Null Cell Identification and Characterization With OKT16: An Anti-p40 Monoclonal Antibody

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A murine monoclonal antibody, OKT16, specific for human lymphocytes of T lineage, was isolated by standard immunization and hybridization techniques. The distribution of the antigen defined by OKT16 was similar to the antigen reactive with monoclonal antibodies 3A1 and WT1. This identity of antigen targets was confirmed in an enzymelinked immunosorbent assay system and by sequential immunoprecipitation. Under reducing conditions, OKT16 reacted with an antigen of 40K daltons; however, under nonreducing conditions this antigen appeared as an 84K-dalton molecule, which suggests that the p40 antigen exists as a disulfide-linked dimer. By indirect immunofluorescence, OKT16 reacted with a greater fraction of nonrosetting, non-B (null) lymphocytes than with antibodies to other T cell-specific proteins. Two-color immunofluorescence demonstrated the coexpression of the T16 antigen and the C3bi receptor on most null cells. The T10 antigen (found on cortical thymocytes and activated peripheral T cells) was restricted to most T16-bearing null cells and expression of the Fc receptor for aggregated IgG (defined by monoclonal antibody 73.1) was restricted to a major subset of T16-bearing null cells. The T cell-specific markers defined by OKT8, OKT11, and OKT17, as well as the monocyte marker defined by OKM5, were expressed by smaller subsets of OKT16-reactive null cells. These studies support by phenotypic analysis the functional heterogeneity ascribed to null cells. The 40K-dalton T16 antigen has the most extensive null cell representation of all the T lineage markers described to date.

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goat antihuman Ig-coated (Tago, Inc, Burlingame, Calif) plastic Petri dishes according to the method of Wysocki and Sato.13 Less than 10% of the nonadherent cells expressed the T3 antigen and less than 20% expressed SmIg by indirect immunofluorescence. These are referred to, therefore, as null cells.

Single-cell suspensions of thymocytes were prepared from fresh thymus tissue obtained from pediatric patients undergoing cardiac surgery.

Enzyme-linked immunosorbent assay (ELISA). Modification of an ELISA system described by Fuccello et al19 was used to determine whether distinct monoclonal antibodies recognized a common molecule. Flat-bottom wells of a polystyrene chloride plate (Dynatech Labs, Alexandria, Va) were filled with 200 μL of goat antimouse IgG and IgM (Tago) at 5 μg/mL in 0.1 mol/L bicarbonate buffer, pH 9.6. After incubation for 18 hours at 4 °C in a humid chamber and subsequent removal of nonadherent antibody, the coated wells were rinsed three times with phosphate-buffered saline (PBS), pH 7.2, containing 0.5% Tween 20 (Sigma, St Louis), and three times with glass-distilled water and then dried at 37 °C for one hour. Wells were filled with the murine monoclonal antibody to be tested (either undiluted culture supernatant, ascites fluid diluted 1:1,000, or purified antibody at 1 μg/mL) for one hour at room temperature in a humid atmosphere and unbound antibody was removed by washing three times with PBS–0.05% Tween 20. Nonidet P-40 (NP40) extract (see below) of sheep erythrocyte rosette-positive (E+) lymphocytes or HSB-2 (T-ALL) cells containing the T16 antigen was added to the antibody-coated wells and allowed to bind for one hour at room temperature in a humid chamber. Wells were cleared of unbound cellular extract by three washes with PBS–0.05% Tween 20. Mouse immunoglobulin (10 μg/mL) was added to each well for 30 minutes to bind to any free goat antimouse Ig and then removed by the rinsing procedure described earlier. Horseradish peroxidase-conjugated OKT16 prepared as described previously14 was diluted in PBS containing 5% (vol/vol) isotype-matched murine monoclonal antibody ascites of an unrelated specificity and was allowed to bind for one hour. Unbound antibody was removed by three washes with PBS–0.05% Tween 20 and 200 μL of HOH (ODP) substrate was added per well. Color was permitted to develop for 20 minutes at room temperature in the dark, and the reaction was stopped by acidification. Optical density was measured at 492 nm using a Titerette Multiskan plate reader (Flow Laboratories, McLean, Va).

Cell surface iodination and extraction. E+ lymphocytes or HSB-2 cells were suspended in PBS at 106 cells per 200 μL and iodinated with 1 mCi Na125I (New England Nuclear, Boston) using iodogen (Pierce Chemical Co, Rockford, Il). Iodinated cells, freed of unincorporated iodine by three washes of PBS with 5 mmol/L NaI, were adjusted to a density of 2.5 × 107 cells per milliliter, and were then extracted for 15 minutes in ice on 10 mL Tris acetate buffer, pH 8.0, containing 0.5% nonidet P40 (Particle Data Laboratories, Ltd, Elmhurst, Il), 1 mg peptatin per milliliter, 2 mmol/L phenylmethylsulfonyl fluoride, and 50 mmol/L iodoacetamide (Sigma, St Louis). Extracts were cleared of nuclei and cell debris by centrifugation at 100,000 g for 45 minutes. Unbound125I was removed by dialysis against three 500-ml changes of the extraction buffer.

Immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Cell extracts were cleared of nonspecifically adsorbing material with (a) aggregates of goat a-mouse IgG and mouse IgG (Cooper Biomedical, Inc, Malvern, Pa) (b) fixed Staphylococcus aureus Cowan I strain (The Enzyme Center, Walden, Mass); and (c) mouse IgG bound to Sepharose 4B beads (Pharmacia, Piscataway, NJ). Purified OKT16 or 3A1 were coupled to CNBr-activated Sepharose 4B beads at 2 mg of antibody per milliliter of packed beads. Antigen was precipitated from up to 200 μL of labeled extracts using 25 μL of packed beads. Nonspecifically adsorbed material was removed as described previously.20 Immunoprecipitates were dissociated by boiling before being loaded onto 12% SDS-polyacrylamide gels as described by Laemmli.21 Reduced samples were dissoluted in buffer containing 10% 2-mercaptoethanol and nonreduced samples were dissolved in buffer containing 20 mmol/L iodoacetamide to prevent artifactual disulfide associations. Gels were stained, dried, and subjected to autoradiography at -70 °C with Kodak XAR film (Eastman Kodak, Rochester, NY) and Cronex lightening plus enhancing screens22 (Dupont, Wilmington, Del).

Immunofluorescence analysis. The percentages of cells expressing a given antigen were assessed by indirect immunofluorescence. In brief, saturating amounts of monoclonal antibodies (ascites diluted 1:100 to 1:1,000) were reacted with 107 cells for 30 minutes at 4 °C in medium containing 15% heat-aggregated human plasma. Cells were washed free of unbound antibody and incubated for 30 minutes at 4 °C with fluorescein-conjugated goat antimouse IgG (Meloy Laboratories, Springfield, Va) diluted in the same medium. After two additional washes, cells (defined as lymphocytes by light scattering properties)17 were analyzed for fluorescence on a Cytofluorogr FC 200/4800A (Ortho Diagnostic Systems, Westwood, Mass). Cells displaying fluorescence intensity above that of control cells stained with P3x63Ag8 ascites (a myeloma producing light and heavy chains of undetermined specificity) were considered positive for antigen expression.

In some experiments, two-color immunofluorescence was used for analysis of cell surface antigen expression. Null cells (107) were first reacted with predetermined saturating amounts of the appropriate monoclonal antibody. This and each subsequent incubation was for 30 minutes at 4 °C in the presence of 15% heat-aggregated human plasma. After two washes to remove unbound antibody, cells were reacted with 100 μL of biotinylated horse antimouse IgG (Vector) diluted 1:20 in RPMI with 25 mmol/L HEPES and 5% fetal calf serum. Excess antisera was washed from the cells, and the cells were suspended in 100 μL of rhodamine-avidin D diluted 1:20 (Vector) in PBS. Cells were washed free of unbound avidin, and 50 μg of mouse IgG (Pel-Freeze Biologicals, Rogers, Ark) were added to occupy any unbound sites on the horse antimouse IgG. Finally, the cells were reacted with fluorescein-conjugated monoclonal antibody of the appropriate specificity and dilution. Two-color fluorescence analysis was accomplished on a system 50H Cytofluorograf linked to a 2150 computer system (Ortho).

RESULTS

Isolation and reactivity patterns of the OKT16 monoclonal antibody. OKT16 was isolated on the basis of its reactivity with all SRBC-rosetting (E+) lymphocytes from peripheral blood. Unlike previously described T lymphocyte markers, however, the T16 antigen was detected on a major portion of nonrosetting (E-) lymphocytes. Further fractionation of the E+ population revealed that the T16 antigen was expressed by 74% ± 11% of SmIg-negative or null cells by indirect immunofluorescence (Table 1). Only 40% ± 11% of null cells expressed the T11 (p50; SRBC receptor) antigen, and the density of T11 antigen on these cells as judged by fluorescence intensity was much lower than that expressed by the E+ population (data not shown). Neither the mature T cell markers, T1 (p67) and T3 (p20), nor the immature thymocyte marker, T6 (p45, 12), were expressed by null cells.

Single-cell suspensions of thymocytes showed a broad range of reactivity with OKT16 (35% to 75%) (Table 1).
Peripheral blood B cells, monocytes, granulocytes, and platelets were consistently nonreactive with OKT16 by indirect immunofluorescence (data not shown). Transformed cell lines of T lineage (HSB-2, JM, HPB-ALL, CEM, MOLT-3, JURKAT, MOLT-4, 8402) were reactive with OKT16, whereas transformed cell lines of B (EB3, RAJI, DAUDI, U266), myeloid (HL60), or erythroid (K562) lineage did not bind OKT16.

**Immunoprecipitation of the T16 antigen.** Immunoprecipitation of an extract from 125I-labeled E' lymphocytes by OKT16 covalently coupled to Sepharose 4B beads, followed by electrophoresis on 12% SDS-polyacrylamide gels, resulted in identification of a 40K-dalton polypeptide in both the presence (Fig 1B, lane 2) and the absence (Fig 1A, lane 1) of 2-mercaptoethanol. Under nonreducing conditions there was precipitated, in addition to the predominant 40K-dalton species, an 84K-dalton molecule. Neither the 40K-dalton nor the 84K-dalton polypeptide was observed if the antibody used for precipitation was normal mouse Ig (Fig 1A, lane 2, and B, lane 1). Reduced precipitates using OKT16 or mouse IgG contained a nonspecifically precipitated molecule of 91K daltons; this species was not present in nonreduced precipitates. The 84K-dalton species precipitated by OKT16 appeared even though 50 mmol/L iodoacetamide was present throughout the cellular extraction procedure and nonreduced samples for SDS-PAGE analysis contained 20 mmol/L iodoacetamide.

**Multiple antibodies detect the T16 (p40) antigen.** Several monoclonal antibodies have been reported that identify a 40K-dalton polypeptide found on peripheral blood T cells. 3A1, a monoclonal antibody originally reported to define a subset of peripheral T cells,\(^{18,19}\) precipitates a 40K-dalton polypeptide from several T cell lines,\(^{20}\) and WT1, a monoclonal antibody that reacts with most prethymic acute lymphoblastic leukemias (ALL), precipitates a 40K-dalton polypeptide from the HSB-2 T-ALL cell line.\(^{21,22}\) We developed an ELISA system to determine whether OKT16 recognized the same antigen as 3A1 and WT1. The monoclonal antibody to be tested was bound to a polyvinylchloride microtiter plate that had been precoated with goat antimouse IgG serum, and cellular extract containing antigen was permitted to react with the monoclonal antibody. Free goat antimouse binding sites were filled with excess mouse Ig before the addition of horseradish peroxidase (HRP)-conjugated OKT16.

As shown in Table 2, when either OKT16, 3A1, or WT1 was the monoclonal antibody coating the plate, the levels of HRP–OKT16 bound were significantly higher than those bound by plates coated with either OKT1, OKT3, or OKT11. The surprising finding that the OKT16–T16 antigen complex was able to find HRP–OKT16 suggests that the 40K-dalton polypeptide identified by immunoprecipitation (Fig 1A) is in fact a dimer of the 40K-dalton antigen. The same method of cellular extraction was used in both procedures. The alternative, although less likely, explanation is that the 40K-dalton protein has two identical epitopes.

**Sequential immunoprecipitation with OKT16 and 3A1.** Additional evidence that OKT16 and 3A1 bind to the same molecule was provided by sequential immunoprecipitation. E' cellular extracts containing antigen were cleared of most nonspecifically absorbing material by immunoprecipitation with mouse IgG coupled to Sepharose 4B beads, and the dissociated immunoprecipitate was subjected to electrophoresis on a 12% SDS-polyacrylamide gel under reducing conditions; detection was by radioautography (Fig 1B, lane 1). A portion of the cleared cellular extract was reacted with
OKT16: NULL CELL CHARACTERIZATION

Table 2. ELISA Assay for Detection of Antibodies Binding T16 (p40) Antigen

<table>
<thead>
<tr>
<th>MoAb on Plate</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT16</td>
<td>0.244 ± 0.015</td>
<td>0.917 ± 0.144</td>
</tr>
<tr>
<td>OKT1</td>
<td>0.062 ± 0.020</td>
<td>0.195 ± 0.106</td>
</tr>
<tr>
<td>OKT3</td>
<td>0.057 ± 0.001</td>
<td>0.195 ± 0.097</td>
</tr>
<tr>
<td>OKT11</td>
<td>0.062 ± 0.001</td>
<td>0.144 ± 0.028</td>
</tr>
<tr>
<td>3A1</td>
<td>0.544 ± 0.026</td>
<td>1.344 ± 0.098</td>
</tr>
<tr>
<td>WT1</td>
<td>0.129 ± 0.012</td>
<td>0.427 ± 0.105</td>
</tr>
</tbody>
</table>

Microtiter wells were coated sequentially with (a) goat antiamouse IgG and IgM, (b) monoclonal antibody, (c) extract containing antigen, (d) mouse Ig. (e) horseradish peroxidase-conjugated OKT16 containing isotype-matched murine monoclonal antibody of an unrelated specificity, and (f) OPD substrate as described in Materials and Methods. Color development was for 20 minutes in experiment 1 and for 30 minutes in experiment 2, which accounts for the relative difference in optical density.

*Optical density at 492 nm; mean of three wells ± SD.

3A1, and a 40K-dalton (reduced) polypeptide was specifically immunoprecipitated (Fig 1B, lane 5). An identical pattern was obtained if OKT16 was reacted with a separate portion of the extract (Fig 1B, lane 2). After three sequential immunoprecipitations with OKT16, no additional material capable of binding OKT16 or 3A1 remained in the extract (Fig 1B, lanes 3 and 4). This result indicates that OKT16 and 3A1 recognize the same polypeptide.

Characterization of null cells by two-color immunofluorescence and OKT16. Null cells represent only 10% to 15% of peripheral blood lymphocytes and yet are responsible for a variety of functions, including natural killer activity, antibody-dependent cell-mediated cytotoxicity, lectin-dependent cellular cytotoxicity, as well as precursors to other functional cell types. We found the T16 antigen expressed on a far greater percentage of null cells than most other T lineage markers. It was of interest, therefore, to examine highly enriched null cells for coexpression of the T16 antigen with other T cell markers, as well as with non-T cell markers present on but not restricted to null cells.

We first analyzed peripheral blood mononuclear cells from which only the SRBC-rosetting cells had been removed to minimize nonselective loss of E- cells. This population was composed of approximately 50% lymphocytes and 50% monocytes by forward-angle v right-angle light-scattering properties and represented about 35% of the total mononuclear cells. Double-marker immunofluorescent analysis of the cells scattering in the lymphocyte region used a three-step staining method to label cells with monoclonal antibody and rhodamine and a single-step method to label cells with fluorescein-conjugated monoclonal antibodies (see Materials and Methods). This lymphocyte population contained less than 5% mature T cells (OKT3 positive) and 65% B cells (OKB2 positive) (Fig 2). OKB2 is expressed by all SmIg+ cells in the peripheral blood.

The T6 antigen was expressed by 23% of these E- lymphocytes, but more important, less than 2% of the cells shared the T16 and B2 antigens. The sum of lymphocytes bearing the T16 and B2 antigens in this experiment was 88%, and in four additional experiments the sum was greater than 85%. The ratio of T16-B2 cells varied from 1:3 to 2:1. We concluded that the T16 antigen was expressed by the majority of T3 negative,
non-B cells and represented a useful null cell marker. All cells bearing the B2 antigen coexpressed Ia, whereas Ia was absent from T16-positive cells (Fig 2).

We exploited the T16 antigen to study coexpression of several T lineage and non-T lineage markers on highly enriched null cells. Mononuclear cells were depleted of monocytes by adherence to plastic. This procedure frequently results in the loss of a significant number of lymphocytes, but these adherent lymphocytes accounted for less than 5% of the total null cells by phenotypic analysis (data not shown). Lymphocyte-enriched mononuclear cells were depleted of T cells by rosetting with SRBC. The E- cells were 97% OKT3 positive. E- null cells were separated from B cells by incubation on antihuman Ig-coated polystyrene dishes. Nonadherent null cells were collected for double-marker analyses and less than 10% OKTI6-positive null cells were lost to the adherent B cell population.

As shown in Fig 3, more than 80% of null cells expressed the T16 antigen and less than 10% expressed the T3 antigen. B cell contamination was less than 5% in this experiment (data not shown), and results from three additional experiments indicate that only 10% to 15% of null cells may not express the T16 antigen. OKM10, an antibody directed toward the C3bi receptor expressed by monocytes and granulocytes,8,9 reacted with all OKT16-positive null cells. The T10 antigen, associated with cortical thymocytes and activated T cells,7 was present on a major subset of T16-positive null cells, whereas the Fc receptor for aggregated Ig of large granular lymphocytes defined by antibody 73.123 was expressed by a smaller subset.

Cells bearing T8, T11, T17, or M5 markers (Table 3) were restricted to the T16-positive population. The densities of these antigens on null cells was low (Fig 3), and increased fluorescence sensitivity might in fact reveal wider distrib-

![Fig 3. Surface marker analysis of null cells by two-color immunofluorescence with OKT16. Null cells were stained and analyzed as described in Fig 2. The percentages of cells stained positive for red but not green fluorescence are indicated along the bottom margin; the percentages of cells stained positive for green but not red fluorescence are indicated along the left margin. Percentages of cells bearing both antigens are reported in the upper right corner.](image-url)
Table 3. Monoclonal Antibodies Used in Null Cell Studies

<table>
<thead>
<tr>
<th>Ab</th>
<th>Specificity</th>
<th>Ag Size*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT6</td>
<td>Cortical thymocytes</td>
<td>49, 12</td>
<td>4</td>
</tr>
<tr>
<td>OKT3</td>
<td>Mature T cells</td>
<td>20</td>
<td>1, 4</td>
</tr>
<tr>
<td>OKT8</td>
<td>Cytotoxic/Suppressor T subset</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>OKT11</td>
<td>E rosette receptor</td>
<td>45</td>
<td>3</td>
</tr>
<tr>
<td>OKT17</td>
<td>T cells; thymocytes</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>OKM16</td>
<td>T, null, thymocytes</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>73.1</td>
<td>LGL, PMN; Fc receptor</td>
<td>50-70</td>
<td>23</td>
</tr>
<tr>
<td>OKM10</td>
<td>C3bi receptor</td>
<td>170, 95</td>
<td>9</td>
</tr>
<tr>
<td>OKM5</td>
<td>Monocytes, platelets</td>
<td>88</td>
<td>8</td>
</tr>
<tr>
<td>OKB2</td>
<td>B cells, PMN</td>
<td>ND</td>
<td>10</td>
</tr>
<tr>
<td>OKIa2</td>
<td>HLA-DR monomorphic Ag</td>
<td>34, 29</td>
<td>—</td>
</tr>
<tr>
<td>OKT10</td>
<td>Cortical thymocytes, activated T</td>
<td>46, 12</td>
<td>4, 7</td>
</tr>
</tbody>
</table>

ND, not determined.

*Reduced mol wt.

OKT6: NULL CELL CHARACTERIZATION

Fig 4. Null cell subset analysis by two color immunofluorescence. Null cells were stained and analyzed as described in Fig 2. The percentages of cells stained positive for red but not green fluorescence are indicated along the bottom margin; the percentages of cells stained positive for green but not red fluorescence are indicated along the left margin. Percentages of cells bearing both antigens are reported in the upper right corner. P3x63Ag8 ascites, a murine IgG monoclonal antibody of undetermined specificity, served as a negative control.

Null cell analysis with subset markers. The presence of several of these antigens on only a fraction of null cells prompted us to study them by double-marker analysis. As shown in Fig 4, only T8+ and T17+ cells constituted a fully overlapping population. T11 and T8 were expressed by largely nonoverlapping populations, as were T11 and T17. The total number of T8-positive cells was reduced by 50% when costained with OKT17, suggesting a steric inhibition by OKT17 (OKT17 was added to the cells before OKT8). In most experiments the total percentage of T8-positive cells was only a fraction of the T17+ population.

M5, an antigen associated with monocytes, platelets, and some null cells, was coexpressed by only a small percentage of T8-, T11-, or T17-positive cells. The fact that most null
cell subset markers identify partially nonoverlapping populations provides evidence for the heterogeneity often associated with these cells.

Figure 5 depicts the proposed relationship of null cell subsets. The results shown are derived from several experiments and are based on populations containing 10% mature T cells and 10% B cells. The precise relationship of 73.1-positive and OKT10-positive cells to cells bearing markers other than T16 is unknown; in the figure these populations have been indicated with a hatched bar. Designation of T8-positive cells is by a dashed line to show the donor variation of this population.

DISCUSSION

The antigen defined by monoclonal antibody OKT16 is a T lineage marker expressed by most peripheral blood E rosette-positive cells and most null cells (ie, lymphocytes that do not form E rosettes and have neither the mature T cell markers identified by OKT3 and OKT1 nor B lymphocyte markers). It is not expressed by peripheral blood B cells, monocytes, granulocytes, or platelets.

Histologic analysis of a variety of lymphoid and nonlymphoid tissues supports the T lineage restriction of the T16 antigen. T lymphocytes in the lymph node and spleen were positive for OKT16, whereas B cells, plasma cells, macrophages, and vascular endothelium were negative. Thymocytes expressed the T16 antigen; the antigen density was lower, however, on cortical thymocytes than on medullary thymocytes. Thymic epithelium did not stain with OKT16. Among the nonlymphoid organs studied with OKT16, reactivity was restricted to the T lymphocytes within the skin and gut. No staining was observed in heart, muscle, testis, pancreas, kidney, or brain (G. Janossy, unpublished observations).

Immunoprecipitation of the T16 antigen from either peripheral blood T cells or the T-ALL established cell line HSB-2 revealed a 40K-dalton polypeptide under reducing conditions. Two previously described monoclonal antibodies, WT1 and 3A1, bind to a common p40 antigen with a tissue distribution similar to that of the T16 antigen. We used an ELISA to show that OKT16 binds to the same molecule as do WT1 and 3A1. Sequential immunoprecipitation with OKT16, followed by 3A1, confirmed this conclusion.

If the antigen immunoprecipitated by OKT16 was analyzed under nonreducing conditions, we detected an 84K-dalton polypeptide in addition to the 40K-dalton protein observed under reducing conditions (Fig 1a). A similar observation was made by Sutherland et al if WT1 was used to precipitate antigen from cell extracts lacking iodoacetamide. However, when these experiments were repeated with a cell extract containing as little as 2 mmol/L iodoacetamide the larger band did not appear, suggesting that the larger protein was an artifactual disulfide-linked dimeric aggregate of the WT1 40K-dalton antigen. The T16 antigen was precipitated from extracts containing 50 mmol/L iodoacetamide and was loaded onto polyacrylamide gels under nonreducing conditions in the presence of iodoacetamide, yet the 84K-dalton protein was consistently observed. Recently, a monoclonal antibody termed LAU-A1, described by Carrel et al, was shown to compete for binding with 3A1. Both LAU-A1 and 3A1 precipitated antigens of 74K daltons under nonreducing conditions and 40K daltons under reducing conditions. It is possible, therefore, that the p40 antigen exists in the membrane as both a monomer and a dimer and that OKT16 recognizes both configurations, whereas WT1 recognizes primarily the monomeric form.

Cell lysates containing 50 mmol/L iodoacetamide were also the source of T16 antigen for the ELISA assay, in which HRP-OKT16 was able to bind to antigen that had been bound by either 3A1, WT1, or OKT16. If the antigen in the lysate possessed a single OKT16-reactive epitope, then no binding of HRP-OKT16 should have occurred when OKT16 was the antibody coating the well due to competition for the epitope. Because HRP-OKT16/OKT16 produced a positive ELISA reaction, the T16 antigen probably exists as a dimer in cell extracts. Our extracts contained more iodoacetamide than previously found necessary to prevent disulfide aggregation but we cannot exclude the possibility of an experimentally induced association of the T16 monomer. The ELISA may provide evidence for a difference in the antigenic specificity of WT1 and OKT16. When WT1 coated the well, the amount of HRP-OKT16 bound was less than that observed with coatings of 3A1 or OKT16. Because HRP-OKT16 reacted with antigen bound by OKT16, at least some of the antigen must have been present as a dimer, providing two T16 epitopes per molecule. A similar result is obtained if OKT9, an antibody directed to the homodimeric transferrin receptor, is both the antibody coating the plate and the antibody conjugated to HRP. WT1 was able to compete weakly with FITC-OKT16 on intact cells (data not shown).
and previous studies have shown that antibodies that are able to compete partially for antigen will exhibit a weak ELISA if the antibodies recognize only a single epitope per molecule.26 Fully competing antibodies result in a negative ELISA. Our extracts clearly contained forms of the antigen capable of binding two OKT16 antibodies. A plausible explanation for the weak ELISA reactivity of WT1 would be that it binds only the monomeric form of the antigen (Sutherland et al25), and because OKT16 and WT1 compete for antigen binding, a weakly positive ELISA would result on monomeric antigens. Alternatively, a weak ELISA could result if the binding affinity of WT1 for the antigen were reduced by the conditions of the assay, or if the dimeric antigen contained only one WT1 epitope formed by the folding of the two chains.

Expression of the T16 antigen by a greater percentage of null cells than any other T lineage marker described to date prompted us to use this marker to analyze the null cell population for containing a variety of monoclonal antibodies. Peripheral blood mononuclear cells depleted only of E rosetting-forming cells were studied by two-color immunofluorescence. This demonstrated the absence of the p40 antigen on B cells and its presence on most T3+, non-B lymphocytes under conditions in which nonselective cell losses were minimized (Fig 2). In fact, the summation of OKB2 (or OKIa2+) and OKT16+ cells within the nonrosetting lymphocyte population always accounted for more than 80%; the ratio of OKB2+ cells to OKT16+ cells, however, varied considerably among individuals. Cells bearing the DR antigen, as defined by OKla2, were almost completely restricted to B cells.

If B cells and adherent monocytes were removed from the nonrosetting population, the residual population of null cells was 80% to 90% reactive with OKT16. Two-color immunofluorescence analysis with OKT16 revealed that all OKT16-reactive null cells bear the C3bi receptor as defined by OKM10. The majority of OKT16-positive null cells also bear the T10 antigen, a marker shared by cortical thymocytes and activated (but not unstimulated) mature T cells. Null cells expressing the Fc receptor for aggregated Ig, identified by monoclonal antibody 73.1, were exclusively within the OKT16-positive population and represented about 80% of that population. Other T lineage markers identified by OKT11, OKT8, and OKT17 were detected on smaller subsets of OKT16-positive null cells, as was the myeloid lineage marker defined by OKM5.

Most null cells bearing T11 did not coexpress the T8, T17, or M5 antigens, and most OKM5-positive null cells did not express T8 or T17, but none of these populations was nonoverlapping (Fig 4). All OKT8+ cells, however, were contained within the OKT17+ population. The phenotypic diversity demonstrated by these monoclonal antibodies directed toward antigens usually associated with either T lymphocyte or monocyte lineage cells is in accordance with the functional heterogeneity within this population. Whereas all NK activity resides within the T3+ population, the function of the T16+T3+T3.1+ cell remains to be established. Perhaps these surface markers will permit a precise definition of cells capable of lectin-dependent cellular cytotoxicity.27

Approximately 70% to 80% of the T3+, T16+, non-B cells that display lymphocyte light-scattering characteristics express the Fc receptor for aggregated Ig. Abo et al28 have shown that 98% of cells bearing this receptor, as defined by Leu-11, are large granular lymphocytes and possess all the natural killer activity of peripheral blood mononuclear cells. Similarly, Perussia and Trinchieri29 have demonstrated that all natural killer, as well as killer cell, activity related to antibody-dependent cell-mediated cytotoxicity is restricted to cells reactive with 73.1 and possessing large granular lymphocyte morphology. It is reasonable to conclude, therefore, that large granular lymphocytes represent a large fraction of the OKT16+ null cells defined by lack of reactivity with OKT3 and OKB2.

Large granular lymphocytes bearing receptors for the Fc region of Ig have been reported to lack MHC-class II (DR, DS) molecules30 and to express the 32K-dalton molecule recognized by OKT8 at a lower density than OKT3+ mature T cells.31,32 We have obtained similar results with the broader null cell population. However, it is possible that null cells express La under certain activation conditions. Previous reports concluded that only some Fc receptor-positive cells or natural killer cells bear the C3bi receptor as detected by the monoclonal antibody OKM1.31,32 Using an antibody directed toward the C3bi receptor binding site (OKM10), we observe that all OKT16+ null cells bear this complement receptor. The epitope of the C3bi receptor reactive with OKM1, however, is either masked or missing on some Fc receptor-positive cells.

The 40K-dalton molecule recognized by OKT16 has been shown by several investigators to be an important diagnostic marker for leukemia classification. A study by Haynes et al33 demonstrated that 3A1 was able to distinguish peripheral blood T cells in cutaneous T cell lymphoma (3A1+) from those in acute lymphoblastic leukemia (3A1–). Vodinelich et al21 reported that WT1 was reactive with 100% of 80 cases of thymic acute lymphoblastic leukemia (ALL) and 18 of 24 cases of presumptive prethymic ALL. Six other T lineage markers were generally not expressed by these 24 cases of presumptive T-ALL. Similarly, Link et al34 found 4H9, also directed toward the p40 antigen, to be superior to other antibodies specific for T lineage markers in identifying acute leukemias and non-Hodgkin's lymphomas of T cell origin.

Vodinelich et al21 have suggested a therapeutic potential in bone marrow transplantation for antibodies reactive with the p40 antigen. They reported the p40 antigen (as detected by WT1) to be absent from BFU-E, CFU-GM, and multipotential progenitors in the bone marrow. Similar results were obtained using OKT16 (L. Filipovich, unpublished observations). OKT16 is, therefore, a potential candidate for removing normal or malignant T cells from donor bone marrow before bone marrow transplantation.

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null cell identification and characterization with OKT16: an anti-p40 monoclonal antibody

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