A Novel Leukocyte Differentiation Antigen: Two Monoclonal Antibodies TM2 and TM3 Define a 120-kd Molecule Present on Neutrophils, Monocytes, Platelets, and Activated Lymphoblasts

By Hitoshi Ohto, Hiroo Maeda, Yoichi Shibata, Rou-Fuie Chen, Yukio Ozaki, Masaaki Higashihara, Akiteru Takeuchi, and Hiroshi Tohyama

We produced two hybridomas by fusion of mouse myeloma cells with splenocytes from a mouse immunized with the THP-1 human monocytoid leukemia cell line. Two cloned hybridoma cell lines, designated as TM2 and TM3, were obtained. They secreted antibodies against a unique cell surface antigen expressed on all normal peripheral blood monocytes, neutrophilic granulocytes, platelets, and mitogen-induced lymphoblasts, some cells from patients with immature-type lymphoid leukemias. However, the antibodies reacted neither with large numbers of peripheral blood lymphocytes nor with red cells. Cross-blocking studies showed that these monoclonal antibodies recognized the same or a nearly positioned antigen epitope. Immunoprecipitation of THP-1 cell extract with TM2 or TM3 under reducing and nonreducing conditions yielded a specific band of mol wt equal to 120,000 daltons. This determinant appeared to be involved in granulocyte chemotaxis, since neutrophilic granulocytes exposed to TM2 or TM3 showed a significant decrease in chemotaxis toward endotoxin-activated serum. These two monoclonal antibodies did not affect O₂⁻ release or luminol-dependent chemiluminescence of neutrophils. Moreover, they did not alter platelet aggregation induced by thrombin. TM2 and TM3 will provide a new reagent in defining the linkage between lymphoid and myeloid differentiation and intermyeloid development.

CONSIDERABLE INTEREST has recently been directed to the membrane glycoproteins that contain the major antigenic determinants which characterize the myeloid cells. Patients with congenital deficiency of unique glycoproteins on mature granulocytes have been shown to have functional abnormalities of neutrophils. Cell surface glycoproteins may change during the course of development and differentiation. By using heteroantisera and xenogeneic monoclonal antibodies (MoAbs) to human myelogenous cells, differentiation stages of human myeloid cells have been characterized. Monoclonal antibodies reactive with both monocytes and granulocytes have been described. Recent biochemical studies have demonstrated the antigenic determinants recognized by some of the MoAbs. However, no antimonyoid murine MoAbs reacting against the common surface antigens of human granulocytes, monocytes, and platelets have been reported.

In the studies reported in this paper, we have characterized two murine MoAbs that recognize a 120,000-mol wt glycoprotein (gp 120) antigen determinant present on the surface of the human neutrophilic granulocyte, monocyte, platelet, and activated lymphoblasts.

MATERIALS AND METHODS

Production of monoclonal antibodies. A male BALB/c mouse was immunized intraperitoneally (IP) with the human monocytoid leukemia cell line THP-1. Four days later the splenocytes were fused with cells of the X63Ag8.653 murine myeloma cell line by the method of Galfre et al with slight modification, as previously described. The supernatants from cultures exhibiting cell growth were tested for the presence of antibodies reactive with THP-1 cells by the solid phase mixed passive hemagglutination method, as previously described (Fig 1). Briefly, culture supernatants were incubated with fixed monolayer THP-1 cells on a 96-well round-bottom plastic plate (Terumo Corp., Tokyo) at room temperature for 30 minutes in a humid chamber. After washing three times with phosphate-buffered saline (PBS), pH 7.2, containing 0.02% Tween-20 using an automatic well washer, Sera Washer (BioTec Corp, Tokyo), one drop of rabbit anti-mouse IgG-coated tanned sheep red blood cell suspension was added and the reactivity of the supernatant was determined according to the sedimentation patterns of sheep red cells. Finally, the selected hybridoma cells were injected IP into BALB/c mice primed with pristane (Aldrich Chemical Co, Milwaukee).

Characterization of monoclonal antibodies and fragmentation of (ab)\(^2\). The two resulting MoAbs, TM2 and TM3, were found to be IgG2b and IgG1 by micro Ouchterlony technique using subclass-specific antisera (Miles Laboratories, Elkhart, Ind). To obtain (ab)\(^2\) fragment of TM3, the precipitate of 40% (NH\(_4\))\(_2\)SO fractionated ascites was resuspended in PBS and purified on Affi-Gel protein A (Bio-Rad, Richmond, Calif). (ab)\(^2\) fragments of TM3 were prepared by digestion with pepsin at PH 4.18 for 18 hours at 37 °C. The (ab)\(^2\) fragments were purified on Affi-Gel protein A, and purity was checked with polyacrylamide gel electrophoresis.

Cell preparation. Peripheral blood mononuclear cells (MNCs) were isolated from heparinized blood by centrifugation on Ficoll-Hypaque. T cells, B cells, null cells, and monocytes were obtained from MNCs using glass adherent technique, rosetting with sheep erythrocytes, nylon wool column, as previously described. Granulocytes were obtained from Ficoll-Hypaque pellet, purified by hydroxyethyl starch (HES) sedimentation, and freed from erythrocytes by hypotonic ammonium chloride lysis.

Leukemia samples. Peripheral blood or bone marrow aspirate cells from patients with leukemia were collected into sterile heparin-containing syringes. MNCs were prepared by Ficoll-Hypaque sedimentation and examined to determine the percentage of leukemic blasts. All patients had at least >60% leukemic cells in the analyzed sample, and usually >90%. Leukemic cells were cryopreserved in 10% dimethylsulfoxide at −120 °C before immunofluorescence assay. Chronic myelogenous leukemia (CML) cells were collected from the Blood Transfusion Service, Tokyo University Hospital; the Blood Transfusion Service, Toranomon Hospital, Tokyo; and the Department of Pediatrics, the First Department of Internal Medicine, and the Second Department of Internal Medicine, Faculty of Medicine, University of Tokyo.

Submitted Aug 20, 1984; accepted April 8, 1985.

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Solid Phase Mixed Passive Haemaggulitation

Tanned Sheep Red Blood Cell

Anti-Mouse IgG

Monoclonal Antibody

Target Cells

Round Bottom Microtiter Plate

Fig 1. Detection of MoAbs by solid-phase mixed passive hemagglutination.

from leukocyte-rich plasma by sedimenting the red blood cells with 5 mL of 10% HES solution in saline to every 5 mL of heparinized blood.

Lymphoma cells were isolated from lymph node tissue removed from patients undergoing open biopsy. The tissue was minced with scissors and forceps in RPMI 1640, and a single cell suspension was obtained by passage of this medium through a mesh screen.

Indirect immunofluorescence assay. One hundred microliters of cell suspension was incubated with an equal amount of 1/100 diluted hybridoma ascitic fluid in a Fischer tube for 30 minutes at 22 °C. After three washes the cells were resuspended in 100 µL of 1/30 diluted fluorescein isothiocyanate (FITC)-conjugated goat antimouse IgG antiserum (Cappel Laboratories, West Chester, Pa) and incubated for an additional 30 minutes at 4 °C. The cells were washed again three times and examined by fluorescence microscopy (Olympus, Tokyo).

Radiolabeling and immunoprecipitation of cellular proteins. Monocytoid cell lines THP-1 and U937 were internally radiolabeled with [35S]-methionine (New England Nuclear, Boston) in methionine-free RPMI 1640 supplemented with 10% fetal calf serum (FCS) at a concentration of 10–6/mL for 16 hours at 37 °C. After labeling, the cells were washed twice with RPMI 1640 medium and suspended in 0.5 mL of the lysis buffer22 containing 0.5% Nonidet P-40 (Sigma Chemical Co, St Louis). The cell lysate was incubated for 30 minutes at 4 °C and centrifuged at 10,000 g for 30 minutes. The supernatant was separated into several aliquots and frozen at –70 °C until used. Immunoprecipitation, using protein A-Sepharose as an indirect immunoadsorbent, was performed as described elsewhere.24

Electrophoresis and fluorography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on discontinuous vertical slab gels according to the Laemmlí procedure.31 Electrophoresis was performed using a 7% SDS-acrylamide gel at 20 mA per gel for four hours. After electrophoresis, the gels were stained with 0.05% Coomassie brilliant blue, 15% acetic acid, and 50% methanol and destained with 7% acetic acid and 25% methanol before fluorography was performed using ENHANCE (New England Nuclear). The dried gels were exposed to Kodak X-Omat AR films (Eastman Kodak, Rochester, NY) at –70 °C for two weeks.

Blocking assay. Immunoglobulins were isolated from ascites by precipitation with 33% saturated ammonium sulfate followed by dialysis against PBS. They were enzymatically labeled with [125I] by the lactoperoxidase-glucose oxidase method.24 Competition experiments were performed using THP-1 cells as target cells. [125I]-labeled MoAb was added to a mixture of cells (5 x 103 in 50 µL of medium) and 50 µL of diluted cold antibody. Unlabeled autologous ascites and medium served as positive and negative controls, respectively. Cells were incubated at 22 °C for 60 minutes, washed five times with PBS containing 0.1% bovine serum albumin (BSA), and the cell-bound radioactivity was counted.

Enzymatic treatment of monocytoid cell line THP-1. A quantity of 2 x 106 cells was suspended in varying concentrations of neuraminidase (Vibrio cholerae, 1 U/mL, Behringwerke AG, Marburg, FRG), trypsin (lyophilized trypsin, 10,200 U/mg, Sigma), or papain (2.1 U/mg, type 2, Sigma) at a final substrate concentration of 5 x 10–3 U/mL per milliliter and incubated for 30 minutes at 37 °C. Papain was activated by the addition of L-cysteine to a final concentration of 5 mmol/L. Treated cells (always >95% viable) were washed three times in FCS containing medium before analysis.

Colony assay after separation by panning method. TM2 and TM3 reactive bone marrow cells were separated by the indirect panning method.25,26 Briefly, 2 x 104 buffy coat cells were incubated at 22 °C for 30 minutes with 1:100 diluted MoAb. The cells were washed three times in alpha medium (Flow Laboratories, Rockville, Md) containing 10% FCS and poured onto polystyrene dishes (60 x 8 mm, Toyoshima, Tokyo) previously coated with goat anti-mouse IgG (Cappel) at a concentration of 1 mg/mL. After 40 minutes at 22 °C, unattached cells were separated as the TM2 and TM3 negative cell population, and adherent cells were removed by pipetting as positive population. The assay for granulocyte/macrophage progenitor cells (CFU-GM) was performed by the single-layer soft agar method of Robinson et al.,27 with minor modifications. Human placental conditioned medium was used as the source of colony-stimulating factors.28 CFU-E- and BFU-E-derived erythroid cells were also assayed similarly.

Chemotaxis inhibition assay. Modified Boyden chambers were used to study the effect of TM2 and TM3 on chemotaxis of neutrophils or monocytes, with slight modification as previously described.26 Cells were pretreated for 30 minutes at 37 °C with diluted MoAb or buffer in Hanks' balanced salt solution (HBSS) before measuring chemotaxis. Endotoxin (Escherichia coli 0127:B8 lipopolysaccharide B, Difco Laboratories, Detroit) -activated serum, in which CsA is primarily concerned, and supernatant from the culture of E coli were used as chemotactic factors. The results were expressed as the number of cells that migrated through the filter.

Luminol-dependent chemiluminescence. Luminol-dependent chemiluminescence of granulocytes with or without MoAbs was measured according to the method reported by Van Dyke et al.31 The stimuli tested included 1 µL of 10–5 mol/L of n-formyl-Methionyl-Leucyl-Phenylalanine (MLP, Sigma), 1 µL of 1 mmol/L A23187 (Ca ionophore, Sigma), and 20 µL of opsonized zymosan (10 mg/mL, Sigma).

Superoxide generation of granulocyte. Superoxide (O2–) generation of granulocytes induced by opsonized zymosan in the presence
or absence of MoAbs was measured by the reduction of ferricyto-
crome C (horse heart type 3, Sigma) as described.31

Platelet aggregation. Platelet aggregation was measured
according to the method of Feinman et al30 on Chrono-Log lumiag-
gregometer (Chrono-Log Corp., Havertown, Pa). The platelet-rich
plasma was adjusted to 5 X 107 platelets per microliter, and reaction
was initiated by adding aggregating agents, such as collagen,
adenosine diphasphonate (ADP), and arachidonic acid in the presence
or absence of MoAbs.

Antisera. An anti–HLA-A,B,C framework MoAb NC6 (IgG1,
kappa), an anti–HLA-DR framework MoAb NC1 (IgG1, kappa), a
MoAb TG8 (IgM, kappa) with specificity for granulocyte mem-
brane antigen, and a MoAb TP80 (IgG1, kappa) with specificity for
platelet glycoprotein IIb/IIIa complex were produced in this labora-
tory.

RESULTS

Tissue distribution of TM2 and TM3 cells. Table 1
summarizes the tissue distribution of cells positive for TM2
and TM3 antibody. The vast majority of peripheral lympho-
cytes and erythrocytes did not bind to the two MoAbs. In
contrast, almost all of the granulocytes, monocytes, and
platelets were stained with TM2 and TM3. Large numbers
of bone marrow cells reacted strongly with these antibodies.
Although the two MoAbs reacted with small numbers of
peripheral lymphocytes, a lot of lymphocytes were included
in positive populations when bone marrow cells were separ-
rated by the indirect panning method. In addition, a majority
of mitogen-activated lymphocytes bound to these antibodies,
40% and 60% of lymphocytes activated with either phytohe-
magglutinin (PHA) (1%) or pokeweed mitogen (PWM) (10
µg/mL) for two to three days were positive. In order to
preclude the possibility that the observed monocyte and
granulocyte staining may have been due to adherent plate-
lets, cells were washed in 0.5 mmol/L EDTA. No changes in
the staining pattern were observed.

Reactivity with leukemia cells and cell lines. Testing
with different types of leukemic cells (Table 2) revealed that
the two MoAbs reacted with cells from 12 of 12 patients with
acute myelogenous leukemia (AML) and acute promyelo-
cytic leukemia (APL) and cells from three of three patients
with acute monocytic leukemia (AMoL) and acute myelo-
monocytic leukemia (AMML). Similarly, cells from nine of
11 (82%) patients with non-T, non-B acute lymphocytic leukemia (ALL) were reactive with these two MoAbs, while
cells collected from the five cases of B lymphoid leukemias
were not reactive. Cells from three of 11 (27%) patients with
T cell malignancies were reactive with antibodies.

The binding of TM2 and TM3 at saturating concen-
trations to a panel of cultured cell lines is shown in Table 3. The
two MoAbs showed similar binding patterns. They reacted
strongly with the monocyteid cell line THP-1 and interme-
tiately with other cells of myeloid origin except K-562
myeloid/erythroid cell line. None of the seven established
lymphoid cell lines were reactive with the two MoAbs.

Enzymatic treatment. Table 4 illustrates the results of
treatment with the proteolytic enzymes trypsin and papain.
It could be seen that the expression of TM2- and TM3-
defined antigen disappeared after proteolysis in contrast to
TG8 antigen. But these sensitivities were not evident at low
enzyme concentrations. Treatment with neuraminidase had
no effect on the degree of antigenic expression.

Biochemical characterization of the membrane anti-
gen. To analyze the membrane component(s) recognized
by these MoAbs, the THP-1 monocyteid cell line was intern-
ally labeled by [35S]-methionine. After detergent solu-
bilization, aliquots of the lysate were treated with TM2 or
TM3 and protein A to precipitate immune complexes. The
isolated antigen was then analyzed by SDS-PAGE and
fluorography. The results are depicted in Fig 2. TM2 and
TM3 precipitated a broad band with mol wt of 110,000 to
140,000 daltons (mean, 120,000 daltons) under both reduc-
ing and nonreducing conditions. The same immunoprecipita-
tion patterns were confirmed using another monocytoid cell
line, U937, as a target cell (data not shown).

Because both MoAbs immunoprecipitated similar poly-
peptides, the similarity of the antigenic determinants recog-

Table 1. Reactivity of Blood Cells with TM2 and TM3 Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Cells Tested</th>
<th>No. of Samples Tested</th>
<th>TM2 No. of Samples Positive</th>
<th>TM3 No. of Samples Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Platelets</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bone marrow cells</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>T lymphocytes*</td>
<td>5</td>
<td>10.0% ± 5.34%§</td>
<td>8.4% ± 4.36%</td>
</tr>
<tr>
<td>B lymphocytes†</td>
<td>5</td>
<td>14.7% ± 7.05%</td>
<td>14.5% ± 5.36%</td>
</tr>
<tr>
<td>Null cells‡</td>
<td>5</td>
<td>32.2% ± 7.33%</td>
<td>32.6% ± 7.75%</td>
</tr>
<tr>
<td>PHA (1%) activated MNC</td>
<td>1</td>
<td>70%</td>
<td>60%</td>
</tr>
<tr>
<td>PWM (75 µg/mL) activated MNC</td>
<td>1</td>
<td>40%</td>
<td>40%</td>
</tr>
</tbody>
</table>

Assay for the reactivity with TM2 and TM3 was performed by indirect immunofluorescence as outlined Materials and Methods.

*OKT11*, 87.7%; Leu7*, 17.3%; NC1*, a MoAb against HLA-DR framework, 8.5%.
†OKT11*, 33.9%; Leu7*, 19.4%; NC1*, 73.3%.
‡OKT11*, 16.7%; Leu7*, 29.6%; NC1*, 29.5%.
§Mean ± SD.
nized was examined in cross-blocking studies. As shown in Fig 3, \(^{125}\)I-labeled TM2 was completely blocked by unlabeled TM3 in binding to the antigenic epitope on the surface membrane of THP-1 and vice versa.

The data suggest that these two MoAb detect the same or a closely positioned epitope, which leads to cross-blocking by steric hindrance.

**Effect on colony formation.** In order to define the bone marrow cells reacting with these MoAbs, CFU-GM was assessed in positive selection experiments by the indirect panning method. The MoAb reactive cells attached to dishes that had been coated with goat anti-mouse IgG were not bound tightly so that they could be recovered as the MoAb reactive population and were cultured in CFU-GM- and CFU-E/BFU-E-derived colonies (Table 5). The majority of CFU-GM were observed in the TM2 and TM3 positive populations; however, CFU-E/BFU-E-derived colonies were observed mainly in the TM3 negative populations. These experiments showed that TM2- and TM3-defined antigen is expressed on CFU-GM progenitor cells but not on erythroid progenitor cells.

**Effect on chemotaxis of neutrophils or monocytes.** Because TM2- and TM3-defined antigen was detected on peripheral blood polymorphonuclear cells and monocytes, we reasoned that this antigen might also be involved in membrane-associated neutrophil or monocyte functions. In a

### Table 2. Reactivity of TM2 and TM3 With Cells From Leukemia/Lymphoma Patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Case No.</th>
<th>Positive Cases (% of Positive Cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TM2</td>
</tr>
<tr>
<td>AML</td>
<td>10</td>
<td>(99, 99, 99, 97, 95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(95, 90, 90, 89)</td>
</tr>
<tr>
<td>APL</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(95, 90)</td>
</tr>
<tr>
<td>AMML</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(97)</td>
</tr>
<tr>
<td>AMoL</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(99, 50)</td>
</tr>
<tr>
<td>CML</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Chronic phase</td>
<td></td>
<td>(95, 95, 89, 83, 45)</td>
</tr>
<tr>
<td>Myeloid crisis</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(97, 93, 89)</td>
</tr>
<tr>
<td>Lymphoid crisis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(95)</td>
</tr>
<tr>
<td>CMML</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(95)</td>
</tr>
<tr>
<td>Erythroleukemia</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(96, 95, 73)</td>
</tr>
<tr>
<td>Malignant reticulosis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(60)</td>
</tr>
<tr>
<td>AUL</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(84, 80)</td>
</tr>
<tr>
<td>Non-T, non-B ALL</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(99, 97, 97, 97, 96)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(92, 90, 89, 80)</td>
</tr>
</tbody>
</table>

| B lymphoma           | 3        | 0                                      | 0                                      |
|                      |          |                                       |                                        |
| B CLL                | 2        | 0                                      | 0                                      |
|                      |          |                                       |                                        |
| Pre-T ALL            | 1        | 1                                      | 1                                      |
|                      |          | (26)                                   | (30)                                   |
| Pre-T lymphoma       | 2        | 1                                      | 1                                      |
|                      |          | (99)                                   | (99)                                   |
| Cortical T ALL       | 1        | 1                                      | 1                                      |
|                      |          | (98)                                   | (99)                                   |
| T ALL                | 2        | 0                                      | 0                                      |
| T lymphoma           | 1        | 0                                      | 0                                      |
| ATL                  | 2        | 0                                      | 0                                      |
| T CLL                | 2        | 0                                      | 0                                      |

Assay for the reactivity with TM2 and TM3 was performed by indirect immunofluorescence as outlined in Materials and Methods. AML, acute myeloblastic leukemia; APL, acute promyelocytic leukemia; AMML, acute myelomonocytic leukemia; AMoL, acute monocytic leukemia; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukemia; AUL, acute unclassified leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; ATL, adult T cell leukemia.
A NOVEL LEUKOCYTE DIFFERENTIATION ANTIGEN

Table 3. Reactivity of Established Cell Lines With Monoclonal Antibodies TM2 and TM3

<table>
<thead>
<tr>
<th>Cells Tested</th>
<th>Positive Cells (%)</th>
<th>TM2</th>
<th>TM3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common ALL type</td>
<td>Reh</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pre B lymphoid</td>
<td>Nalm-6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Immature B lymphoid</td>
<td>SD</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B lymphoid</td>
<td>Raji</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T lymphoid</td>
<td>Mol-3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myeloid</td>
<td>KG-1</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>ML-1</td>
<td>98</td>
<td>97</td>
</tr>
<tr>
<td>Promyelocytic</td>
<td>HL-60</td>
<td>98</td>
<td>95</td>
</tr>
<tr>
<td>Myeloid/erythroid</td>
<td>K 562</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Monoblastoid</td>
<td>U 937</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Assay for the reactivity with TM2 and TM3 was performed by indirect immunofluorescence as outlined Materials and Methods.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*SD cell line was established from a patient with Burkitt’s lymphoma by R-F Chen.

chemotaxis system driven by the chemoattractant endotoxin, both MoAbs TM2 and TM3 blocked the movement of cells through a micropore filter (Fig 4). Seventy-eight percent or 54% of inhibition of neutrophil chemotaxis occurred at 1:100 dilution of TM2 or TM3, respectively, whereas these MoAbs failed to inhibit the neutrophil chemotaxis toward supernatant from the culture of E. coli. TM3 F(ab')2 fragments behaved analogously on neutrophil chemotaxis driven by endotoxin-activated serum. Similarly, monocyte chemotaxis toward endotoxin-activated serum was inhibited by 56% or 43% at 1:1,000 dilution of TM2 or TM3, respectively.

Effect on luminol-dependent chemiluminescence of neutrophils. TM2 and TM3, at various concentrations, neither enhanced non blocked chemiluminescence caused by fMLP (Fig 5), A23187 (data not shown), and opsonized zymosan (data not shown).

Effect on platelet aggregation. TM2 and TM3 MoAbs had no effect on platelet aggregation induced by ADP (Fig 6), arachidonic acid (data not shown), or collagen (data not shown), although these MoAbs reacted with almost all platelets.

DISCUSSION

In the present study, we describe two MoAbs, TM2 and TM3, which are mainly generated to myeloid cells. The antigen to which the antibodies bind is a 110,000- to 140,000-dalton (mean, 120,000-dalton) polypeptide. This antigen has a widespread distribution on hematopoietic cell populations—granulocytes, monocytes, and platelets—and is also found on immature lymphoid cell populations.

MoAbs reactive with human granulocytes and monocytes have been reported: OKM1,6 MO1,10 TM15.34 MO4 binds to both monocytes and platelets.35 However, no MoAb that is reactive with granulocytes, monocytes, and platelets—and is also found on immature lymphoid cell populations.

MoAbs reactive with human granulocytes and monocytes have been reported: OKM1,6 MO1,10 TM15.34 MO4 binds to both monocytes and platelets.35 However, no MoAb that is reactive with granulocytes, monocytes, and platelets—and is also found on immature lymphoid cell populations.

TM2 and TM3 bound to all myeloid (KG-1, ML-1, and HL60) and monocytoid (U937 and THP-1) cell lines tested but not myeloid/erythroid (K562) cell line. K562 cells are considered to contain cells capable of expressing markers erythroid precursors as well as very immature myeloid cells.36 It has been demonstrated in the mouse that a type of hematopoietic colonies is consisting of granulocytes, macrophages, megakaryocytes, and yet lacking erythroid ele-
Table 5. Recovery of CFU-GM and CFU-E/BFU-E Colonies in the Fractions of TM2- and TM3-Treated Bone Marrow Cells

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Fraction</th>
<th>CFU-GM*</th>
<th>CFU-E†</th>
<th>BFU-E†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7 14</td>
<td>Day 7 14</td>
<td>Day 7 14</td>
</tr>
<tr>
<td>NC1†</td>
<td>+</td>
<td>19 9 25 3</td>
<td>0 0 0 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>4 2 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TM2</td>
<td>+</td>
<td>67 42 ND ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>3 0.5 ND ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TM3</td>
<td>+</td>
<td>48 36 7 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>15 13 88 5</td>
<td></td>
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</tr>
</tbody>
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ND, not determined.
*Colonies per 2.5 x 10⁵ cells plated.
†Colonies per 5 x 10⁶ cells plated.
‡NC1, a MoAb against HLA-DR framework.

Fig 3. (A) Blocking of the binding [125I]-labeled TM2 antibody to THP-1 cells by preincubation with unlabeled ascites fluid containing TM2 (○), TM3 (○), or TG8 (Δ) (a MoAb that mainly binds to granulocytes). (B) Blocking of the binding [125I]-labeled TM3 antibody to THP-1 cells by preincubation with unlabeled ascites fluid containing TM2 (■), TM3 (○), or TG8 (Δ).

Fig 4. (A) Effect of TM2 on chemotaxis of neutrophils or monocytes. Neutrophil chemotaxis toward endotoxin-activated serum (—○—), or E colic-cultured media (—■—), monocytes toward endotoxin-activated serum (—□—). (B) Effect of TM3 on chemotaxis of neutrophils or monocytes. Neutrophil chemotaxis toward endotoxin-activated serum (—○—) or E colic-cultured media (—●—), monocytes toward endotoxin-activated serum (—□—). (——○—): Effect of TM3 F(ab')2 fragments on neutrophil chemotaxis driven by endotoxin-activated serum. Equivalent concentrations of immunoglobulin were used.

These colonies could be distinguished from granulocyte-erythrocyte-macrophage-megakaryocyte (GEMM) colonies because the former lacked the erythroid elements. Coexpression of antigen between monocytes, granulocytes, and platelets may suggest a differentiative link among the monocyte, granulocyte, and platelet-megakaryocyte lineages.

Because TM2 and TM3 are reactive with the KG-1 cell line, which is considered to represent myeloid precursors,38 we have studied the expression of TM2- and TM3-defined antigen on CFU-GM cells, using the indirect panning method. We found that CFU-GM progenitor cells expressed the TM2- and TM3-defined antigen, but TM3 failed to react with BFU-E/CFU-E progenitor cells. These results confirmed that TM2- and TM3-defined antigen is uniformly expressed on myeloid hematopoietic cells but not on erythroid hematopoietic cells.

It is unlikely that these antibodies bound nonspecifically to cells by the receptor for the Fc fragment of IgG. These antibodies did not bind B lymphocytes or B lymphoid cell lines. Moreover, the IgG antibody-coated erythrocyte (EA) rosette formation with granulocytes was not inhibited by these MoAbs (data not shown).
cells subsequently lose this antigen through their maturation and differentiation. However, mature T and B lymphocytes still have potency to express the antigen again when T and B lymphocytes are transformed by some mitogens.

MoAbs directed against the same antigenic determinants display differences in their reaction patterns. Recently, it has been shown that two mouse anti-human myeloid MoAbs recognize the same carbohydrate structure, 3-fucosyl-N-acetyllactosamine, although these two MoAbs have different reactivities against neutrophils, eosinophils, and monocytes. It was also revealed that the majority (9/12) of the independently isolated MoAbs reacting against the surface antigens of neutrophilic granulocytes and their precursor cells, but not reacting with other normal cells, had immunoprecipitated the same glycoproteins (mol wt 145 kd and 105 kd). Cross-competition experiments and different reactivity patterns with cell lines and bone marrow cells already confirmed the diversity of the antigenic sites recognized by these MoAbs on the two glycoproteins of granulocyte. The binding activity of labeled TM2 or TM3 against THP-1 cells was completely blocked in cross-competition assays. These experiments suggest that the molecules recognized by TM2 and TM3 are closely associated. However, TM2 and TM3 MoAbs are distinct from "granulocyte specific" MoAbs, because TG8, which is previously obtained for anti-granulocyte-specific MoAbs, had no blocking effect on TM2 or TM3 MoAb binding activity with THP-1 cells.

Fig 5. Effect of TM2 on luminol-dependent chemiluminescence (CL) of neutrophilic granulocyte. (A) No activation of CL was caused by TM2 at 1/100 dilution. (B) CL caused by 10^{-18} mol/L fMLP. (C) CL caused by 10^{-14} mol/L fMLP. TM2 was added to a neutrophilic-rich suspension three minutes beforehand.

The present results also showed that several T, B, and null cell lines were not reactive with TM2 and TM3. According to the analysis of established T cell lines by Minowada et al., CCRF-CEM, which represent early T blast cells, was negative. Reh, which represents cells at a level of lymphoid precursor, was also negative with TM2 and TM3. However, the antigen was expressed on many cells from patients with cALL and some cells from patients with T lymphoid malignancies. These results suggest that TM2- and TM3-defined antigen is also expressed on early stages of T and B lymphoid lineage. This observation was confirmed on normal lymph node tissue sections by Dr. Mon (University of Tokyo, personal communication, Feb 1984). Many lymphocytes in germinal center, where immature lymphocytes are observed normally, were specifically stained with TM2 and TM3 by peroxidase-antiperoxidase enzyme immunostain. Interestingly, lymphoblasts, which were transformed by PHA or PWM in vitro culture, expressed TM2- and TM3-defined antigen by indirect immunofluorescence assay.

We presume at present that TM2 and TM3 positive cells in these lymphoid malignancies belong to the cells representing the early stage level in maturity and that lymphoid

Fig 6. Effect of TM2 on ADP-induced platelet aggregation. (A) No aggregation was induced by TM2 at 1/100 dilution. (B) Platelet aggregation induced by ADP. (C) Platelet aggregation induced by ADP. TM2 was added to a platelet-rich plasma four minutes beforehand.
and the complement receptor type 3-associated OKM 1/Mac-1/Mo 1 molecule were found to be structurally and antigenetically related. Furthermore, MoAb 60.3 and OKM1 are different but proposed to recognize a family of multimeric surface antigens since neither 60.3 nor OKM1 react normally with cells from a patient with recurrent bacterial infections. It cannot be resolved at present whether MoAbs TM2 and TM3 recognize a component of a LFA-1 family antigen complex.

In conclusion, TM2 and TM3 are unique MoAbs which define an antigen that is expressed on almost entire stages of neutrophilic granulocyte, monocyte, and megakaryocyte-platelet lineages and is transiently expressed on early stages of T cell and B cell lineages but not on erythrocyte lineage. These TM2 and TM3 MoAbs are of value in studying the linkage between myeloid and lymphoid differentiation and hematopoietic development of neutrophilic granulocytes, monocytes, and megakaryocytes.

ACKNOWLEDGMENT

We thank Dr Keiya Ozawa for his help with the CFU-GM assay. We acknowledge the excellent technical assistance of Ranko Hirata and Machiko Okuyama.

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A NOVEL LEUKOCYTE DIFFERENTIATION ANTIGEN


A novel leukocyte differentiation antigen: two monoclonal antibodies TM2 and TM3 define a 120-kd molecule present on neutrophils, monocytes, platelets, and activated lymphoblasts

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