Acute Myeloid Leukemia M4 With Bone Marrow Eosinophilia (M4Eo) and inv(16)(p13q22) Exhibits a Specific Immunophenotype With CD2 Expression

By Henk J. Adriaansen, Peter A.W. te Boekhorst, Anne M. Hagemeijer, C. Ellen van der Schoot, H. Ruud Delwel, and Jacques J.M. van Dongen

Extensive immunologic marker analysis was performed to characterize the various leukemic cell populations in eight patients with inv(16)(p13q22) in association with acute myeloid leukemia with abnormal bone marrow eosinophilia (AML-M4Eo). The eight AML cases consisted of heterogeneous cell populations; mainly due to the presence of multiple subpopulations, which varied in size between the patients. However, the immunophenotype of these subpopulations was comparable, independent of their relative sizes. Virtually all AML-M4Eo cells were positive for the pan-myeloid marker CD13. In addition, the AML were partly positive for CD2, CD11b, CD11c, CD14, CD33, CD34, CD36, CDw65, terminal deoxynucleotidyl transferase (TdT), and HLA-DR. Double immunofluorescence stainings demonstrated coexpression of the CD2 antigen and myeloid markers and allowed the recognition of multiple AML subpopulations. The CD2 antigen was expressed by immature AML cells (CD34+, CD14−) and more mature monocytic AML cells (CD34−, CD14+), whereas TdT expression was exclusively found in the CD34+, CD14− cell population. The eight AML-M4Eo cases not only expressed the CD2 antigen, but also its ligand CD58 (leukocyte function antigen-3). Culturing of AML-M4Eo cell samples showed a high spontaneous proliferation in all three patients tested. Addition of a mixture of CD2 antibodies against the T11.1, T11.2, and T11.3 epitopes diminished cell proliferation in two patients with high CD2 expression, but no inhibitory effects were found in the third patient with low frequency and low density of CD2 expression. These results suggest that high expression of the CD2 molecule in AML-M4Eo stimulates proliferation of the leukemic cells, which might explain the high white blood cell count often found in this type of AML.

From the Departments of Immunology and Hematology, University Hospital Dijkzigt/Erasmus University, Rotterdam; the Department of Cell Biology and Genetics, Erasmus University, Rotterdam; the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam; and the Dr Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.

Submitted September 14, 1992; accepted January 9, 1993.

Address reprint requests to H.J. Adriaansen, MD, PhD, Department of Immunology, Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1993 by The American Society of Hematology.
MATERIALS AND METHODS

Patients and cytomorphology. Four children (<16 years of age) and four adults were diagnosed with AML-M4Eo (Table 1). In all cases, more than 50% of blast cells were found. The diagnosis of AML-M4Eo was based on cytomorphology of peripheral blood (PB) and bone marrow (BM) aspirates stained for May Grünwald Giemsa and cytochemistry (Sudan Black B, myeloperoxidase, and α-naphthyl acetate esterase), according to the revised criteria of the FAB group.3

Cell samples were obtained after informed consent of the patients, according to the guidelines of the Medical Ethics Committee of the Erasmus University/University Hospital, Rotterdam, The Netherlands.

Cytogenetic studies. Cytogenetic studies of BM cells were performed on PB samples (patients E.K., N.S., A.K., J.M., D.W.) or BM samples (patients E.E., M.B., M.V.) from the eight AML patients at initial diagnosis. Mononuclear cells (MNC) were isolated by Ficoll Paque (density, 1.077 g/cm3; Pharmacia, Uppsala, Sweden) density centrifugation. These MNC samples were frozen and stored in liquid nitrogen.

Immunologic marker analysis. MNC were incubated as described with optimally titrated monoclonal antibodies (MoAbs).28 Several MoAbs were conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE), which enabled us to perform double-marker immunophenotyping.

Table 1. Clinical Features and Immunologic Marker Analysis of Eight AML-M4Eo Patients

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)/sex</td>
<td>1/M</td>
<td>14/M</td>
<td>10/F</td>
<td>38/F</td>
<td>26/M</td>
<td>63/F</td>
<td>13/F</td>
<td>37/M</td>
</tr>
<tr>
<td>WBC count (×10⁹/L)</td>
<td>24.9</td>
<td>165</td>
<td>77.1</td>
<td>194</td>
<td>75.9</td>
<td>142</td>
<td>10.3</td>
<td>69.2</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CNS leukemia</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>Cell sample</td>
<td>PB</td>
<td>BM</td>
<td>BM</td>
<td>PB</td>
<td>PB</td>
<td>BM</td>
<td>PB</td>
<td>PB</td>
</tr>
</tbody>
</table>

Immunologic markers

<table>
<thead>
<tr>
<th>Single IF stainings</th>
<th>CD2 (T11)</th>
<th>CD11a (3B13)</th>
<th>CD11b (Leu-15)</th>
<th>CD11c (Leu-M5)</th>
<th>CD13 (My7)</th>
<th>CD14 (My4)</th>
<th>CD15 (VM-D5)</th>
<th>CD19 (Bb)</th>
<th>CD19 (Leu-12)</th>
<th>CD20 (B1)</th>
<th>CD33 (My9)</th>
<th>CD34 (Bi-3C5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)/sex</td>
<td>1/M, E.E.</td>
<td>M.B.</td>
<td>N.S.</td>
<td>A.K.</td>
<td>J.M.</td>
<td>M.V.</td>
<td>D.W.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count (×10⁹/L)</td>
<td>24.9</td>
<td>165</td>
<td>77.1</td>
<td>194</td>
<td>75.9</td>
<td>142</td>
<td>10.3</td>
<td>69.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS leukemia</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell sample</td>
<td>PB</td>
<td>BM</td>
<td>BM</td>
<td>PB</td>
<td>PB</td>
<td>BM</td>
<td>PB</td>
<td>PB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Immunologic markers

<table>
<thead>
<tr>
<th>Single IF stainings</th>
<th>CD2 (T11)</th>
<th>CD11a (3B13)</th>
<th>CD11b (Leu-15)</th>
<th>CD11c (Leu-M5)</th>
<th>CD13 (My7)</th>
<th>CD14 (My4)</th>
<th>CD15 (VM-D5)</th>
<th>CD19 (Bi)</th>
<th>CD19 (Leu-12)</th>
<th>CD20 (B1)</th>
<th>CD33 (My9)</th>
<th>CD34 (Bi-3C5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)/sex</td>
<td>1/M, E.E.</td>
<td>M.B.</td>
<td>N.S.</td>
<td>A.K.</td>
<td>J.M.</td>
<td>M.V.</td>
<td>D.W.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count (×10⁹/L)</td>
<td>24.9</td>
<td>165</td>
<td>77.1</td>
<td>194</td>
<td>75.9</td>
<td>142</td>
<td>10.3</td>
<td>69.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS leukemia</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell sample</td>
<td>PB</td>
<td>BM</td>
<td>BM</td>
<td>PB</td>
<td>PB</td>
<td>BM</td>
<td>PB</td>
<td>PB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data represent percentage positivity per MNC.

Abbreviation: NA, not available.
analysis. We used the B-cell markers CD10 (VIL-A1, Dr W. Knapp, Vienna, Austria), CD19 (Leu-12, Becton Dickinson, San Jose, CA), and CD20 (B1, Coulter Clone, Hialeah, FL); the T-cell markers CD2 (T11 and T11 FITC, Coulter Clone; 6G4 [T11.1], 4B2 [T11.2], and HK27 [T11.3], Dr R. W. A. van Liem, Amsterdam, The Netherlands), CD3 (Leu-4, Leu-4 FITC, and Leu-4 PE, Becton Dickinson), CD4 (Leu-3 PE, Becton Dickinson), CD7 (3A1, American Type Culture Collection, Rockville, MD), and CD8 (Leu-2 PE, Becton Dickinson); the myeloid monocary markers CD13 (My7, My7 PE, Coulter Clone), CD14 (My4, My4 FITC, and My4 PE, Coulter Clone), CD15 (VIM-D5, Dr W. Knapp; Leu-M1 FITC, Becton Dickinson), CD33 (My9, My9 FITC, and My9 PE, Coulter Clone), and CDw65 (VIM-2, Dr W. Knapp; BMA-0210 FITC, Professor Dr W. Ax, Behring Diagnostica, Marburg, Germany); the erythroid marker glycophorin A (VIE-G4, Dr W. Knapp); the platelet markers CD42a (FMC25, Dr H. Zola, Bedford Park, Australia) and CD61 (C17, Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands); the non-lineage-specific markers CD1a (T13B3, Dr R. W. A. van Liem; CLB LFA1/2, Central Laboratory of the Blood Transfusion Service), CD11b (CLB mon3/1, Central Laboratory of the Blood Transfusion Service; Leu-15 PE, Becton Dickinson), CD11c (Leu-M5, Becton Dickinson), CD18 (CLB54, Dr R. W. A. van Liem; CLB LFA1/1, Central Laboratory of the Blood Transfusion Service), CD34 (BL-3C5, Seralab, Crawley Down, UK; HPCA-2 FITC and HCPA-2 PE, Becton Dickinson), CD34+ FITC, Dr R. Kurrle, Behring Diagnostica), CD36 (OKM5, Ortho Diagnostic Systems, Raritan, NJ), CD54 (BBL-4, British Biotechnology, Oxford, UK), CD58 (TS2/9, Dr T. Schumacher, Netherlands Cancer Institute, Amsterdam, The Netherlands), and HLA-DR (L243, L243 FITC, and L243 PE, Becton Dickinson). In case of unconjugated MoAbs, we used an FITC-conjugated goat anti-mouse immunoglobulin (lg) antiserum (Central Laboratory of the Blood Transfusion Service) as a second-step reagent.

Fluorescent labelings of surface membrane antigens were measured with a FACSscan flow cytometer using FACSscan-research software (Becton Dickinson). All MoAbs as defined by forward and sideward scatter patterns were gated; only debris was excluded from analysis. For double-membrane stainings, only directly conjugated (FITC or PE) MoAbs were used. Expression of all markers tested in double IF stainings was also tested in single IF stainings using direct or indirect FITC labeling. As negative controls in all experiments, we used either unconjugated or isotype-matched irrelevant MoAbs, conjugated with FITC or PE.

The expression of terminal deoxynucleotidyl transferase (TdT) was detected as described by use of a rabbit anti-TdT antisera and a FITC-conjugated goat anti-rabbit Ig antisera (Supertechs, Bethesda, MD).21 Double IF staining for TdT and several surface membrane antigens, ie, CD2 (T11), CD7 (3A1), CD10 (VIL-A1), CD13 (My7), CD14 (My4), CD15 (VIM-D5), CD19 (Leu-12), CD33 (My9), CD34 (BL-3C5), CDw65 (VIM-2), and HLA-DR (L243), was performed as described previously.20 The binding of the MoAbs on the surface membrane was demonstrated by use of a tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse Ig antisera (Central Laboratory of the Blood Transfusion Service). The TdT IF labelings were analyzed on Zeiss fluorescence microscopes (Zeiss, Oberkochen, Germany), equipped with phase-contrast facilities.29

Culture systems. MNC from three of the eight AML patients (M.B., A.K., J.M.) were cultured. Before culturing, T-cell depletion was performed by use of CD3 (OKT3; Ortho Diagnostic Systems) and magnetic cell separation (MACS system, Miltenyi Biotec, Bergisch-Gladbach, Germany) as described.31 For all cell culture experiments, MNC were adjusted to a final concentration of 5 × 10^5 cells/mL. Cells were cultured in a serum-free medium as described.31 MoAbs added in optimal concentrations were CD2 (a mixture of 6G4 [T11.1], 4B2 [T11.2], and HK27 [T11.3]), CD18 (CLB54), and/or CD58 (TS2/9). Control cultures did not contain MoAbs. For measurement of [3H]-thymidine incorporation, MNC were cultured in 96-well flat-bottom tissue culture plates (10^5 cells/well; Costar, Cambridge, MA). Incubation was performed for 3 days at 37°C, with 100% relative humidity and a PCO2 of 5%.

[3H]-thymidine incorporation. MNC were cultured for 3 days. [3H]-thymidine (specific activity, 6.7 Ci/mmol; Amersham International, Amersham, UK) pulsing was for 6 hours using 0.5 μCi/well. After the 6-hour pulse, the cells were harvested using an automatic cell harvester (Skatron, Lier, Norway). [3H]-thymidine incorporation was measured with a Betaplate Liquid Scintillation Counter (LKB Wallac, Turku, Finland). Each determination was performed in triplicate.

RESULTS

Cytomorphology and cytogenetics. The eight leukemias were classified as AML-M4eo. Cytogenetics at initial diagnosis showed an inv(16)(p13q22) in 60% to 100% of the metaphases in the eight cases studied. In patient M.V., a trisomy 22 was seen in 18% of the metaphases in addition to inv(16).

Immunologic marker analysis. The results of the immunologic marker analysis are summarized in Tables 1 through 3 and in Figs 1 and 2. With the exception of patient E.K., the MNC samples contained more than 80% of leukemic blasts. The PB MNC sample of patient E.K. enclosed approximately 10% of CD3+ T cells, approximately 25% of CD19+ B cells, and approximately 65% of leukemic blasts. The MNC sample of patient M.V. contained approximately 10% of CD3+ T cells, whereas the other six AML samples contained less than 5% of CD3+ T cells (Table 1).

Virtually all AML cells were positive for the pan-myeloid marker CD13. Double IF staining allowed the recognition of immature myeloid cells (CD13+, CD34+) and more mature monocytic cells (CD13+, CD14+) within the CD13+ cell population (Fig 1, Table 1). In addition, small subpopulations of CD14+, CD34+ and probably also CD14-, CD34- cells were present (Fig 2). Only in patient E.E. and M.B. were greater than 10% CD14+, CD34+ cells detected (Table 1). In general, the CD14+ cells had the strongest CD13 fluorescence intensity (Fig 1). In each AML, the CD33+ cell population was less prominent than the CD13+ cell population (Table 1). The difference between these two pan-myeloid markers was caused by the fact that within the immature CD34+ subpopulation CD33 expression was lower than CD13 expression (Table 1), whereas the percentages CD13+, CD14+ cells and CD33+, CD14+ were comparable (Figs 1 and 2, Table 1). Although a considerable part of the leukemic cells was HLA-DR+, a relatively large fraction of the CD34+ cells was HLA-DR- (Table 1). The myeloid markers CD11b, CD11c, CD15, CD36, and CDw65 were especially expressed by the more mature leukemic cells.

In all eight MNC samples, the percentages of CD2+ cells exceeded the CD3 and CD7 percentages (Tables 1 and 2). Table 2 summarizes the results of double IF stainings for CD2 and several other differentiation markers. In addition to a small CD3+, CD2+ T-cell population, the majority of the CD2+ cells expressed CD13, CD33, and HLA-DR (Table 2, Fig 1). Despite some patient-to-patient variation, CD2 expression was found in both immature (CD34+) and more...
mature monocytic (CD14+) leukemic subpopulations (Table 2, Fig 2).
Four AML samples were tested for CD4 and CD8 expression, which showed weak CD4 reactivity on a portion of the leukemic cells. The leukemic cells were positive for the leukocyte function antigen (LFA) CD11a/CD18 (LFA-1) and its ligand CD54 (ICAM-1), as well as CD58 (LFA-3) (Table 1).

TdT+ cells were detected in all eight MNC samples. The percentages of TdT+ cells varied from 0.1% (patient E.K.) to 40% (patient E.E.) (Table 1). To establish the precise immunophenotype of the TdT+ cells and to prove their leukemic origin, we performed extensive double IF stainings for TdT and a series of membrane-bound differentiation marker (Table 3). The precursor B-cell markers CD10 and CD19 were detected on only a few TdT+ cells. The far majority of the
AML-M4eo with INV(16) exhibits CD2+ phenotype

Fig 2. Dot-plot analysis of four double IF labelings in patients J.M. and M.V. Green (FITC) fluorescence is shown on the X-axis. Red (PE) fluorescence is shown on the Y-axis. (Top left) CD2 (T11 FITC), CD34 (HPCA-2 PE); (top right) CD2 (T11 FITC), CD14 (My4 PE); (bottom left) CD14 (My4 FITC), CD34 (HPCA-2 PE); (bottom right) CD14 (My4 FITC), CD33 (MY9 PE).

TdT+ cells were positive for HLA-DR and the myeloid markers CD13, CD33, and CDw65, and to a lesser extent for the CD15 antigen. Virtually all TdT+ cells were CD34+, while only a few CD14+, TdT+ cells could be detected, indicating that TdT was particularly expressed by the immature AML cells (Table 3). These results indicate that the majority of the TdT+ cells represented leukemic cells, independent of the relative size of the TdT+ subpopulation.

In three patients (M.B., A.K., and M.V.) both BM and PB samples were tested at diagnosis. The relative size and the immunophenotype of the various subpopulations in the corresponding cell samples of these three patients were comparable.

Culture of AML cells. MNC from three of the eight AML samples were cultured with or without MoAbs against LFA (CD2, CD18, and CD58). Because of shortage of cells, the culture experiments were performed only once, but each culture was performed in triplicate. Before culture, the AML samples were depleted of T cells with CD3 MoAb using magnetic cell separation. After T-cell depletion, the percentage of CD3+ T cells in these samples was less than 1%. The results of the culture experiments are shown in Fig 3. High spontaneous proliferation was observed in all three AML cases. Addition of CD2 MoAb to the culture medium inhibited the proliferative response in patients M.B. and J.M., but in patient A.K. the CD2 MoAb did not influence the proliferation, which was probably caused by the relatively low frequency and low density of CD2 expression in this patient (Fig 1, Table 1). Addition of CD18 or CD58 MoAb did not result in significantly higher proliferative responses in patient M.B. and J.M. However, in patient A.K., addition of CD18 and CD58 MoAb or a mixture of CD2, CD18 and CD58 MoAb

Table 2. Immunologic Marker Analysis of CD2+ Cells

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3 (Leu-4)</td>
<td>18</td>
<td>8</td>
<td>12</td>
<td>7</td>
<td>13</td>
<td>6</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>CD13 (My7)</td>
<td>78</td>
<td>94</td>
<td>84</td>
<td>93</td>
<td>83</td>
<td>92</td>
<td>74</td>
<td>95</td>
</tr>
<tr>
<td>CD14 (My4)</td>
<td>52</td>
<td>25</td>
<td>32</td>
<td>18</td>
<td>20</td>
<td>58</td>
<td>57</td>
<td>56</td>
</tr>
<tr>
<td>CD33 (My9)</td>
<td>62</td>
<td>62</td>
<td>68</td>
<td>64</td>
<td>35</td>
<td>94</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>CD34 (HPCA-2)</td>
<td>4</td>
<td>83</td>
<td>68</td>
<td>43</td>
<td>78</td>
<td>13</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>HLA-DR (L243)</td>
<td>62</td>
<td>78</td>
<td>92</td>
<td>78</td>
<td>92</td>
<td>92</td>
<td>89</td>
<td></td>
</tr>
</tbody>
</table>

Data represent percentage positivity for the various markers per CD2+ cells as determined by double IF staining.
resulted in an increased proliferative response, probably related to stimulation by the CD18 and CD58 MoAb and absence of inhibition by the CD2 MoAb (Fig 3).

DISCUSSION

In this study, we could show that AML-M4Eo with inv(16)(p13q22) is associated with a specific immunophenotype with CD2 expression. The eight leukemias consisted of heterogeneous cell populations mainly caused by the presence of multiple subpopulations, which varied in size between the patients. However, the immunophenotype of these subpopulations was comparable, independent of their relative size. Not only the presence or absence of various immunologic markers was comparable, but also the fluorescence intensity (Fig 1). Whereas a close association between a specific chromosome aberration and a particular (immuno)phenotype is well known in acute lymphoblastic leukemia, this has not been frequently observed in AML, except for the typical monocytic phenotype.\textsuperscript{3,4} It was not reported whether the other four AML contained less than 20% of TdT+ cells,\textsuperscript{4,14} as occurs in the majority of TdT+ AMLs.\textsuperscript{30} Reports on immunologic marker analysis of AML-M4Eo with a chromosome 16 aberration are scarce and only a minimal number of markers have been used.\textsuperscript{21-26} If tested, CD13 was found to be positive.\textsuperscript{21,23} In addition, expression of CD14, CD33, and HLA-DR has been reported, confirming the monocytic phenotype.\textsuperscript{21,23,25} Paietta et al described one patient with an AML-M4Eo with inv(16) in whom the cells were CDw65\textsuperscript{+} and TdT\textsuperscript{+}, but CD14\textsuperscript{−}. It is not clear whether this leukemia indeed differs from our eight cases, or whether the difference can be explained by the presence of a relatively small (<20%) CD14\textsuperscript{+} monocytic subpopulation, comparable with patient N.S. in our study. Hogge et al found 20% to 25% TdT positivity in two of six patients with either an inv(16) or a t(16;16).\textsuperscript{41} It was not reported whether the other four AML contained less than 20% of TdT+ cells,\textsuperscript{33} as occurs in the majority of TdT+ AMLs.\textsuperscript{30} This may be explained by the heterogeneous composition of most AML, which can only be characterized properly, if multiparameter analysis is performed.\textsuperscript{41,42} In addition, small leukemic subpopulations may be missed, if rigid cut-off values of 15% to 25% positivity are used, which is often the case in routine immunologic marker analysis. Therefore, we performed detailed immunophenotyping by use of multiple double IF stainings in our series of AML-M4Eo patients.

Virtually all AML-M4Eo cells were positive for the pan-myeloid marker CD13. In addition, the AMLs were partly positive for CD2, CD11b, CD11c, CD14, CD33, CD34, CD36, CDw65, TdT, and HLA-DR. The double IF stainings demonstrated coexpression of the CD2 antigen and myeloid markers and allowed us to recognize multiple AML subpopulations (Figs 1 and 2). Within the CD13\textsuperscript{+} cell population, immature cells (CD34\textsuperscript{−}, CD14\textsuperscript{+}) and more mature monocytic cells (CD34\textsuperscript{−}, CD14\textsuperscript{+}) could be identified. The CD2 antigen was expressed by immature cells, as well as more mature monocytic cells, whereas TdT expression was exclusively found in the CD34\textsuperscript{−}, CD14\textsuperscript{−} subpopulation. Although the relative size of the TdT\textsuperscript{+} subpopulation varied from 0.1% to 40% among patients, our extensive double IF stainings demonstrated the homogeneous leukemic immunophenotype of this subpopulation in all eight patients (Table 3). Based on the double IF staining results obtained in our AML-M4Eo cases, a hypothetical diagram of the subpopulations in this type of AML is shown in Fig 4.

![Fig 3. Influence of the addition of CD2, CD18, and/or CD58 MoAb on the in vitro proliferation in three AML-M4Eo patients. Each determination was performed in triplate. Results are expressed as the mean ± SEM.](image-url)
AML-M4Eo with Inv(16) Exhibits CD2 + Phenotype

Virtually all AML-M4Eo cells express CD13. Most cells are positive for either CD34 (immature subpopulation) or CD14 (more mature subpopulation), whereas CD14 +, CD34 + cells and CD14 -, CD34 - cells are scarce. CD33, HLA-DR, and CD2 are expressed on a part of the cells in both subpopulations. TdT expression is restricted to the CD34 + subpopulation. This diagram is based on the results of double IF staining experiments given in Tables 1 through 3.

The consistent expression of the CD2 antigen in our eight AML cases was detectable by MoAbs against the three CD2 (T11.1, T11.2, and T11.3) epitopes. In one AML (D.W.), the expression of the T11.1 epitope was confirmed by the ability to form rosettes with sheep red blood cells (data not shown). These results are in line with the report by Ball et al., who demonstrated CD2 mRNA in a case of AML-M4Eo. The negativity for other T-cell markers, such as CD3 and CD7, as well as the coexpression of CD2 and myeloid markers, argues against T-cell lineage committed. According to the literature, CD2 expression can be found in 6% to 21% of AML cases. So far, an association between CD2 expression and a specific type of AML has only been reported in one patient (D.W.), CD18 and CD58 MoAbs might indeed induce some additional cell proliferation (Fig 3). In such cases, it is not known whether binding of CD18 and CD58 MoAbs directly induces cell proliferation or whether this is indirectly caused by other mechanisms such as the production of interleukin-1.

Finally, it is intriguing to speculate about some unique clinical and biologic characteristics of AML-M4Eo, which might be related to the expression of the CD2 and CD58 antigens. The proliferation-inducing effect of the CD2-CD58 mediated cell-cell contact may contribute to the high WBC count in AML-M4Eo. In addition, based on the distinct expression of the CD58 antigen on endothelial cells, Plunkett et al. speculated that CD2-CD58 interaction may support extravasation of activated T lymphocytes at sites of immune reaction. Therefore, it is intriguing that patients with AML-M4Eo frequently have enlarged lymph nodes, hepatomegaly, and/or splenomegaly. Furthermore, in AML-M4Eo, a relatively high incidence of CNS leukemia has been observed, manifesting as leptomeningeal disease and intracerebral myeloblastomas. Also, in most of our patients, a high WBC count, hepatosplenomegaly, and/or CNS leukemia were observed (Table 1). Whether expression of the CD2 and CD58 antigens induces high WBC counts and facilitates dissemination of leukemic cells to lymphoid tissues and the CNS needs further investigation.

ACKNOWLEDGMENTS

We gratefully acknowledge Professor Dr R. Benner and Dr H. Hoijkkaas for their continuous support; Drs K. Höhl, A.C.J.M. Hordiet, P. Sonneveld and M.B. van 't Veer for sending patient material and providing clinical information; Drs W. Axt, W. Knapp, R. Kurre, R.A.W. van Lier, and T. Schumacher for kindly providing monoclonal antibodies; P.W.C. Adriaansen-Soeting, J.G. te Marvelde, A.C. Amo, and R. R. Wierenga-Wolf for excellent technical assistance; T. W. van Os for preparation of the figures; and A.D. Koppershoek for secretarial support.

REFERENCES

leukemias with chromosomal abnormalities involving the 21q22 region identified by their in vitro responsiveness to interleukin-5. Leukaemia 5:687, 1991


46. Mirro R, Antoun GR, Zipf TF, Melvin S, Stass S: The E rosette-associated antigen of T cells can be identified on blasts from patients with acute myeloblastic leukemia. Blood 65:363, 1985


Acute myeloid leukemia M4 with bone marrow eosinophilia (M4Eo) and inv(16)(p13q22) exhibits a specific immunophenotype with CD2 expression

HJ Adriaansen, PA te Boekhorst, AM Hagemeijer, CE van der Schoot, HR Delwel and JJ van Dongen