Identification and Characterization of a Differentiation Antigen in Human Neutrophils and Monocytes

By Ryushi Nozawa, Hisayo Kato, Teruyo Ito, and Takeshi Yokota

Human promyelocytic leukemia (HL-60) cells were induced to differentiate into macrophage-like cells by treatment with 10⁻⁷ mol/L 1,25-dihydroxyvitamin D₃ (VD₃). A monoclonal antibody (MoAb, 60B8), reactive with the particulate of the differentiated cells but not of the untreated cells, was isolated. The antigen recognized by the MoAb became apparent two days after VD₃ treatment, and its concentration increased and peaked on day 6. Human neutrophils, followed by monocytes and differentiated HL-60 cells, showed the greatest abundance of the antigen. Monocytes cultured for eight days in vitro lost the antigen. No 60B8 antigen was seen in other blood cells. The MoAb precipitated two polypeptides with an apparent molecular weight (mol wt) of 15,000 (15 k) and 13 k in the detergent-solubilized, ³⁵S-methionine-labeled lysate of the differentiated HL-60 cells. Double-sandwich type enzyme-linked immunosorbent assay (ELISA) devised for the quantitative assay of 60B8 antigen indicated that some 2% to 5% of neutrophil protein was 60B8 antigen. This antigen was not exposed on the neutrophil cell surface, since the cells were not stained immunofluorescently with either monoclonal antibody, unless they had become permeable. The neutrophil membrane and the granules were separated on the Percoll density gradient, and the antigen was found localized in the plasma membrane-rich fraction. These findings suggested that 60B8 antigen is a novel differentiation antigen for phagocytic cells.

© 1988 by Grune & Stratton, Inc.

Materials and Methods

Cells and cultures. Human cells were cultivated in F12 medium supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (100 μg/mL each of aminopenicillin and kanamycin sulfate) in a 3% CO₂ incubator. Mouse myeloma P3U1 cells were cultivated in RPMI 1640 medium similarly supplemented.

Enzyme assays. NADPH oxidase activity in intact cells was assayed in 2 mL of Hanks solution containing 80 μmol/L ferricytochrome C, 100 μg/mL phorbor myristate acetate (PMA), and 10⁴ cells, with and without 300 U/mL of superoxide dismutase (SOD). The SOD-inhibitable reduction of cytochrome C at 550 nm was recorded at room temperature by a Hitachi U-3200 spectrophotometer (Hitachi, Ibaraki, Japan). Reduced cytochrome C was calculated as \( E_{550} = 21,000 \text{ cm}^{-1} \text{ mol}^{-1} \text{ L}^{-1} \). NADPH oxidase in a cell-free system was estimated in the presence of sodium dodecyl sulfate (SDS) according to the method of Bromberg and Pickel using VD₃-differentiated HL-60 cells. Alkaline PMase was assayed with 1 mL of 1 mmol/L phenylmethylsulfonyl fluoride as substrate in 1 mL of 1 mmol/L MgCl₂, 50 mmol/L sodium barbital buffer at pH 10.5. Samples were incubated for 60 minutes at 37°C, and the reaction was terminated by the addition of ice-cold barbital buffer. β-glucuronidase was assayed at 37°C for six hours by liberation of phenolphthalein from 1 mmol/L phenolphthalein β-glucuronic acid in 100 mmol/L sodium acetate buffer at pH 4.4. The assay was terminated by the addition of 400 mmol/L glycine buffer at pH 10.5. Lactoferrin was measured by double-sandwich type enzyme-linked immunosorbent assay (ELISA). Lysozyme was measured using the decrease in turbidity at 450 nm of 0.2 mg/mL Micrococcus lysodeikticus in a 67 mmol/L Na-phosphate buffer, pH 6.2, at room temperature using egg white lysozyme as the standard. Myeloperoxidase (MPO) was assayed in 10 mmol/L Na-phosphate buffer, pH 6.2, containing 125 mmol/L H₂O₂, 1.5 mmol/L o-dianisidine, and 0.05% Triton X-100. Activity was measured by the absorbance at 460 nm using purified human MPO as the standard.

MoAb production. HL-60 cells, either treated or untreated with 10⁻⁷ mol/L VD₃, for four days, were suspended at 5 x 10⁶ cells/mL in a sucrose buffer (0.34 mol/L sucrose, 1 mmol/L MgCl₂, and 0.5 mmol/L phenylmethylsulfonyl fluoride [PMSF] in 5 mmol/L K-phosphate buffer, pH 7.0). They were disrupted with a Branson sonifier (Danbury, CT) at 30 W with 40 x 1 second pulses, and centrifuged at 480 g for 15 minutes. The postnuclear fraction was further centrifuged at 27,000 for 30 minutes. The pellets were then suspended in the sucrose buffer (particulate fraction). All of the above procedures were performed at 4°C. A male (6-week-old) BALB/c mouse was injected subcutaneously (SC) and intraperitoneally (IP) with 250 μg protein of the particulate fraction of VD₃-treated cells in a complete Freund’s adjuvant, and this protein was assayed by the Lowry method with bovine serum albumin (BSA) as the standard. The animal was then administered boosters on days 21, 42, and 49 with injections of 100 to 250 μg protein in an incomplete Freund’s adjuvant. Cell fusion between spleen cells of the immunized mouse and the P3U1 cells was performed three days after the fusion.
DIFFERENTIATION ANTIGEN IN PHAGOCYTES

1289

Din Lilly in polyethylene glycol. Hybrid secreting antibody reactive with the particulate fraction of VD3-treated cells but not that of untreated HL-60 cells. Reactive IgG was identified by ELISA and cloned by limiting dilution. Ascitic fluid was prepared by injecting 1 x 10^7 cells from selected clones into pristane-primed BALB/c mice. Antibodies were then purified from the ascites by ammonium sulfate precipitation and DEAE-cellulose chromatography. Purified MoAb 60B8 was conjugated with CNBr-activated Sepharose 4B (IgG 10 mg/g resin) according to the method recommended by the manufacturer's leaflet (Pharmacia, Tokyo). A subclass of antibodies was determined by the Ouchterlony method.

ELISA for MoAb selection and antigen assay. An immunoplate (Nunc, X) was coated with 0.1 to 5 µg particulate protein or with Triton X-100 lysate in 0.1 mL of the coating buffer (15 mmol/L Na2CO3, 35 mmol/L NaHCO3, and 0.05% Na2SO4, pH 9.6). The plate was washed three times with 0.05% Tween 20 in Puck's saline G and incubated for 60 minutes at room temperature with 0.5% BSA (Sigma) in saline G. The plate was washed again and incubated for 60 minutes with affinity-purified alkaline PMase-conjugated rabbit anti-mouse IgG, and was then washed with Tween solution. Finally, 0.1 mL of substrate solution for alkaline PMase was added to each well. The solution was incubated at room temperature for 0.5 to 2 hours and its absorbancy at 405 nm was recorded by an ELISA analyser (Toyo Soda, Tokyo).

Preparation of Triton X lysate. Cells were rinsed twice with saline G and treated for ten minutes on ice in a lysis buffer (0.5% Triton X-100 in PBS, 1 mmol/L PMSF, and 10 µg/mL each of days were washed, and the pellets were collected. The pellets were reconstituted in Triton X-lOO in PBS, I mmol/L PMSF, and 10 µg/mL each of calf serum, Tokyo). At 405 nm was recorded by an ELISA analyser (Toyo Soda, Tokyo). Preparative centrifugation was performed at 7 x 10^9 cells/mL in a Beckman standard fluorescent microscope.

Immuno precipitation. HL-60 cells treated with VD3 for two days were washed, resuspended in a methionine-free medium, and incubated overnight with 2 µCi/mL of [35S]-methionine. A lysate of [35S]-labeled cells was prepared as described above. A cell lysate of peripheral blood neutrophils was also prepared without isotope labeling. The following procedures were essentially the same as reported previously. Briefly, the lysate (0.1 to 0.3 mL) was incubated with 50 µL of MoAb in culture medium for 60 minutes on ice with constant agitation. The 8 µL of affinity purified sheep anti-mouse IgG was added, and the incubation was continued for an additional 60 minutes. Finally, 60 µL of a 10% (wt/vol) solution of HCHO-fixed Staphylococcus aureus (Zrysorbin) was added for 30 minutes at 4°C. This was followed by two washings with an HSA buffer, two more washings with detergent solution, and a final washing with an HSA buffer. The precipitate was suspended in 40 µL of electrophoresis sample buffer and boiled for two minutes. As a control, mouse IgG was used instead of MoAb. In some experiments, Sepharose beads conjugated with MoAb 60B8 or mouse IgG were used. In that case, anti-mouse IgG and Zrysorbin were not added.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli, using 2-mm thick slab gels with 12.5% or 7.5% resolving gels. For 35S-labeled samples, the gels were processed by the same procedure as previously described and exposed overnight on Kodak X-Omat AR film at -80°C.

Preparation of blood cells. Mononuclear cells were isolated after the centrifugation of heparinized blood on Mono-Poly resolving medium. Cells were suspended in saline G and centrifuged at 300 g for five minutes. The upper two-thirds of the turbid supernatant containing platelets was collected. The pellets were washed twice by centrifugation in the same manner, suspended in the medium, and plated on plastic culture dishes. After a 30-minute incubation at 37°C, cells adherent to the plastic were washed several times with warm medium (monocytes). The plating of nonadherent cells was repeated three times, and the final nonadherent cells (lymphocytes) were collected. Polymorphonuclear cells were collected at the second interface on Mono-Poly medium, and contaminating RBCs were punctured by a hypotonic treatment. The RBCs at the bottom of the Mono-Poly medium were also collected. The approximate cell and platelet purities were as follows: RBC and platelets, 100%; monocytes and neutrophils, >90%; lymphocytes, 70% to 80%.

Indirect immunofluorescence. Neutrophils were sandwiched between a coverslip and a coverslip at room temperature for ten minutes, and the cells on the coverslip were fixed in a fixation buffer (1% paraformaldehyde in PBS) at room temperature for 15 minutes. Cells were then washed in PBS, dipped into acetone (-80°C) for 30 seconds, and again washed in PBS. Cells treated or untreated with acetone were incubated with 1% BSA in PBS for 60 minutes, then incubated with appropriately diluted MoAb 60B8 or polyclonal rabbit anti-60B8 protein in PBS and washed three times with high-salt PBS (0.65 mol/L NaCl). The cells were further incubated for 30 minutes with affinity purified fluorescein isothiocyanate (FITC)-conjugated F(ab')2, anti-mouse IgG + IgM or anti-rabbit IgG that had been preadsorbed in a batch procedure with fixed neutrophils, and washed four times with high-salt PBS. As a control, cells were stained with mouse IgG or rabbit preimmune serum instead of anti-60B8 antibody. In some experiments, neutrophils were activated with 100 ng/mL PMA for five minutes at 37°C before fixation. Cells were then photographed under a Zeiss standard fluorescent microscope.

Subcellular fractionation of neutrophils. Neutrophils were suspended in a relaxation buffer at 7 x 10^8 cells/mL and briefly sonicated as the HL-60 cells were. The postnuclear fraction was layered on a discontinuous gradient of 37.4% and 97.4% Percoll in warm medium (monocytes). The plating of nonadherent cells was repeated three times, and the final nonadherent cells (lymphocytes) were identified by ELISA and centrifugation in the same manner, suspended in the medium, and plated on plastic culture dishes. After a 30-minute incubation at 37°C, cells adherent to the plastic were washed several times with warm medium (monocytes). The plating of nonadherent cells was repeated three times, and the final nonadherent cells (lymphocytes) were collected. Polymorphonuclear cells were collected at the second interface on Mono-Poly medium, and contaminating RBCs were punctured by a hypotonic treatment. The RBCs at the bottom of the Mono-Poly medium were also collected. The approximate cell and platelet purities were as follows: RBC and platelets, 100%; monocytes and neutrophils, >90%; lymphocytes, 70% to 80%.

RESULTS

Isolation of MoAbs reactive with particulate of differentiated HL-60 cells. HL-60 cells (2 x 10^5 cells/mL) were treated with 10^{-7} mol/L VD3. Cell growth decreased two days after, and stopped four days after the treatment. Round HL-60 cells converted to cells with a few pseudopod-like

From www.bloodjournal.org by guest on September 24, 2017. For personal use only.
projections. No such morphological change occurred with 0.1% ethanol, a solvent of VD₃. When phagocytic cell markers were assayed in the treated HL-60 cells (Fig 1), NADPH oxidase activity in intact cells became apparent two days after treatment and reached a plateau four days after treatment, whereas alkaline PMase, β-glucuronidase, and lysozyme activity in Triton X-100 lysate of cells increased from day 6. MPO decreased with VD₃ treatment, and no lactoferrin was detected in the lysate of the treated cells. Thus, HL-60 cells differentiated with VD₃ to phagocytic cells with macrophage-like marker enzyme activity. Day-4 cells were collected and gently sonicated in the sucrose buffer. The postnuclear fraction of the sonicate was further centrifuged at 27,000 g. A BALB/c mouse was immunized with 27,000 g precipitate (particulate fraction), and spleen cells were fused with mouse myeloma P3U1 cells. Hybrids that secreted antibodies reactive with the particulate of VD₃-treated cells but not of untreated cells were isolated and cloned. Two MoAbs (60B5 and 60B8) survived the selection and were found to belong to the IgG1 subclass.

Expression of 60B8 antigen in the differentiated HL-60 cells. Expression of the antigen of MoAb 60B8 was examined during VD₃ treatment (Fig 2). Particulate fractions were prepared in each case on the day of assay, and the amount of 60B8 antigen was assayed by ELISA. The antigen appeared on day 2, continued to increase, and peaked on day 6. No antigen was detected either in the 27,000 g supernatant of differentiated cells (day 4 and day 10) or in the particulate fraction of ethanol-treated cells (not shown in Fig 2). Mouse IgG was not reactive with any of the particulates. Thus, 60B8 antigen was induced as early as NADPH oxidase after VD₃ treatment. The antigen of MoAb 60B5 was induced with almost the same kinetic pattern as that of MoAb 60B8. The effect of MoAb 60B8 on NADPH oxidase activity was then examined. The sonicate of the differentiated HL-60 cells (day 4) was assayed for the SOD-inhibitable reduction of cytochrome C, and the cytochrome was reduced by the addition of SDS and NADPH, as reported previously. Preincubation of the sonicate with DEAE-cellulose-purified MoAb 60B8 (open symbols) or 1 μg of mouse IgG (closed symbols). Each symbol represents the average of three determinations. The bars represent SD.

60B8 antigen in human cells. The expression of 60B8 antigen in human peripheral blood cells was examined by ELISA (Table 1). An ELISA plate was coated with Triton X-100 lysate of blood cells (0.1 and 1 μg protein per well). The richest sources of 60B8 antigen were neutrophils and then monocytes. However, the amount of the antigen in monocytes was far smaller than that in neutrophils. The differentiated HL-60 cells (day 6) contained some of the antigen, but less than the monocytes. No appreciable amount of the antigen was detected in RBC, platelets or lymphocytes. After monocytes were cultured in vitro for eight days, they exhibited macrophage morphology, but these cells had little or no 60B8 antigen. Other human cells, normal skin fibroblasts, SV40-transformed WI-38 cells (VA-4), neuroblastoma (IMR-32), melanoma (A-375), B cell lymphoma (Raji), T cell lymphoma (Molt 4F and Jurkat), and myelogenic leukemia (K-562) showed no 60B8 antigen content.

Determination of molecular weight (mol wt) of 60B8 antigen. To determine the mol wt of 60B8 and 60B5 antigens, HL-60 cells treated with VD₃ for two days were labeled with ³⁵S-methionine, and detergent-lysate was prepared. The lysate from VD₃-untreated, isotope-labeled HL-60 cells was also prepared. Both lysates were incubated with MoAbs or mouse IgG, and the antigen precipitated was separated on SDS-PAGE followed by DMSO-PPO fluorography. MoAb 60B8 precipitated two polypeptides (apparent mol wt, 15 k and 13 k) in the lysate of VD₃-treated cells (Fig 3A, lanes 2, 5). No precipitate was formed in the lysate of
Table 1. Expression of 60B8 Antigen in Human Blood Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Lysozyme Protein Coated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 g</td>
</tr>
<tr>
<td>HL-60, day 0</td>
<td>0.036 ± 0.001</td>
</tr>
<tr>
<td>HL-60, day 6†</td>
<td>0.045 ± 0.001</td>
</tr>
<tr>
<td>Neutrophils‡</td>
<td>0.150 ± 0.008</td>
</tr>
<tr>
<td>Monocytes, day 0</td>
<td>0.045 ± 0.001</td>
</tr>
<tr>
<td>Monocytes, day 8§</td>
<td>0.047 ± 0.009</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.042 ± 0.007</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.039 ± 0.001</td>
</tr>
<tr>
<td>RBCs</td>
<td>0.041 ± 0.004</td>
</tr>
</tbody>
</table>

An immunoplate was coated with the indicated concentrations of Triton X lysozyme protein from the blood cells of healthy donors. The ELISA assay was performed as mentioned in Materials and Methods. The values in parentheses are for samples incubated with mouse IgG instead of MoAb 60B8.

*Average of three determinations ± SD.
†Incubated with VD3 for six days.
‡Obtained from three different donors.
§Incubated for eight days as described in the text.

Fig 3. SDS-PAGE of 60B8 antigen. (A) 35S-methionine-labeled lysates of untreated (lane 1) and VD3-treated (lane 2) HL-60 cells were precipitated with MoAb 60B8-Sepharose. The same lysate of VD3-treated cells were incubated with MoAb 60B5 (lane 3), mouse IgG (lane 4), and MoAb 60B8 (lane 5) followed by precipitation with S. aureus. The precipitate was analyzed on SDS-PAGE in the presence of DTT and visualized by fluorography. (B) Human neutrophil lysates were precipitated with mouse IgG or MoAb 60B8-Sepharose and analyzed on SDS-PAGE with and without DTT. Lane 1, mouse IgG with the lysate, + DTT; lane 2, mouse IgG, + DTT; lane 3, MoAb 60B8 with lysate, + DTT; lane 4, MoAb 60B8 with lysate, – DTT; lane 5, MoAb 60B8, + DTT; lane 6, MoAb 60B8, – DTT; lane 7, marker proteins with the same mol wt as A. Proteins were stained with coomassie brilliant blue.
60B8 protein of HL-60 cells. The plate was then incubated with 1 to 300 ng of 60B8 protein per well, and then MoAb 60B8. The other ELISA procedures were the same as described in Materials and Methods. Under these assay conditions the amount of 60B8 protein was determined to be in the range of 3 to 300 ng (Fig 4). Triton X-100 lysates of seven healthy donors were prepared, and concentration of the 60B8 protein in each lysate (1 μg protein) was determined. The concentrations of 60B8 protein were thus found to be 3.9% ± 1.0% (2.5% to 5.5%) of total neutrophil protein.

**Indirect immunofluorescence.** Neutrophils were stained with MoAb 60B8 and FITC-conjugated (Fab')2 anti-mouse IgG. No cells were stained unless they had first been treated with acetone, suggesting that the epitope of 60B8 antigen for the MoAb was not exposed on the outer cell surface of neutrophils. Neutrophils were then stained with polyclonal rabbit anti-60B8 protein and with FITC-anti-rabbit IgG. The cells were stained when they became permeated with acetone (Fig 5A). Neutrophils pretreated with 100 ng/mL of 60B8 protein in each lysate (1 μg protein) was determined. The concentrations of 60B8 protein were found to be 3.9% ± 1.0% (2.5% to 5.5%) of total neutrophil protein.

**Fig 4.** Dose-response curve of 60B8 protein in double-sandwich type ELISA. The 60B8 protein was affinity-purified from Triton X-100 lysate of human neutrophils. Protein concentration was assayed by Bio-Rad Protein Assay Kit using bovine immunoglobulin as the standard.

**Fig 5.** Indirect immunofluorescence of neutrophils. Cells were not activated (A) or activated with PMA (B-D). After the fixation with paraformaldehyde, cells were permeated with acetone, incubated with polyclonal rabbit anti-60B8 or preimmune serum, and stained with FITC-anti-rabbit-IgG. (A, B) Treated with acetone and anti-60B8 serum, (C) treated with acetone and pre-immune serum, and (D) treated with anti-60B8 serum (magnification x1,000).

97.4%). Three bands were visible after the centrifugation, as previously reported. The α (lowest) band was identified as azur granules with MPO and β-glucuronidase activity (Table 2). The β (middle) band was formed of specific granules with an enriched lactoferrin content. Lysozyme was found evenly in both azur and specific granules. The top γ band contained plasma membrane with alkaline PMase activity. The 60B8 antigen was found exclusively in the γ band, indicating that the protein was localized in the plasma membrane of neutrophils.

**DISCUSSION**

Human promyelocytic leukemia HL-60 cells were induced by VD₃ to differentiate into macrophage-like cells, and a MoAb (60B8) immunoreactive with the particulate of VD₃-treated cells, but not of untreated HL-60 cells, was established. We attempted to isolate MoAbs to recognize an early induced phagocytic cell marker like NADPH oxidase (Fig 1). As expected, 60B8 antigen was induced by VD₃ as early as the oxidase was (Fig 2). A preliminary test was conducted to discover whether MoAb 60B8 is inhibitory for NADPH

**Table 2. Localization of 60B8 Protein in Human Neutrophils**

<table>
<thead>
<tr>
<th>Fraction (Band)</th>
<th>Alkaline PMase (U/mg Protein)</th>
<th>β-glucuronidase (μg Phenolphthaëin/mg Protein)</th>
<th>MPO (U/mg Protein)</th>
<th>α</th>
<th>β</th>
<th>γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>0.15 ± 0.01</td>
<td>406.9 ± 0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>0.24 ± 0.02</td>
<td>80.0 ± 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ</td>
<td>1.33 ± 0.07</td>
<td>87.7 ± 6.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lactoferrin (μg/mg Protein)</th>
<th>Lysozyme (μg/mg Protein)</th>
<th>MPO (U/mg Protein)</th>
<th>60B8 Antigen (A₅₆₆/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7 ± 0.0</td>
<td>25.7 ± 3.1</td>
<td>36.8 ± 0.8</td>
<td>−0.007 ± 0.018</td>
</tr>
<tr>
<td>10.6 ± 0.1</td>
<td>26.0 ± 2.1</td>
<td>3.6 ± 0.1</td>
<td>−0.007 ± 0.008</td>
</tr>
<tr>
<td>0.5 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>2.0 ± 0.1</td>
<td>0.096 ± 0.028</td>
</tr>
</tbody>
</table>

Neutrophils were fractionated at discontinuous Percoll gradient into α, β, and γ bands (beginning at the bottom).

*Average of three determinations ± SD.
DIFFERENTIATION ANTIGEN IN PHAGOCYTES

oxidase activity, but the MoAb did not affect the activity. This result does not necessarily mean that 60B8 antigen is not correlated with NADPH oxidase, which forms an electron transfer chain in the plasma membrane.²⁰ Further studies on the possible correlation of 60B8 antigen with the oxidase system are in progress.

The molecular nature, expression in human cells and subcellular localization of 60B8 antigen were then studied. The antigen apparently comprised two polypeptides, heavy and light, both of which are present in human neutrophils and VD₃-differentiated HL-60 cells (Fig 3). The antigen exhibited several protein bands on SDS-PAGE in the absence of DTT (Fig 3B), probably by forming artificial intra- and intermolecular disulfide bonds. It is difficult to judge which is the native form of 60B8 protein in the hydrophobic environment of the plasma membrane. Furthermore, it is unknown whether the heavy polypeptide differs from the light one, or the light polypeptide is a partially cleaved product of the heavy one. A study to determine the amino acid sequence of these polypeptides, which is in progress, could solve this question. Among the human cells tested, only peripheral blood neutrophils and monocytes revealed any presence of 60B8 antigen (Table 1), demonstrating that it is a phagocyte-specific antigen. The antigen was far more abundant in neutrophils than in monocytes, and 2% to 5% of the neutrophil protein seemed to be a 60B8 protein (Fig 4). Therefore, it is expected that the antigen plays a specific role in phagocytic cells, especially in neutrophils. It would be of interest to find a person whose 60B8 antigen is defective or varied, for it is likely that such a person would have neutrophils with some dysfunctions.

Recently, numerous surface antigens of human monocytes were demonstrated by a MoAb technique.¹¹⁻²⁰ Many antigens like 60B8 were lost when monocytes were cultivated on an artificial substratum such as plastic. However, some of them remained in the macrophages cultivated in circumstances more closely resembling physiological conditions, in the collagen matrix.²⁵ Expression of 60B8 antigen in the macrophages grown on collagen and in tissue macrophages is under investigation. The 60B8 protein is probably localized in the plasma membrane of monocytes, as demonstrated in neutrophils (Table 2). However, the protein would not be identical with such monocytic surface antigens, since the molecular weights of those antigens are much larger than mol wt of 60B8. The 60B8 antigen was not exposed on the outer cell surface (Fig 5), quite unlike the known surface antigen,¹¹⁻²⁰ and this fact further supports our assertion that 60B8 antigen is a novel differentiation antigen for phagocytic cells.

REFERENCES

4. Massey V: The microsorbitation of succinate and the extinction coefficient of cytochrome C. Biochim Biophys Acta 34:255, 1959
23. Raff HV, Picker LJ, Stobo JD: Macrophage heterogeneity in man. A subpopulation of HLA-DR-bearing macrophages required...


Identification and characterization of a differentiation antigen in human neutrophils and monocytes

R Nozawa, H Kato, T Ito and T Yokota

Updated information and services can be found at:
http://www.bloodjournal.org/content/71/5/1288.full.html

Information on similar topics can be found in the following Blood collections.