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Intracellular Location of T200 and Mo1 Glycoproteins in Human Neutrophils*

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Mo1 (CD11b), a glycoprotein heterodimer that is involved in cellular adhesion processes and functions as the C3bi receptor of human myeloid cells, and T200 (CD45), a panleukocyte glycoprotein family whose function is still not well understood, increased their expression in the plasma membrane of human neutrophils after exposure to various stimuli which induce degranulation, such as formylmethionylleucylphenylalanine or calcium ionophore A23187. This increment in the expression of both molecules shows a good correlation with the release to the extracellular environment of gelatinase, a marker for an intracellular organelle named "tertiary granule" (Mollinedo, F., and Schneider, D. L. (1984) *J. Biol. Chem.* 259, 7143–7150). Flow cytometry studies indicate that at least 50% of the total Mo1 and T200 molecules are located in intracellular organelles. Furthermore, the subcellular distribution of Mo1 and T200 glycoproteins in resting human neutrophils was investigated by immunoprecipitation of the radiolabeled membrane proteins obtained from the distinct subcellular fractions. Both Mo1 and T200 were mainly localized in tertiary or specific intracellular granules, which were resolved from the azurophilic granules as well as from the cell membrane fraction. These findings suggest that the mobilization of intracellular Mo1 and T200 to the plasma membrane may regulate early events occurring upon neutrophil activation.

The leukocyte-common antigen (LCA), or T200 (CD45), is a family of high molecular weight cell surface glycoproteins expressed by cells of hematopoietic origin, except by erythroid mature cells (1–3). In human peripheral blood mononuclear cells, the T200 complex is comprised of four members with molecular weights of 220,000, 205,000, 190,000, and 180,000 (4–6), whereas in human neutrophils, only the lower molecular weight members are present. Although most speculation has centered on the possibility that T200 plays some role in T- or B-cell differentiation, NK and CTL activities, and other T-cell functions (7–13), its cellular function remains to be elucidated.

The leukocyte surface also contains a heterodimeric glycoprotein family formed by a common β subunit of 95 kDa (CD18) noncovalently associated with distinct α subunits of 177, 165, and 150 kDa designated as LFA-1 (CD11a), Mo1 or Mac-1 (CD11b), and gp150 (CD11c), respectively (5, 14–17). The Mo1 molecule functions as the receptor for C3bi opsonized particles (18–20) and plays a role in several aspects of cellular adhesion, such as adherence, spreading, chemotaxis (21), and neutrophil aggregation (22). Recent evidence (23–27) indicates that distinct stimuli mobilize intracellular stores of Mo1 to the cellular surface, suggesting that increased surface expression of Mo1 mediates neutrophil adherence to endothelial cells and other substrates, as well as neutrophil aggregation. Previous reports (24, 25, 28) supported a specific granule location for the intracellular pool of Mo1 and gp150. However, the observation that up-regulation of Mo1 can occur under conditions which do not induce secretion of specific granule contents (27) suggests a putative intracellular Mo1 localization in another cytoplasmic organelle.

The presence of a tertiary granule in human neutrophils has been recently reported (29–31), in addition to the azurophilic (primary) and specific (secondary) granules previously described (32, 33). The tertiary granule contains part of the neutrophil respiratory burst machinery (30) and gelatinase as a granule marker (29, 31). Isolation of this tertiary granule is difficult to achieve due to its nearly identical density to specific granules, and certain fractionation conditions using sedimentation velocity are required to resolve each organelle (29–31).

Here, we show that different stimuli induce a rapid increase in neutrophil surface expression of Mo1 and T200 glycoproteins, which correlates with gelatinase release to the extracellular environment. Furthermore, by subcellular fractionation and immunoprecipitation studies, we have found that Mo1 and T200 molecules are constituents not only of neutrophil plasma membranes, but also of intracellular granules, which in fact contain a high proportion of these glycoproteins.

MATERIALS AND METHODS

Chemicals—$Na^{125}I$ was purchased from Amersham Corp. Acrylamide, bisacrylamide, ammonium persulfate, and TEMED[1] were from Bio-Rad. Protein A-Sepharose CL-4B was from Pharmacia LKB Biotechnology Inc. The ionophore A23187 was from Behring Diagnostics. Dextran, cytochalasin B, formylmethionylleucylphenylalanine (FMLP), and all the substrates and reagents for enzymatic assays were purchased from Sigma. All other chemicals were of the best quality available from Merck.

Monoclonal Antibodies—Monoclonal antibodies were used as hybridoma culture supernatants. Anti-Mo1 chain (CD11b) monoclonal antibody was Bear 1 (34). Anti-T200 (CD45) monoclonal antibody

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[1] The abbreviations used are: TEMED, *N,N,N′,N′*-tetramethylethylenediamine; FMLP, formylmethionylleucylphenylalanine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate.

was D3/9 (11), which immunoprecipitates all members of the T200 complex. Anti-β_2-microglobulin monoclonal antibody was FG2/2 (35). P3x63 myeloma culture supernatant was used as negative control.

Neutrophil Isolation—Neutrophils were prepared from fresh human blood as described (30, 36), with a slight modification of the procedure. After sedimentation of erythrocytes in 1.3% (w/v) dextran at room temperature, the leukocyte-rich supernatant was siphoned off and centrifuged at 300 × *g*. The pellet was gently resuspended in phosphate-buffered saline, loaded into a layer of Lymphoprep (Nyegaard & Co., A/S, Oslo, Norway), and centrifuged at 400 × *g* for 40 min. The neutrophil-enriched pellet was subsequently purified by hypotonic lysis of erythrocytes, giving a purity higher than 98%.

Subcellular Fractionation of Neutrophils—Neutrophils were fractionated after cell disruption in a Potter-Elvehjem homogenizer as described (30), with a slight modification of the procedure. A part of the postnuclear fraction (6 ml) was layered onto a 27-ml, 15–40% (w/w) continuous sucrose gradient with a 1-ml cushion of 60% sucrose and centrifuged in an SW 27 rotor at 76,000 × *g* for 15 min at 4 °C in a Beckman L8-70B ultracentrifuge. Subsequently, fractions (the first one of 6 ml and the remaining seven of 4 ml) were collected by pumping 60% sucrose into the bottom. Each fraction was diluted with 50 mM Tris-HCl, pH 8.0, 100 mM NaCl and centrifuged at 70,000 × *g* for 90 min at 4 °C in a 30-type rotor. The pellets, representing the membranous fractions, were resuspended in 50 mM Tris-HCl, pH 7.5, and stored at −70°C.

Neutrophil Activation—Cells were resuspended at 3 × 10^6 neutrophils/ml in Hepes/glucose buffer (150 mM NaCl, 5 mM KOH, 10 mM Hepes, 1.2 mM $MgCl_2$, 1.3 mM $CaCl_2$, 5.5 mM glucose, pH 7.5) and preincubated at 37 °C for 5 min with 5 μg/ml cytochalasin B. Then, FMLP or ionophore A23187, at the concentrations indicated in the respective figures, were added as stimulating agents for 10 min at 37 °C. Subsequently, cells were pelleted by centrifugation at 300 × *g* for 10 min. Supernatants were saved to determine the release of enzyme markers for each cytoplasmic granule, and the sedimented cells were saved to carry out the measurement of different cell-surface antigens by flow cytometry. Cells held at 4 °C or incubated at 37 °C in the presence of 5 μg/ml cytochalasin B, but in the absence of any stimulus, were run in parallel and processed as described above.

Enzyme Activities and Protein Determinations—Lysozyme, β-glucuronidase, alkaline phosphatase, and gelatinase activities were assayed as described (36). Protein determination was carried out by the Bradford method (37) using a Bio-Rad kit and bovine serum albumin as a standard.

Immunofluorescence Flow Cytometry—Cells at 2 × 10^6/ml (100 μl) were incubated with 100 μl of monoclonal antibody for 30 min at 4 °C, washed twice with phosphate-buffered saline, and suspended for 30 min at 4 °C in 100 μl of fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Kallestad Laboratories, Inc., Austin, TX) previously diluted 1:40 in phosphate-buffered saline. Then, cells were washed three times and subjected to immunofluorescence flow cytometry in an EPICS-C cytofluorometer (Coulter Científica, Móstoles, Spain). Fluorescence data were collected both on log and linear scales. Specific linear fluorescence was obtained by subtracting control fluorescence in which the first monoclonal antibody was substituted by the myeloma P3x63 IgG1.

Radiolabeling, Immunoprecipitation, and Electrophoresis—Membrane proteins (50 μg) from the subcellular fractions were solubilized in 200 μl of borate/saline buffer (0.01 M sodium borate, pH 8.2, 0.14 M NaCl) containing 0.2% Triton X-100 and radioiodinated (0.2 mCi of $Na^{125}I$) in solution using IODO-GEN (Pierce Chemical Co.) (38). After overnight dialysis against phosphate-buffered saline, samples (2 × 10^6 cpm) were precleared with protein A from *Staphylococcus aureus* coupled to Sepharose. For immunoprecipitation, equal amounts of input radioactivity of ^{125}I-labeled proteins from each membrane fraction were mixed with 100 μl of monoclonal antibody. To isolate immune complex, 100 μl of 187.1 rat anti-mouse κ chain monoclonal antibody followed by 30 μl of protein A coupled to Sepharose were added. Immunoprecipitates were processed as previously described (39), and samples were subjected to sodium dodecyl sulfate (SDS), 10% polyacrylamide gel electrophoresis and autoradiography with enhancing screens (40).

Statistical Analyses—Unless otherwise indicated, the results given are the mean ± S.E. of the number of experiments indicated.

RESULTS

Translocation of Mo1 and T200 to the Plasma Membrane and Enzyme Release during Cell Stimulation—Flow cytometry experiments showed that Mo1 and T200 molecules were overexpressed at the cell surface upon neutrophil stimulation with FMLP (Fig. 1), suggesting mobilization of an intracellular pool of these molecules. To test this possibility, the effect of temperature, FMLP, and calcium ionophore A23187 on Mo1 and T200 surface expression was studied by flow cytometry (Fig. 2). Controls of cells maintained at 4 °C in the absence of cytochalasin B and any stimulus were run in parallel. Warming of neutrophils from 4 to 37 °C in the presence of cytochalasin B resulted in a significant increase of both Mo1 and T200 cell-surface expression. Cytochalasin B interferes with the function of cytoplasmic microfilaments, inhibiting the ingestion of particles and facilitating the fusion of intracellular granules with the cell surface upon cell stimulation (41). Subsequent stimulation of neutrophils with 10^{-7} M FMLP or ionophore A23187 (1 or 3 μM) caused a further increase in Mo1 and T200 surface expression. Stimulation with higher concentrations of A23187 did not further enhance the cell-surface expression of T200 and Mo1 molecules. Stimulation of neutrophils with FMLP or ionophore A23187 in the presence of the protein synthesis inhibitor cycloheximide (200 μg/ml) did not alter the above-described up-regulation of Mo1 and T200 cell-surface expression (data not shown). In contrast, the expression of β_2-microglobulin at the cell surface was not affected under the above experimental conditions (Figs. 1*B* and 2), in agreement with previous reports (28, 42–44). Parallel experiments were conducted to examine the degree of degranulation of the three types of intracellular granules described in human neutrophils. As shown in Table I, gelatinase was significantly secreted upon cell preincubation

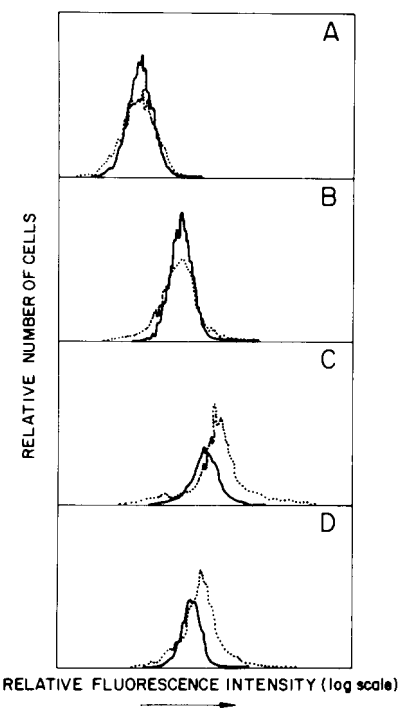

FIG. 1. **Effect of FMLP on surface antigen expression of human neutrophils.** Neutrophils were incubated at 4 °C in the absence of any stimulus (control) or with 10^{-7} M FMLP at 37 °C in the presence of cytochalasin B (5 μg/ml) as described under "Materials and Methods." Resting (——) and stimulated (· · · ·) cells were then chilled at 4 °C, labeled with the corresponding monoclonal antibodies and FITC fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin, and subjected to immunofluorescence flow cytometry. Profiles represent cells labeled with monoclonal antibodies specific for the following surface antigens: P3x63 negative control (*A*), β_2-microglobulin (*B*), Mo1 (*C*), and T200 (*D*).

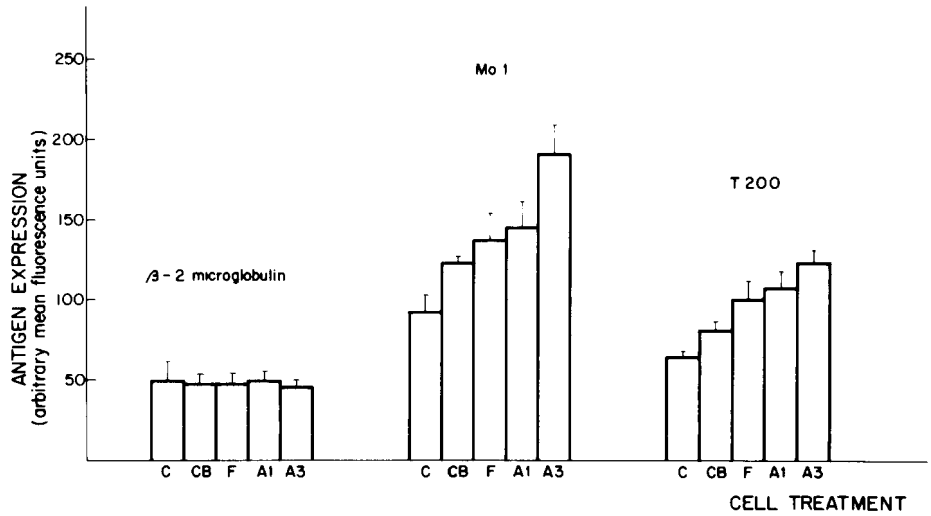

FIG. 2. **Comparison of β_2-microglobulin, Mo1, and T200 antigen expression in resting and activated human neutrophils.** Cell-surface antigen expression was monitored by immunofluorescence flow cytometry in cells held at 4 °C (*bar C*), in cells incubated at 37 °C in the presence of cytochalasin B (5 μg/ml) (*bar CB*), in cells incubated with 10^{-7} M FMLP at 37 °C in the presence of cytochalasin B (5 μg/ml) (*bar F*), in cells incubated with 1 μM A23187 at 37 °C in the presence of cytochalasin B (5 μg/ml) (*bar A1*), or in cells incubated with 3 μM A23187 at 37 °C in the presence of cytochalasin B (5 μg/ml) (*bar A3*). Cell treatments with the activating agents and immunofluorescent staining with the corresponding monoclonal antibodies specific for β_2-microglobulin, Mo1, and T200 were as described under "Materials and Methods."

TABLE I

Release of enzyme markers from human neutrophils upon incubation with different stimuli

Neutrophils were incubated with the stimuli listed below as described under "Materials and Methods." Then, the cellular supernatants were assayed for the granule enzyme markers: gelatinase (tertiary granules), lysozyme (specific granules), and β-glucuronidase (azurophilic granules). Results are shown as mean ± S.E. of the percent of the total cellular enzyme activity released to the extracellular medium of three independent experiments. The total cellular enzyme content was measured after cellular disruption by addition of 0.2% (v/v) Triton X-100. Cytochalasin B (CB) was used at 5 μg/ml.

Treatment	Gelatinase	Lysozyme	β-Glucuronidase
4 °C	11.3 ± 5.9	9.5 ± 2.8	4.3 ± 0.6
37 °C + CB	48.3 ± 6.5	19.1 ± 3.9	6.9 ± 0.8
37 °C + CB + FMLP (10^{-7} M)	66.9 ± 5.0	39.3 ± 3.0	15.4 ± 0.3
37 °C + CB + A23187 (1 μM)	82.4 ± 2.3	32.6 ± 4.6	10.0 ± 0.8
37 °C + CB + A23187 (3 μM)	88.1 ± 1.9	63.7 ± 6.9	15.8 ± 3.8

at 37 °C in the presence of cytochalasin B, indicating fusion of tertiary granules with the plasma membrane. A low release of lysozyme, a marker for specific granules, was also observed. Additional cell stimulation with FMLP, a complete secretagogue, or with ionophore A23187, an agent that has been shown to trigger the discharge of the specific granule contents to the extracellular environment (45, 46), induced a distinct release of the three granule markers (Table I). Gelatinase was almost totally secreted under the highest stimulation conditions, where the maximum exposure of the antigens at the cell surface was achieved. Lysozyme was partially released, whereas β-glucuronidase (a marker for azurophilic granules) was weakly secreted, indicating that azurophilic granules were not fused appreciably with the cell surface under the experimental conditions used. Control experiments showed no release of lactate dehydrogenase activity (data not shown), indicating cellular integrity. These results indicated that only tertiary and specific granules could account for the increased expression of Mo1 and T200 at the cell surface stimulation.

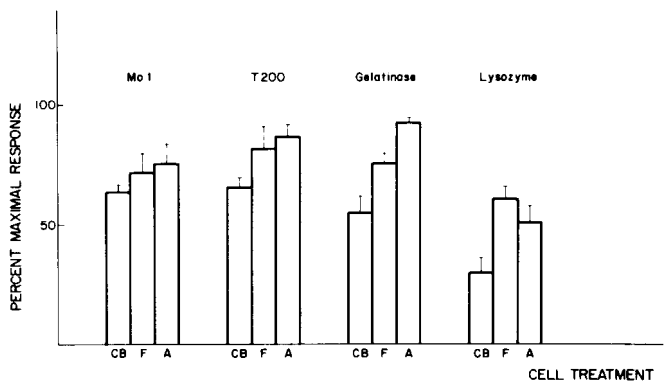

FIG. 3. **Stimulation of antigen expression and granule marker release in human neutrophils by different stimuli.** Neutrophils were incubated at 37 °C in the presence of cytochalasin B (5 μg/ml) (*bar CB*), with 10^{-7} M FMLP at 37 °C in the presence of cytochalasin B (5 μg/ml) (*bar F*), or with 1 μM A23187 at 37 °C in the presence of cytochalasin B (5 μg/ml) (*bar A*) before Mo1 and T200 cell-surface expression as well as enzyme release were measured. Histograms represent the percent of maximum response obtained after the different cell treatments. The maximum response was defined as the amount of activity measured after cells were incubated with 3 μM A23187 at 37 °C in the presence of cytochalasin B (5 μg/ml), as indicated for Fig. 2 and Table I.

In Fig. 3, the effects of temperature, FMLP, and ionophore A23187 on Mo1 and T200 expression as well as on gelatinase and lysozyme release are shown. The data are expressed as the percent of maximum response defined as that observed in the presence of cytochalasin B and 3 μM A23187. There was a good correlation between Mo1 and T200 expression and gelatinase release, suggesting that the additional Mo1 and T200 expression at the cell surface was probably due to the discharged tertiary granules, whose membranes were incorporated into the plasma membrane as a result of the membrane fusion event, which is integral to the degranulation process.

Immunoprecipitation of Mo1 and T200 in Subcellular Fractions—Subcellular fractionation studies were carried out in

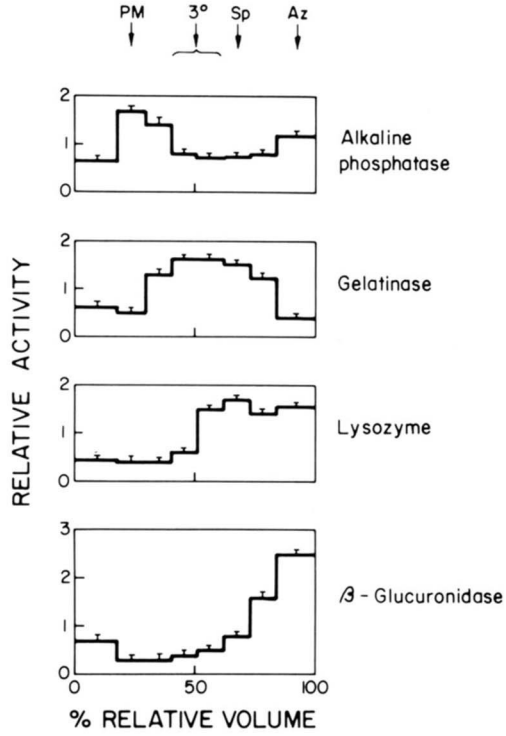

FIG. 4. **Relative activity distributions of marker enzymes after gradient centrifugation of neutrophil postnuclear fractions.** Postnuclear fractions from resting human neutrophils were separated by rate zonal centrifugation at 76,000 × *g* for 15 min. The sucrose gradients were prepared and assayed as described under "Materials and Methods." Plots of relative activity (*versus* percent volume) are given, where relative activity is the percent activity in a fraction divided by the percent volume collected in that fraction (30). The first fraction (*top* of each gradient, *left*) represents the enzymes remaining in the sample layer and cytosol. Values are shown as mean ± S.E. of three independent determinations. The percentages of recovered activities were higher than 80% for all the assayed enzyme markers. *PM*, plasma membrane; *3°*, tertiary granules; *SP*, specific granules; *AZ*, azurophilic granules.

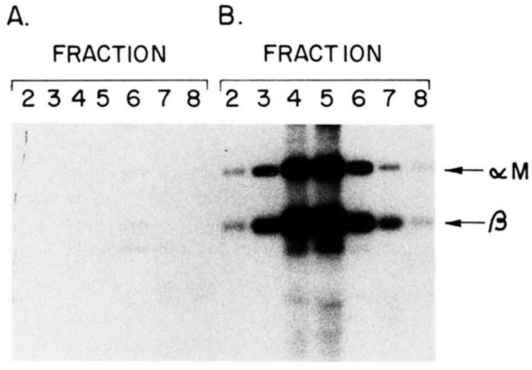

FIG. 5. **Immunoprecipitation of Mo1 from ^{125}I-labeled lysates of membrane obtained from neutrophil subcellular fractions.** Solubilized membrane proteins, isolated from the subcellular fractions shown in Fig. 4, were iodinated as described under "Materials and Methods." Lysates from the different subcellular fractions indicated were immunoprecipitated with P3x63 myeloma culture supernatant as negative control (*A*) and with anti-Mo1 (*B*). Immunoprecipitates were then subjected to SDS, 10% polyacrylamide gel electrophoresis under reducing conditions and autoradiography. The positions of the α (*αM*) and β subunits of the Mo1 glycoproteins are indicated.

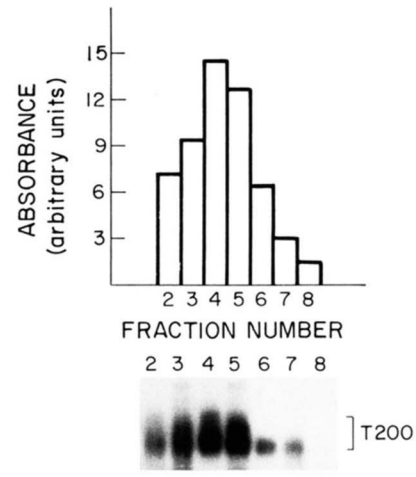

FIG. 6. **Immunoprecipitation of T200 from ^{125}I-labeled lysates of membranes obtained from neutrophil subcellular fractions.** Solubilized membrane proteins, isolated from the subcellular fractions shown in Fig. 4, were iodinated as described under "Materials and Methods." Lysates from the different subcellular fractions indicated were immunoprecipitated with anti-T200. Immunoprecipitates were then subjected to SDS, 10% polyacrylamide gel electrophoresis under reducing conditions and autoradiography. Densitometric analyses were carried out from the autoradiographs.

order to assess further the intracellular locations of Mo1 and T200 in resting neutrophils. Postnuclear fractions obtained from resting cells were separated by rate zonal sedimentation under conditions that resolved cytosol (lactate dehydrogenase), plasma membrane (alkaline phosphatase), tertiary (gelatinase), specific (lysozyme), and azurophilic (β-glucuronidase) granules, as shown in Fig. 4. Lysozyme, although present in both specific and azurophilic granules, was shown to be a reliable marker for specific granules (30). Tertiary granules (fractions 4 and 5) were resolved from specific granules (fractions 5 and 6), azurophilic granules (fraction 8), and the plasma membrane (fraction 2). To examine the intracellular location of Mo1 and T200 glycoproteins, immunoprecipitation experiments of radiolabeled membrane proteins from the distinct subcellular fractions were carried out with monoclonal antibodies anti-Mo1 and anti-T200. As shown in Fig. 5, Mo1 was present in high amounts in fractions 4 and 5, corresponding to the location of tertiary granules. Likewise, T200 glycoproteins were also immunoprecipitated from internal organelles, demonstrating the intracellular location of these molecules (Fig. 6). As evidenced by densitometric measurements of immunoprecipitates, T200 is highly present in fractions 3–5, which are enriched in tertiary granules.

DISCUSSION

We have demonstrated for the first time the localization of T200 in the membranes of cytoplasmic granules in resting human neutrophils. Evidence for this intracellular location has been obtained with two distinct experimental approaches. First, upon exposure to various experimental conditions and degranulating stimuli, the cell-surface expression of T200 increases by up to 2-fold, as determined by flow cytometry. Second, subcellular fractionation and immunoprecipitation studies show T200 to be present in the membranes obtained from fractions enriched for tertiary granules.

We have found a good correlation between increased cell-surface Mo1 and T200 expression and gelatinase release upon cell stimulation. This increase was rapid and was not dependent on protein synthesis. Also, we have found enhanced cell-surface expression of Mo1 and T200 glycoproteins under mild

experimental conditions that only induced fusion of tertiary granules with the plasma membrane, such as simple heating in the presence of cytochalasin B (36). Furthermore, we have found (by immunoprecipitation experiments with monoclonal antibodies anti-Mo1 and anti-T200) that a high proportion of the total cellular Mo1 and T200 was located in fractions highly enriched in tertiary granules. All of this evidence indicates that the up-regulation of Mo1 and T200 upon cell activation is due to the translocation of a large, preformed, intracellular pool of these molecules, very likely the membranes of the tertiary granules, to the cell surface. Nevertheless, we cannot rule out that some of the Mo1 and T200 molecules are located in the specific granules because of the partial overlapping between tertiary and specific granules in the subcellular fractionation studies and of the reported specific granule heterogeneity (47). In this regard, the results herein reported are in agreement with those recently described indicating that most of Mo1 glycoprotein is not localized in peroxidase-positive granules (azurophilic granules) (48). On the other hand, experimental conditions which result in maximum Mo1 and T200 expression also cause the release of substantial amounts of specific granule contents.

Interestingly, we have observed by flow cytometry an increase of up to 2-fold in Mo1 and T200 cell-surface expression upon cell stimulation, indicating that at least 50% of the total Mo1 and T200 molecules are intracellularly located. This value could be underestimated if we consider that the prolonged incubation time required for sample preparation in flow cytometry studies can induce fusion of tertiary granules with the plasma membrane. As a matter of fact, the rapid fusion of tertiary granules with the cell surface under very mild experimental conditions is well documented (29, 31, 36, 49). This readiness of the tertiary granule to degranulate can explain previous results showing variable increases in cell-surface Mo1 expression over basal levels, ranging from 2 to 10-fold (23–27). In this context, we can also suggest that part of the Mo1 and T200 molecules localized in the plasma membrane in subcellular fractionation studies may originate from granule disruption during the purification procedure, giving rise to granule membranes that cosediment with the plasma membrane fraction. Thus, we envisage that most of the Mo1 and T200 molecules could be intracellularly located in resting neutrophils.

Previous subcellular fractionation studies have suggested a location of Mo1 in the specific granules of resting human neutrophils (24, 25, 28). However, gelatinase activity was not measured in these studies; and specific and tertiary granules have almost identical densities, as they are difficult to resolve from each other except under certain fractionation conditions where sedimentation velocity is used (29–31). Furthermore, two recent reports showed suggestive evidence for an Mo1 localization in gelatinase-rich granules. Petrequin *et al.* (43) found increased amounts of Mo1 on the plasma membrane of enucleated neutrophils or cytoplasts, as compared to the cell surface of resting cells. Mollinedo (36) reported a significant fusion of tertiary granules with the plasma membrane during preparation of neutrophil cytoplasts. A strong correlation between Mo1 cell-surface expression and fusion of gelatinase-containing organelles with the plasma membrane upon cellular activation under different experimental conditions has been reported (27).

The gelatinase-containing granules are prone to fuse with the plasma membrane upon gentle stimulation and precede fusion of specific and azurophilic granules (29, 49). In this regard, the predominant presence of Mo1 and T200 in these organelles may have very important physiological consequences, specially in the early events of neutrophil activation. Since Mo1 has been implicated in cellular adhesion functions (21, 22, 50) and as the C3bi receptor (18–20), its location in the membrane of the tertiary granule seems to play a pivotal role in regulating diapedesis, chemotaxis, migration into inflammatory sites, and phagocytosis. Upon appropriate cell stimulation, the tertiary granule would fuse with the cell surface, secreting gelatinase into the environment and exposing additional Mo1 molecules at the plasma membrane. The increased expression of Mo1 would enhance neutrophil attachment to surfaces, facilitating neutrophil chemotaxis. The released gelatinase could partially degrade connective tissue to facilitate neutrophil mobility. Moreover, the parallel increased expression of Mo1 and the release of gelatinase could mediate the neutrophil passage through capillary walls into the tissue. Furthermore, the up-regulation of Mo1, acting as C3bi receptor, would enhance recognition and killing of microbes opsonized with C3bi. In the case of T200, the significance of its intracellular location is more speculative due to the lack of information about its function. In this regard and considering the special features of the tertiary granules, it could be envisaged that T200 might play a role in the early neutrophil responses to stimulation.

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REFERENCES

1. Trowbridge, I. S. (1978) *J. Exp. Med.* **148,** 313–323
2. Dalchau, R., Kirkley, J., and Fabre, J. W. (1980) *Eur. J. Immunol.* **10,** 734–744
3. Omary, M. B., Trowbridge, I. S., and Battifora, H. A. (1980) *J. Exp. Med.* **152,** 842–852
4. Banga, J. P., Guarnotta, G., Harte, A., Pryce, G., Campbell, M. A., Quartey-Papafio, R., Lydyard, P. M., and Roitt, I. M. (1984) *Scand. J. Immunol.* **19,** 11–21
5. Cobbold, S., Hale, G., and Waldmann, H. (1987) in *Leukocyte Typing III* (McMichael, A. J., Beverly, P. C. L., Cobbold, S., Crumpton, M. J., Gilks, W., Gotch, F. M., Hogg, N., Horton, M., Ling, N., Maclerman, J. C. M., Mason, D. Y., Milstein, C., Spiegelhatter, D., and Waldmann, H., eds) pp. 788–803, Oxford University Press, Oxford
6. Cebrián, M., Carrera, A. C., de Landázuri, M. O., Acevedo, A., Bernabeu, C., and Sánchez-Madrid, F. (1987) in *Leukocyte Typing III* (McMichael, A. J., Beverly, P. C. L., Cobbold, S., Crumpton, M. J., Gilks, W., Gotch, F. M., Hogg, N., Horton, M., Ling, N., Maclerman, J. C. M., Mason, D. Y., Milstein, C., Spiegelhatter, D., and Waldmann, H., eds) pp. 823–826, Oxford University Press, Oxford
7. Newman, W., Fast, L. D., and Rose, L. M. (1983) *J. Immunol.* **131,** 1742–1747
8. Targan, S. R., and Newman, W. (1983) *J. Immunol.* **131,** 1149–1153
9. Harp, J. A., Davis, B. S., and Ewald, S. J. (1984) *J. Immunol.* **133,** 10–15
10. Ledbetter, J. A., Rose, L. M., Spooner, C. E., Beatty, P. G., Martin, P. J., and Clark, E. A. (1985) *J. Immunol.* **135,** 1819–1825
11. Bernabeu, C., Carrera, A. C., de Landázuri, M. O., and Sánchez-Madrid, F. (1987) *Eur. J. Immunol.* **17,** 1461–1466
12. Martorell, J., Vilella, R., Borche, L., Rojo, I., and Vives, J. (1987) *Eur. J. Immunol.* **17,** 1447–1451
13. Mittler, R. S., Greenfield, R. S., Schacter, B. Z., Richard, N. F., and Hoffman, M. K. (1987) *J. Immunol.* **138,** 3159–3166.
14. Springer, T., Galfre, G., Secher, D. S., and Milstein, C. (1979) *Eur. J. Immunol.* **9,** 301–306
15. Sánchez-Madrid, F., Nagy, J., Robbins, E., Simon, P., and Springer, T. A. (1983) *J. Exp. Med.* **158,** 1785–1803
16. Lanier, L. L., Arnaout, M. A., Schwarting, R., Warner, N. L., and Ross, G. D. (1985) *Eur. J. Immunol.* **15,** 713–717

17. Springer, T. A., Miller, L. J., and Anderson, D. L. (1986) *J. Immunol.* **136,** 240–245
18. Beller, D. I., Springer, T. A., and Schreiber, R. D. (1982) *J. Exp. Med.* **156,** 1000–1009
19. Arnaout, M. A., Todd, R. F., III, Dana, N., Melamed, J., Schlossman, S. F., and Colten, H. R. (1983) *J. Clin. Invest.* **72,** 171–179
20. Wright, S. D., Rao, P. E., Van Voorhis, W. C., Craigmyle, L. S., Lida, K., Talle, M. A., Westberg, E. F., Goldstein, G., and Silverstein, S. C. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 5699–5703
21. Todd, R. F., III, and Arnaout, M. A. (1986) in *Leukocyte Typing II* (Reinherz, E. L., Haynes, B. F., Nadler, L. M., and Bernstein, I. D., eds) Vol. 3, pp. 95–108, Springer-Verlag, New York
22. Arnaout, M. A., Hakim, R. M., Todd, R. F., III, Dana, N., and Colten, H. R. (1985) *N. Engl. J. Med.* **312,** 457–462
23. Springer, T. A., Thompson, W. S., Miller, L. J., Schmalstieg, F. C., and Anderson, D. C. (1984) *J. Exp. Med.* **160,** 1901–1918
24. Todd, R. F., III, Arnaout, M. A., Rosin, R. E., Crowley, C. A., Peters, W. A., and Babior, B. M. (1984) *J. Clin. Invest.* **74,** 1280–1290
25. O'Shea, J. J., Brown, E. J., Seligmann, B. E., Metcalf, J. A., Frank, M. M., and Gallin, J. I. (1985) *J. Immunol.* **134,** 2580–2587
26. Miller, L. J., Bainton, D. F., Borregaard, N., and Springer, T. A. (1987) *J. Clin. Invest.* **80,** 535–544
27. Petrequin, P. R., Todd, R. F., III, Deval, L. J., Boxer, L. A., and Curnutte, J. T., III (1987) *Blood* **69,** 605–610
28. Arnaout, M. A., Spits, H., Terhorst, C., Pitt, J., and Todd, R. F., III (1984) *J. Clin. Invest.* **74,** 1291–1300
29. Dewald, B., Bretz, M., and Baggiolini, M. (1982) *J. Clin. Invest.* **70,** 518–525
30. Mollinedo, F., and Schneider, D. L. (1984) *J. Biol. Chem.* **259,** 7143–7150
31. Mollinedo, F., Manara, F. S., and Schneider, D. L. (1986) *J. Biol. Chem.* **261,** 1077–1082
32. Bainton, D. F., Ullyot, J. L., and Farquhar, M. G. (1971) *J. Exp. Med.* **134,** 907–934
33. Baggiolini, M., and Dewald, B. (1985) *Int. Arch. Allergy Appl. Immun.* **76,** Suppl. 1, 13–20
34. Keizer, G. D., Borst, J., Figdor, C. G., Spits, H., Miedema, F., Terhorst, C., and De Vries, J. E. (1985) *Eur. J. Immunol.* **15,** 1142–1148
35. Bernabeu, C., Morago, G., de Landauzuri, M. O., Carreira, J., and Sánchez-Madrid, F. (1986) *Immunologia* **5,** 83–90
36. Mollinedo, F. (1986) *Biochim. Biophys. Acta* **861,** 33–43
37. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254
38. Fraker, P. J., and Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80,** 849–857
39. Sánchez-Madrid, F., Davignon, D., Martz, E., and Springer, T. A. (1982) *Cell. Immunol.* **73,** 1–11
40. Laskey, R. A., and Mills, D. (1977) *FEBS Lett.* **82,** 314–316
41. Zurier, R. B., Hoffstein, S., and Weissmann, G. (1973) *Proc. Natl. Acad. Sci. U. S. A.* **70,** 844–848
42. Berger, M., O'Shea, J., Cross, A. S., Folks, T. M., Chused, T. M., Brown, E. J., and Frank, M. M. (1984) *J. Clin. Invest.* **74,** 1566–1571
43. Petrequin, P. R., Todd, R. F., III, Smolen, J. E., and Boxer, L. A. (1986) *Blood* **67,** 1119–1125
44. Bjerrum, O. W., Bjerrum, O. J., and Borregaard, N. (1987) *J. Immunol.* **138,** 3913–3917
45. Hoffstein, S., Soberman, R., Goldstein, I., and Weissmann, G. (1976) *J. Cell Biol.* **68,** 781–787
46. Wright, D. G., Bralove, D. A., and Gallin, J. I. (1977) *Am. J. Pathol.* **87,** 273–284
47. Pérez, H. D., Marder, S., Elfman, F., and Ives, H. E. (1987) *Biochem. Biophys. Res. Commun.* **145,** 976–981
48. Bainton, D. F., Miller, L. J., Kishimoto, T. K., and Springer, T. A. (1987) *J. Exp. Med.* **166,** 1641–1653
49. Mollinedo, F., and Schneider, D. L. (1987) *FEBS Lett.* **217,** 158–162
50. Rosen, H., and Gordon, S. (1987) *J. Exp. Med.* **166,** 1685–1701