 (3·4–13·6)	97·3%; 28·6 (17·1–45·7); 3·4 (2·8–9·9)
BM monocytic precursors	98·2%; 33·5 (20·5–58·5); 5·0 (3·7–13·5)	97·5%; 30·2 (18·3–48·9); 3·7 (3·0–11·1)
BM CD34$^+$ cells	<0·5%	<0·5%
PB mobilized CD34$^+$ cells	<0·2%	<0·2%

BM, bone marrow; PB, peripheral blood.

*Data are expressed as a mean of positive cases. Cells from 15 healthy adult subjects were analysed.

† Range for ABC and MESF values is referred to positive cases.

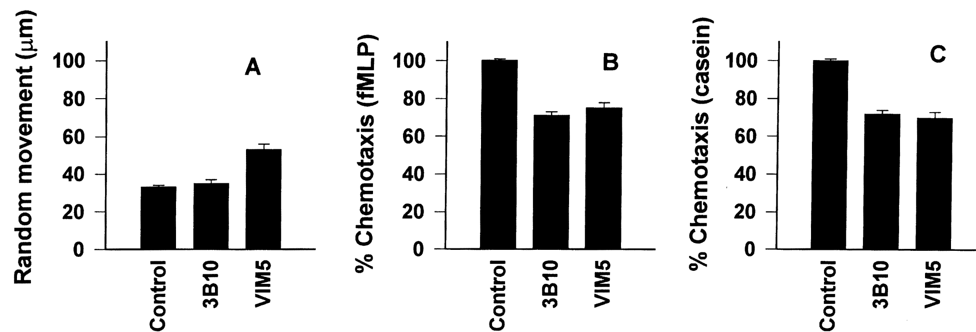

Fig 1. Effect of anti-UPA-R McAbs on neutrophil locomotion. Random locomotion was assayed in the absence of chemotaxins (panel A). Directed locomotion or chemotaxis was evaluated by adding fMLP 10^{-6} M (panel B) or casein 2 mg/ml (panel C) to the lower compartment. The actual control of chemotaxis induced by fMLP or casein was 78 ± 3 μm or 87·6 ± 2 μm, respectively. Each value represents the average ±SEM from eight to 10 separate experiments carried out in triplicate.

McAbs were found to be positive in over 99% of both monocytes and neutrophil granulocytes. Using the reference VIM5 and 3B10 UPA-R McAbs, the number of receptors expressed by monocytes and neutrophil granulocytes was found to be similar, whereas resting B and T lymphocytes were unreactive with both UPA-R McAbs.

As far as the cytofluorimetric expression of UPA-R in bone marrow cells is concerned, we found that myeloid precursors (promyelocytes, myelocytes and metamyelocytes) showed intermediate levels of UPA-R (range of ABC/cell using 3B10: $3{\cdot}4\times10^3$ to $13{\cdot}6\times10^3$ ABC), as demonstrated by one- (using the QIFIKIT assay), two- and three-colour fluorescence analysis (CD13/FITC, UPA-R/PE, CD45/PerCP; HLA-DR/FITC, CD87/PE, CD45/PerCp). In contrast, about 40% of bone marrow neutrophils (mean 42·1% ±13·4 SD) lacked surface UPA-R. Monocytes and their precursors showed similar levels of UPA-R (ABC range $3{\cdot}7\times10^3$ to $13{\cdot}5\times10^3$), as assessed by single and multicolour fluorescence experiments based on the use of the following McAbs: CD45/FITC, UPA-R/PE, CD33/PerCp or CD4/PE-Cy5).

The progenitor cell compartment was defined by the immunophenotypic expression of CD34 antigen. The flow cytometry analysis of purified $CD34^+$ cells obtained from the bone marrow or peripheral blood (collected after G-CSF-induced mobilization of peripheral blood stem cells) of healthy adult subjects showed that >99% of the cells lacked the UPA-R (Table I).

Regarding the reactivity of the two CD87 McAbs used in this study, a positive correlation between expression of VIM5 and 3B10 McAbs in normal cells was found ($r = 0{\cdot}95$).

Detection of cytoplasmic UPA-R

Fixation and permeabilization of normal monocytes and neutrophils increased the amount of detectable UPA-R in all cases examined ($n = 3$). The total amount of UPA-R detected by flow cytometry in the two cell populations was, respectively, 1·8 and 1·3 times higher than its surface expression evaluated on unfixed cells (mean MESF/cell for surface UPA-R of monocytes: $9{\cdot}6\times10^3$; mean MESF/cell for monocyte cyUPA-R: $17{\cdot}4\times10^3$; mean MESF/cell for surface UPA-R of neutrophils: $11{\cdot}1\times10^3$; mean MESF/cell for neutrophil cyUPA-R: $14{\cdot}6\times10^3$), thus revealing intracellular UPA-R pools.

Functional studies

Effects of anti-UPA-R McAbs on neutrophil locomotion. To determine whether neutralization of UPA-R by specific McAbs affects neutrophil locomotion, random movement, chemokinesis and chemotaxis were assessed.

When neutrophils were pretreated with saturating concentrations of McAbs 3B10 or VIM5 for 20 min at 37°C, VIM5 influenced random movement with an increment of about 60% ($P < 0{\cdot}01$) of the basal value of the control (Fig 1A). In addition, when equal concentrations of the chemotaxin fMLP was present in both upper and lower chambers, non-oriented locomotion was significantly activated, whereas the treatment with 3B10 McAb had no effect on cell chemokinesis, as detected by checkerboard analysis (not shown). In similar experiments we evaluated the effect of the treatment with anti-UPA-R on chemotaxis induced by both fMLP and casein.

As shown in Figs 1B and 1C, the two antibodies reduced, in a dose–response way, the chemotaxis activated by both fMLP and casein ($P < 0{\cdot}05$); 3B10 and VIM5 McAbs had no intrinsic chemotactic activity.

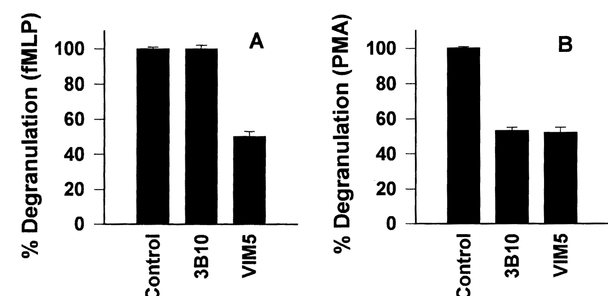

Fig 2. Effect of anti-UPA-R McAbs on neutrophil degranulation. Granule secretion was induced by fMLP 10^{-6} M (panel A) or PMA 100 ng/ml (panel B). The actual control of lysozyme release by fMLP or PMA was 45 ± 4% or 52 ± 5% of release/3×10^6 cells/10 min, respectively. Each value is the average ±SEM from eight to 10 separate experiments carried out in triplicate.

Table II. Flow cytometry quantification of expression levels of UPA-R in acute leukaemic blasts of myeloid and B-lymphoid lineage.

AML FAB subtype‡	3B10 McAb (% positive cases;* MESF/cell × 10^3; ABC/cell × 10^3: mean (range §))	VIM5 McAb (% positive cases;* MESF/cell × 10^3; ABC/cell × 10^3: mean (range))
AML M0 ($n = 7$)	57%; 9·6 (1·5–26·2); 2·0 (1·0–6·9)	43%; 14·0 (0·8–31·4); 2·9 (1·1–7·8)
AML M1 ($n = 12$)	67%; 10·3 (1·8–36·1); 2·5 (1·2–13·6)	50%; 12·9 (1·0–30·2); 2·7 (1·0–11·5)
M2 ($n = 20$)	70%; 8·4 (1·1–36·2); 2·4 (1·1–14·3)	50%; 11·4 (1·4–31·1); 2·6 (1·3–12·9)
M3 ($n = 4$)§	100%; 12·2 (2·1–30·2); 3·0 (1·4–10·1)	75%; 12·8 (1·2–35·1); 3·3 (1·2–11·0)
M4 ($n = 11$)	91%; 21·9 (1·5–53·1); 5·6 (1·2–17·3)	81%; 13·9 (1·2–64·2); 4·5 (1·0–19·5)
M5 ($n = 20$)	100%; 52·7 (6·1–220); 12·9 (2·9–56·4)	100%; 61·2 (5·5–231); 14·4(2·2–60·4)
B-lineage ALL ($n = 24$)	12%; 3·1 (1·3–6·0); 2·0 (0·9–2·8)	12%; 2·3 (1·5–4·6); 1·4 (0·9–2·0)
Biphenotypic acute leukaemia ($n = 3$)	66%; 2·5 (1·7–3·2); 1·3 (0·9–1·5)	33%; 3·0; 1·4

*Data are expressed as mean percentages of positive cases. † ABC range is referred to positive cases. ‡ FAB (French–American–British) subtype. § Cell positivity was evaluated on leukaemic promyelocytes.

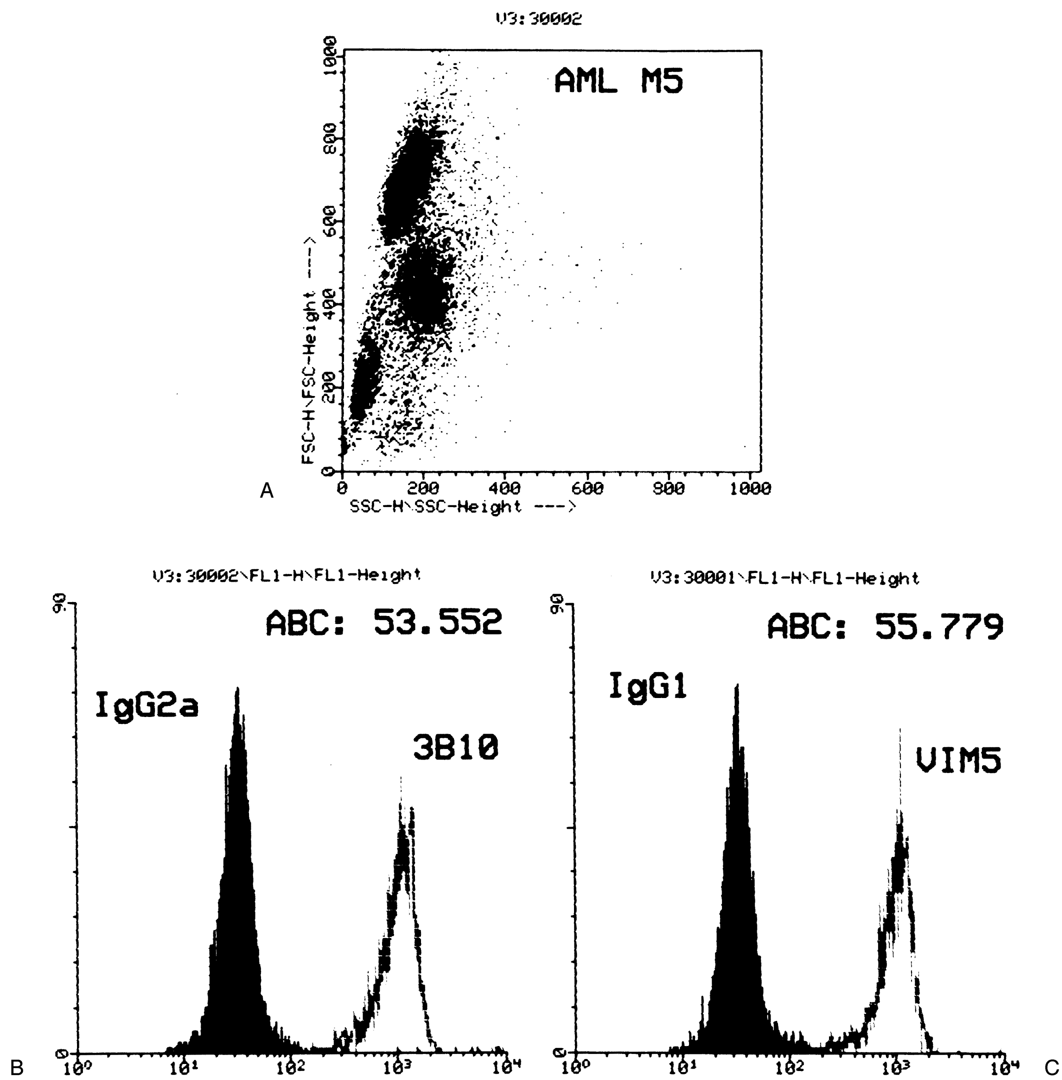

Fig 3. Flow cytometry analysis of UPA-R on blasts from a patient with AML M5. (A) Dot plot showing the light scattering characteristics of the cells analysed. Histogram distribution of cells stained with 3B10 (B) and VIM5 (C) McAb. IgG2a and IgG1 = isotypic controls; ABC = antibody binding capacity.

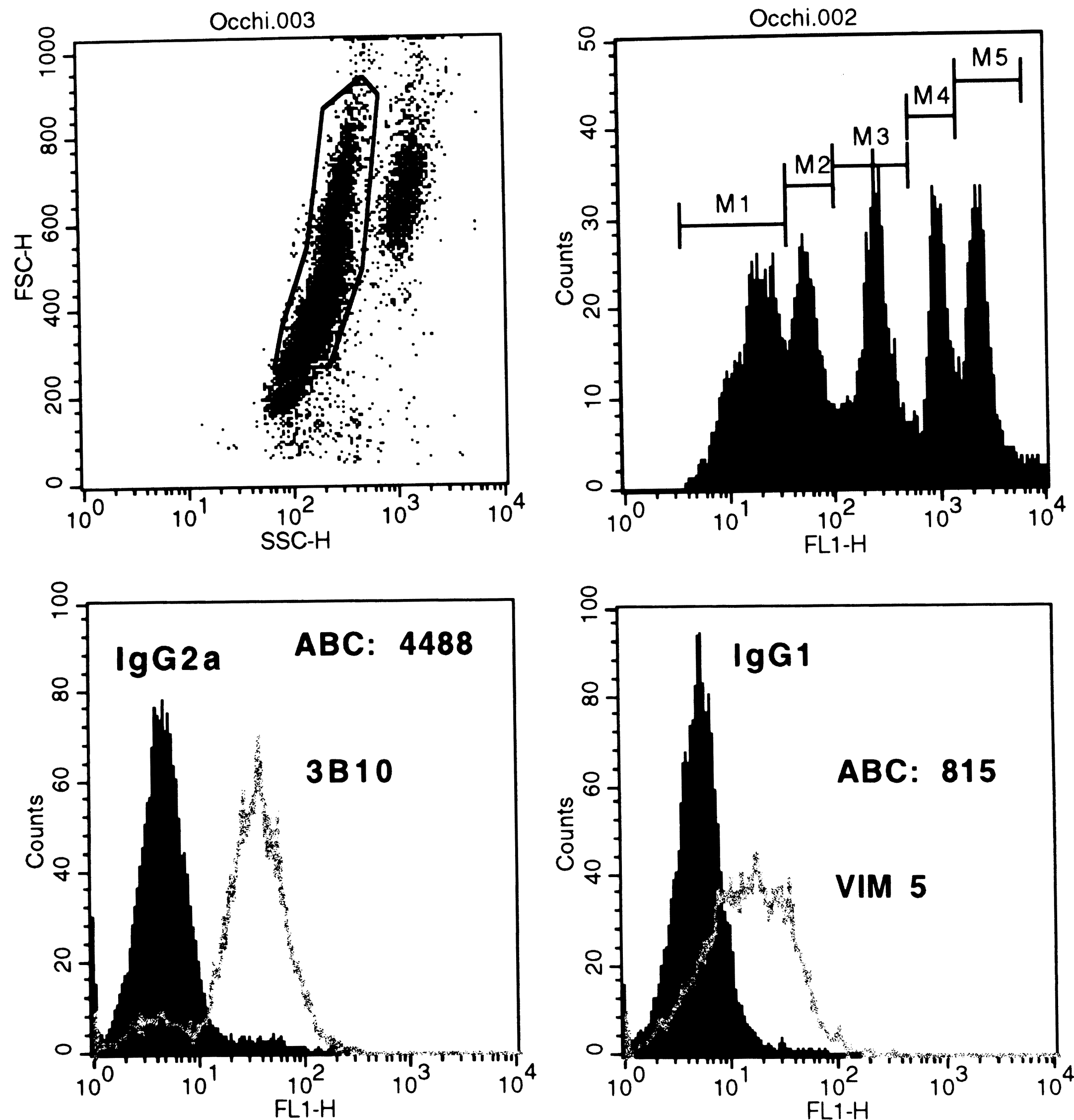

Fig 4. Quantitative analysis of UPA-R on leukaemic cells from a patient with AML FAB M0. Upper left panel: dot plot generated by combining side scatter versus forward scatter (logarithmic scale); upper right panel: calibration curve generated using Dako QIFIKIT microbeads. Histogram distribution of cells stained with 3B10 (lower left panel) and VIM5 (lower right panel) McAb. A discordant reactivity of the two UPA-R McAbs was observed. IgG2a and IgG1= isotypic controls; ABC = antibody binding capacity.

Effects of anti-UPA-R on enzyme degranulation. The capacity of anti-UPA-R McAbs to affect the release of lysozyme from specific granules was assessed. As shown in Fig 2A, the response to fMLP stimulation seemed dependent on McAb specificity, since only VIM5 exhibited a 50% inhibition ($P<0{\cdot}05$) in enzyme exocytosis, whereas the response to PMA stimulation (Fig 2B) showed a significant reduction ($P<0{\cdot}01$) in degranulation in cells treated with 3B10 or VIM5 McAbs. The two McAbs did not affect enzyme release from granules, in the absence of stimuli.

Effects of anti-UPA-R on neutrophil superoxide production. To elucidate whether neutrophil respiratory burst depended on the presence of UPA-R, cells were treated with McAbs and then stimulated by fMLP and PMA for 5 min and 10 min, respectively. No difference in superoxide anion generation was seen in these Mc-Ab-treated cells (not shown).

Quantitative analysis of UPA-R expression in acute leukaemic blasts

Acute myeloid leukaemia. Cell positivity for UPA-R varied significantly from case to case, depending on the FAB subtype, type of anti-UPA-R McAb used, clinical phase of the disease, and type of the sample analysed (peripheral blood *v* bone marrow). By looking at the expression of CD87 in the whole series of AML patients ($n=74$), we found that 81% of AML patients showed reactivity for the 3B10 McAb, and VIM5 McAb was positive in 69% of the cases examined

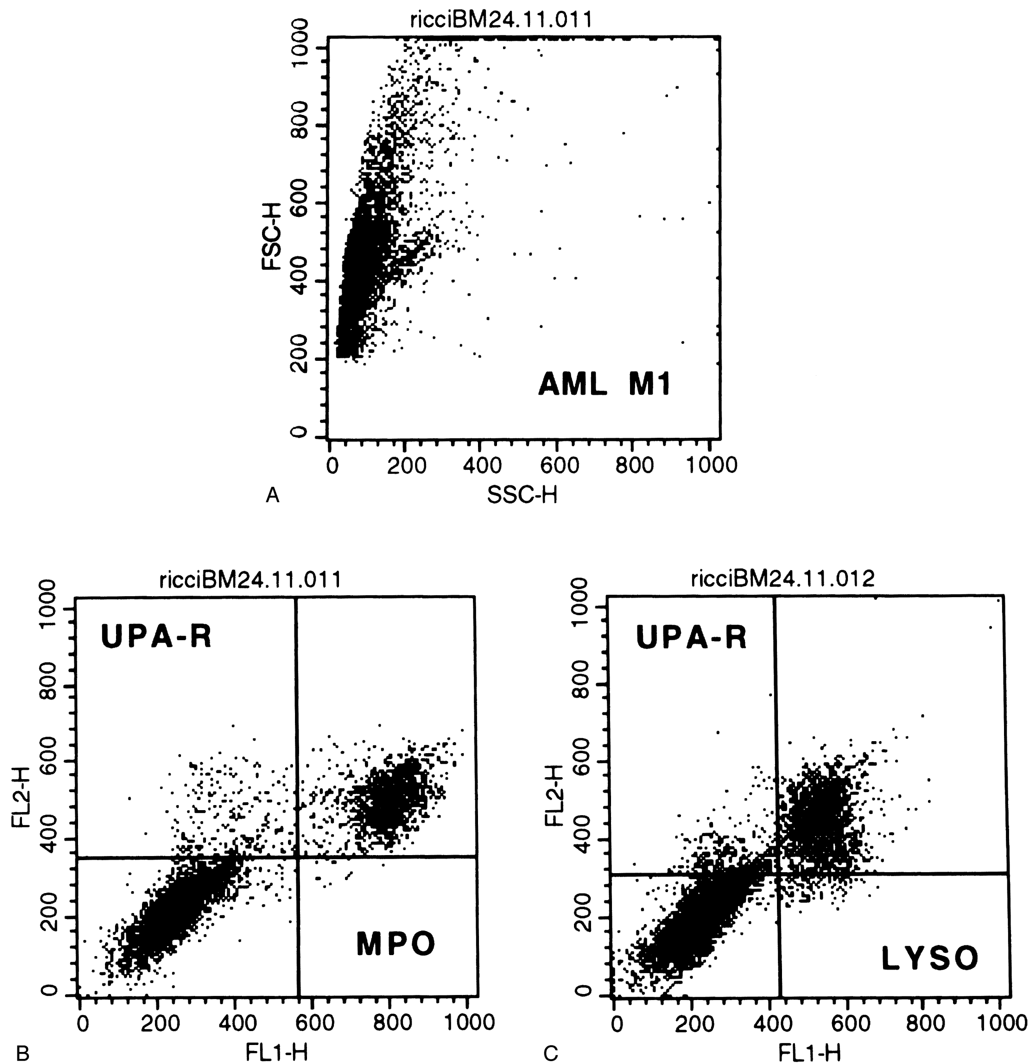

Fig 5. Flow cytometry dot plots of AML cells (FAB M1). Myeloperoxidase (MPO) and UPA-R (VIM5 McAb) antigen dual staining showing a strong positivity for the two markers in 51% of the cells (B). (C) Two-colour fluorescence analysis using lysozyme/FITC and UPA-R-VIM 5/PE showed a weak-intermediate expression for lysozyme on leukaemic cells derived from the same patient described in panels A and B. Panel A shows the light-scattering characteristics of the same cells.

(Table II). Among the positive cases (range of ABC/cell: $1{\cdot}0\times10^3$ to $60{\cdot}4\times10^3$) the number of UPA-R$^+$ blasts was similar, with 3B10 and VIM5 McAbs (mean percentage of positivity: $76{\cdot}6\pm39{\cdot}1$ SD, and $69{\cdot}2\pm36{\cdot}6$ SD, respectively), irrespective of the FAB subtype (Table II).

The highest expression of UPA-R was seen in patients classified as M5 FAB subvariety (mean ABC/cell for the 3B10 McAb: $12{\cdot}9\times10^3$; mean ABC/cell for VIM5 McAb: $13{\cdot}4\times10^3$) (Fig 3), whereas the lowest expression was found in the M0 FAB category (mean ABC for 3B10: $2{\cdot}0\times10^3$) (Fig 4). Two- and three-colour analyses further revealed that >70% UPA-R$^+$ blasts from >60% patients belonging to the M1–M2 FAB subtype showed expression for CD34, c-kit, HLA-DR, CD13, CD33, CD38, MPO^{high}, lysozyme^{low} antigens (Fig 5). Within the M4 and M5 FAB subtypes, UPA-R$^+$ blasts co-expressed CD11c, CD14, CD13^{low}, CD33^{high}, lysozyme^{high}, myeloperoxidase (MPO)$^{low/negative}$ in most cases (Fig 6).

Regarding the reactivity of the two CD87 McAbs used in this study, a positive correlation between the expression of VIM5 and of 3B10 McAbs in leukaemic cells was found ($r=0{\cdot}87$). However, in five cases (AML, $n=4$; ALL, $n=1$) the number of UPA-R$^+$ blasts detected by the two CD87 McAbs was significantly different (Fig 4).

The quantitative analysis of UPA-R in acute leukaemia further showed that the number of receptors expressed by leukaemic cells from 6/74 (8·1%) AML patients was above the highest values seen in normal samples (>15×10^3 ABC/cell; range: $22{\cdot}6\times10^3$ to $60{\cdot}4\times10^3$). All these cases were classified as AML, M5 FAB subtype (Table II, Fig 3).

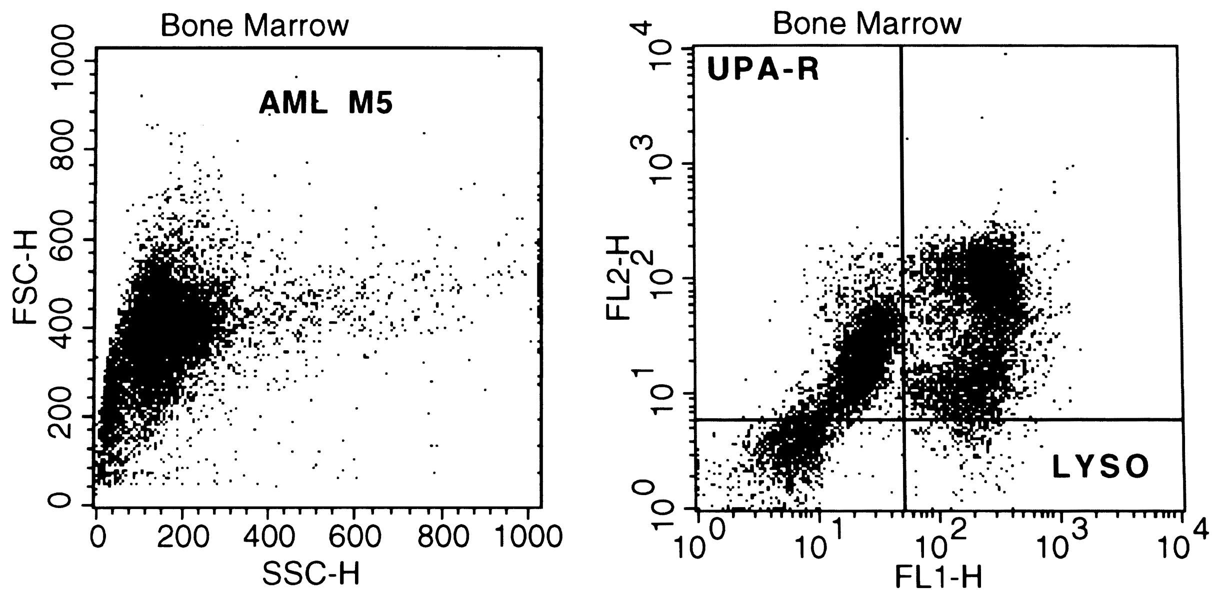

Fig 6. Flow cytometry dot plots of AML bone marrow cells (FAB M5). Left panel: light scattering properties of bone marrow cells from the AML patient. Right panel: lysozyme and UPA-R (VIM5 McAb) antigen dual staining showing a strong positivity for the two markers in 48% of the cells. The same cells (UPA-R$^+$/Lyso$^+$) resulted MPO negative. Lyso = lysozyme McAb.

Furthermore, CD34 antigen was expressed (>20%) by CD87$^+$ leukaemic blasts from 16 patients (20%) with AML. Coexpression of CD34 and UPA-R was associated with FAB M5 morphology (three cases), FAB M0 (two cases), FAB M1 (five cases), FAB M2 (three cases) and FAB M4 (two cases).

Interestingly, in six AML patients, a comparative analysis of UPA-R expression between blood and bone marrow blasts revealed the number of UPA-R expressed by peripheral blood leukaemic cells to be significantly higher (mean ABC/cell for 3B10 McAb: $14{\cdot}7 \times 10^3$; range ABC/cell: $6{\cdot}2$–$45{\cdot}2 \times 10^3$) than that of bone marrow cells (mean ABC/cell: $5{\cdot}8 \times 10^3$; range ABC/cell: $3{\cdot}5$–$24{\cdot}1 \times 10^3$). A similar pattern was observed for the percentage of positivity for UPA-R, which was higher in blood blasts (95%) than in bone marrow cells (61%), despite the higher proportion of acute leukaemic blasts (as assessed by morphology and multicolour analysis with a flow cytometer) in the bone marrow.

Furthermore, in five AML patients (FAB M1: two cases; M4: two cases; M5: one case), relapsed disease following induction-consolidation chemotherapy was characterized by a marked increase in the number of UPA-R (mean pre-treatment ABC values for 3B10 McAb: $3{\cdot}9 \times 10^3$/cell; mean ABC values for 3B10 McAb in relapsed disease: $7{\cdot}1 \times 10^3$/cell). No change in the FAB morphology was noticed in relapsed disease.

The statistical analysis of our data did not show any correlation between number of UPA-R, cell cycle phases, and/or DNA content of AML cells (data not shown).

Clinical and cytogenetic characteristics of AML with higher expression of UPA-R. On the basis of the expression levels for CD87 in leukaemic cells, it was possible to identify 14 AML patients whose blasts had UPA-receptors of more than 12×10^3/cell. 57% (8/14 cases) of these patients had FAB M5 morphology, whereas the remaining 43% were classified as M1 ($n = 1$), M2 ($n = 2$), M3 ($n = 1$), or M4 ($n = 2$) FAB categories; they showed multiple mucocutaneous infiltrative lesions ($n = 10$), hepatosplenomegaly ($n = 11$), generalized peripheral lymphadenopathies ($n = 3$), musculo-skeletal involvement ($n = 2$), infiltration of Waldeyer ring, tonsillitis, pharyngitis ($n = 4$), central nervous system and/or meningeal involvement ($n = 4$) and a higher leucocyte count (>40×10^9/l; mean 59×10^9/l). The frequency of these clinical manifestations was significantly higher in the patient group with bright expression of UPA-R$^+$, compared to AML cells with lower levels of protease (Table III).

Clonal chromosome changes were seen in all but one of the 14 AML patients with UPA-R^{bright} phenotype (>12×10^3 ABC/cell), seven of whom had complex karyotypes (three or more concomitant chromosome abnormalities). Unidentified marker chromosomes were found in four patients (29%). The 13 cases with karyotype abnormalities included six cases with involvement of chromosome 11 (11 q−; t(9;11), +11); four with abnormalities of chromosome 5 (−5; 5q+), three with involvement of chromosome 13 and/or chromosome 3 (3q−, inv 3), or chromosome 10 (10p, +10). Aberrations of chromosomes 2, 4, 8, 9,14, 15, 16, 17 and 22 were seen in only one or two patients.

In contrast, only 71% of 60 AML patients having either low or no expression for UPA-R showed abnormal karyotypes, of which seven (11%) had complex karyotypes.

The incidence of chromosome changes in the AML group with high UPA-R expression was significantly higher than that of the UPA-R$^{dim/neg}$ AML subgroup ($P < 0{\cdot}001$). Furthermore, since it has been shown that the CD87 gene is localized on chromosome 19, band q13, we

Table III. Patient characteristics in UPA-$R^{+\ bright}$ ($>12\times10^3$ ABC/cell) and UPA-$R^{neg/low}$ ($<12\times10^3$ ABC/cell) AML.

	UPA-$R^{+\ bright}$ ($>12\times10^3$ ABC/cell) AML	*P* value	UPA-$R^{neg/low}$ ($<12\times10^3$ ABC/cell) AML
No. of cases	14		60
Male/female	8/6	ns	34/26
WBC $\times10^9$/l (median)	59	0·0001	14
Plt $\times10^9$/l (median)	41	ns	47
Hb (g/dl) (median)	8·1	ns	7·8
FAB M5 subtype (*n* (%))	8 (57%)	0·01	12 (20%)
Multiple mucocutaneous infiltrative lesions (*n* (%))	10 (71%)	0·001	12 (20%)
Hepatosplenomegaly (*n* (%))	11(79%)	0·005	20 (33%)
Generalized lymphadenopathy (*n* (%))	3 (21%)	0·01	1 (1·6%)
CNS involvement (*n* (%))	3 (21%)	0·001	None
Meningeal involvement (*n* (%))	4 (29%)	0·001	1 (1·6%)
Musculo-skeletal involvement (*n* (%))	2 (14%)	0·01	None
Waldeyer's ring involvement (*n* (%))	4 (29%)	0·001	2 (3%)

ns = not significant.

looked for possible correlations between UPA-R expression and involvement of chromosome 19. Abnormalities of chromosome 19 were solely observed in the UPA-R negative/low AML group (mean ABC/cell: $1·6\times10^3$/cell); range: $1·0–2·5\times10^3$/cell), where 5/21 cases (25%) revealed this aberration either alone (+19: four cases; inversion-insertion (19) (q13 p13 p12): one case) or in combination with other chromosome changes. On the contrary, none of the AML patients having higher UPA-R expression showed alterations of chromosome 19.

The frequencies of the more common chromosome aberrations in UPA-$R^{+bright}$ *v* UPA-$R^{neg/low}$ AML are given in Table IV.

Detection of cytoplasmic UPA-R. Fixation and permeabilization of leukaemic cells from AML patients increased the amount of detectable UPA-R in 70% of the cases studied. Using 3B10 McAb, the total amount of UPA-R detected by flow cytometry was 3·3 times higher than its surface expression evaluated on unfixed cells (mean MESF/cell for surface UPA-R: $2·9\times10^3$/cell; mean MESF/cell for UPA-R cytoplasmic pool: $9·7\times10^3$/cell), thus revealing intracellular UPA-R pools.

Table IV. Cytogenetic features in UPA-$R^{+\ bright}$ ($>12\times10^3$ ABC/cell) and UPA-$R^{neg/low}$ ($<12\times10^3$ ABC/cell) AML.

	UPA-$R^{+\ bright}$ ($>12\times10^3$ ABC/cell) AML ($n=14$)		UPA-$R^{-neg/low}$ ($<12\times10^3$ ABC/cell) AML ($n=60$)	
Karyotype	No. of cases (FAB category)	%	No. of cases (FAB category)	%
Overall frequency of chromosome abnormalities	13/14	93%	43/60	71%
Normal	1/14	7%	17/60	29%
t(8;21)(q22;q22)	—	—	4/60 (M2)	7%
t(15;17) (q21;q21)	1/14 (M3)	7%	3/60 (M3)	5%
11q−/t(9;11)	4/14 (M5)	28%	6/60 (M5)	10%
13q abnormalities	1/14 (M5)	7%	5/60 (M1, M2, M4)	8%
−5/5q−	4/14 (M0, M1, M4)	29%	9/60 (M0, M1, M4)	15%
−7/7q−	1/14 (M1)	7%	8/60 (M0, M1, M2, M4)	13%
+8	1/14 (M5)	7%	8/60 (M0, M1, M2, M4, M5)	13%
Complex karyotype *	7/14 (M0, M1, M5)	50%	7/60 (M0, M1, M4, M5)	11%
Abnormalities of chromosome 19†	—	—	5/60 (M1, M2, M4, M5)	8%

* In several cases the single chromosome aberrations were associated with multiple chromosome changes, thus forming a complex karyotype.

† +19; inv ins(19)(q13;p13;p12), as additional aberration within a complex karyotype.

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Acute lymphoblastic leukaemia. 12% (3/24) of ALL cases were UPA-R positive (Table II). However, the number of receptors expressed by leukaemic blasts from all ALL cases was extremely low ($<3\times10^3$ ABC/cell). One patient showed co-expression of myeloid (CD13 and CD33) and lymphoid markers (CD10, CD19, CD79α, CD22), and was classified as common-type ALL with minimal phenotypic deviation, according to the EGIL criteria. The other two cases had a pro-B phenotype (CD19$^+$, cyCD22$^+$, CD79α^+, CD34$^+$, CD10$^-$, cμ^-, SIg$^-$); however, lymphoid blasts were CD13$^+$ in one patient.

Biphenotypic leukaemias. Three cases were classified as byphenotypic acute leukaemia: these comprised two cases with a myeloid and B-lymphoid phenotype and one case with a T-lymphoid and myeloid phenotype. All these cases had scores higher than 2 for the lymphoid and myeloid lineages, according to the criteria of the EGIL group. Two out of three cases were UPA-R positive using 3B10 McAb, but the level of antigen expression was low (mean ABC/cell $1{\cdot}2\times10^3$) (Table II).

DISCUSSION

The cellular expression and clinical significance of the UPA receptor was investigated in acute leukaemic blasts and normal haemopoietic cells through the use of a flow cytometer and appropriate standards, which enabled a reliable measurement of antibody binding capacity and MESF values in the various cell types.

In previous studies it has been shown that UPA-R may be involved in cellular extravasion and in metastasis formation (Cao *et al*, 1995; Todd & Petty, 1997). In leukaemia, overexpression of UPA-R and/or other components of this system was found to consume plasma inhibitors, producing a tendency to haemorrhage (Todd *et al*, 1995; Lijnen, 1996; Bennett *et al*, 1997). The evidence that UPA-R and its ligand UPA are involved in the metastatic potential of tumour cells derives from a wide range of observations, including (i) a close relationship between oncogenic transformation and UPA synthesis, and between UPA and cellular invasion as well as metastasis in several model systems (melanoma, neuroblastoma, breast, colon and prostate carcinoma, etc.); (ii) the inhibition of these processes by anticatalytic McAbs to UPA; and (iii) the histochemical localization of UPA at the invasive fronts of tumours (Bu *et al*, 1994; Lijnen, 1996; Todd *et al*, 1995). However, little is known about the cellular expression and clinical significance of the UPA-receptor in leukaemic cells from patients with acute leukaemia (Knapp *et al*, 1994).

In this study we have demonstrated that both neutrophil granulocytes and monocytes taken from the peripheral blood of healthy subjects had detectable levels of UPA-R, whereas resting B and T lymphocytes lacked CD87 in all the cases examined. In healthy subjects, reactivity for UPA-R McAb was also detected on bone marrow CD34-negative myelomonocytic precursors (promyelocytes, myelocytes, metamyelocytes and promonocytes), whereas BM and mobilized peripheral blood CD34$^+$ progenitors were UPA-R negative, supporting the concept that UPA-R expression is a stage-specific feature of late myelomonocytic cells. The lack of UPA-R in a significant number of bone marrow neutrophil granulocytes may be explained by the peculiar homing characteristics of this cell subset, possibly correlated to an unfinished degranulation of surface proteins.

In a second set of experiments we attempted to confirm whether UPA-R had a role in mediating neutrophil functions (Gyetko *et al*, 1994, 1995). Oriented locomotion induced by different chemotaxins, such as fMLP and denatured casein, was significatively diminished when UPA-R was neutralized by McAbs. Similarly, lysozyme release by granules stimulated with fMLP or PMA was markedly blocked. The inhibitory effect does not depend on either the presence of membrane agonist receptor or the intracellular pathway activated by chemotaxins. In fact, although fMLP possesses at least two receptor isoforms (Ye & Boulay, 1997) (each one able to induce a specific biological response), denatured peptides or proteins, such as casein, could recognize neutrophil surface by aspecific hydrophobic interactions. These results, however, further support those of Gyetko *et al* (1994), indicating that UPA-R has a structural and functional role in the cytoskeleton reorganization required for cell activation.

The observation that UPA-R, in our hands, seemed not to be involved in NADPH oxidase activation was not in contrast with the findings of Cao *et al* (1995) who showed the priming effect of UPA of fMLP-triggered superoxide generation, since in our assay conditions, cytochalasin B was added to potentiate the ability of fMLP to produce superoxide anion, and probably the priming effect mediated by UPA-R ligands was dimmed. Moreover, the amount of O_2^- produced was determined at 5 min, and not at 30 min, when stimulated with fMLP. This observation, together with the lack of UPA-R involvement in NADPH activation, may exclude a receptor–receptor association able to block the second messenger pathway, but may support the hypothesis that UPA-R is involved in the cytoskeletal reorganization required for cell movements, namely locomotion and degranulation (Gyetko *et al*, 1994).

As far as the expression of UPA-R in blast cells is concerned, we found that 81% of AML patients showed reactivity for the anti-UPA-R 3B10 McAb, but only 67% for VIM5 McAb. The number of positive cases and the level of expression was significantly higher in AML patients belonging to the FAB M5 subgroup, thus supporting the concept that a close association between monocytic commitment and expression of UPA-R may exist. Experiments of two- and three-colour fluorescence further showed that FAB M1–M3 AML were characterized by low-intermediate expression for UPA-R and lysozyme, and bright-intermediate expression for MPO, whereas the M5 FAB subvariety had high-intermediate levels of UPA-R and lysozyme, and little or no expression of MPO. On the basis of these data, it can be postulated that the combined use of UPA-R, MPO and lysozyme McAbs may provide useful information for the diagnosis of acute leukaemia, and for the distinction of myeloid from monocyte leukaemias (Knapp *et al*, 1994; Lanza *et al*, 1997b).

Interestingly, since the co-expression of UPA-R and CD34 was not found in normal haemopoietic cells, this marker combination could be used for detecting leukaemic cells in

remission bone marrows and in peripheral blood leukaphersis products. Furthermore, the number of UPA-R expressed by leukaemic cells from 8·1% of AML patients was above the highest values seen in normal samples, making it a feasible marker, even alone, for monitoring the minimal residual disease (MRD) in a subset of AML. Concerning the expression of these markers in AML with minimal evidence of myeloid differentiation, we found that four out of seven cases of FAB M0 AML were positive for the UPA-R McAb. Only one of them showed reactivity for the anti-MPO McAb, and none for lysozyme, thus indicating that CD87 may have a role in the recognition of FAB M0 AML.

We have also shown that the great majority of leukaemic blasts from AML patients had intracellular stores for UPA-R, as demonstrated by the comparison of surface expression evaluated on unfixed cells and total antigen content in permeabilized cells. This finding is in agreement with the observation that, in resting neutrophils, UPA-R are stored in three distinct intracellular compartments (secretory vesicles, specific granules and gelatinase granules) (Plesner *et al*, 1994; Borregaard & Cowland, 1997). Functional studies have also shown that, after activation, the intracellular pools of UPA-R may translocate to the cell surface (Plesner *et al*, 1994; Borregaard & Cowland, 1997). Our own studies provide evidence that both neutrophils and monocytes contain an intracellular pool of UPA-R detectable by flow cytometry on permeabilized cells.

Another interesting finding, which may have clinical implications, is represented by the marked increase in the number of UPA-R receptors in relapsed disease after induction-consolidation chemotherapy, thus indicating that the quantitative analysis of this receptor may be of value for the monitoring of AML patients.

No correlation was found between number of UPA-R, kinetic status and DNA content, whereas the frequency of chromosome abnormalities was much higher in AML cells overexpressing UPA-R than in AML blasts having little or no expression of UPA-R, indicating that the expression of this protease may increase the aggressiveness of the disease.

In contrast, only 12% (3/24) of ALL patients were CD87 positive; however, the degree of positivity was considerably weaker in ALL blasts than in AML cells, allowing us to speculate that this molecule may help in the distinction of acute myeloid leukaemias from lymphoid malignancies. However, the specificity of this marker for the myeloid commitment of the blasts needs to be confirmed using larger numbers of acute leukaemia patients. Furthermore, two out of three byphenotypic leukaemias and one out of three cases of AML with minimal phenotypic deviation showed positivity for CD87. The diagnostic usefulness of UPA-R McAbs in the classification of byphenotypic and undifferentiated acute leukaemias deserves careful evaluation in prospective future studies.

A close analysis of our data further shows a certain variability in the reactivity of leukaemic cells with the two UPA-R McAbs. The significance of this discrepancy is uncertain; nevertheless, it is possible to speculate that this group of McAbs recognizes a distinct epitope of the molecule (Todd *et al*, 1995). Competitive binding assays and extensive immunoprecipitation studies have confirmed the recognition of different epitopes in close proximity of, or within, the UPA binding domain of the urokinase receptor (Garni-Wagner & Todd, 1995); the biological relevance of such a finding is still under evaluation.

A possible correlation between UPA-R expression and the clinical features of acute leukaemias was observed in patients having a number of UPA-receptors superior to 12×10^3 ABC/cell who, irrespective of the FAB category, showed a greater tendency to cutaneous and tissue infiltration, together with a higher leucocyte count, as compared to AML patients whose blasts were UPA-R negative or dimly positive. Since proteolytic enzymes, such as UPA, play a key role in the dissolution of the extracellular matrix and in facilitating the cell egress from the bone marrow, it is conceivable that the expression of the UPA-R could contribute to the invasive properties and, possibly, the metastatic potential of leukaemic cells in a subset of AML. Further evidence in favour of the role of UPA-R in facilitating leukaemic cell extravasation from the bone marrow into the circulation derives from the demonstration that both the percentage of positivity and the surface antigen density of UPA-R was significantly higher in peripheral blood AML cells than in bone marrow blasts.

Furthermore, the bright expression of UPA-R on AML blasts was associated with a high incidence of chromosome abnormalities, most of which were complex. Interestingly, within M5 AML patients, who were cytogenetically characterized by either the t(9;11) or 11q− abnormalities, two groups could be recognized, i.e. those who showed a strong expression of UPA-R and those whose blast cells were dimly positive for UPA-R. However, since in the M5 FAB group, patients carrying 11q− abnormalities have a variable clinical outcome, It can be speculated that the combination of higher UPA-R positivity, abnormalities of chromosome 11 and M5 FAB morphology may identify a peculiar subset of AML characterized by distinct morpho-immunological and cytogenetic features, and a more aggressive clinical course. Moreover, although the incidence of complex karyotypes was significantly higher in the AML patient group with UPA-R^{bright} phenotype in comparison with that of UPA-$R^{neg/dim}$ AML, no correlation was found between FAB category and the occurrence of complex chromosome aberrations.

In conclusion, cellular UPA-R expression was variable, ranged from undetectable (ALL and a minority of AML) to very high intensities in M5 AML, and was also documented in >50% M0 AML, thus supporting the concept that UPA-R may be considered a specific and rather sensitive marker to detect leukaemic cells committed to the myeloid lineage. As a consequence, the inclusion of this marker for the immunophenotype diagnosis of acute leukaemia is recommended, even if a closer analysis of its expression in a large prospective study is needed before drawing any definitive conclusion on this matter.

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