A cell line, designated RS4:11, was established from the bone marrow of a patient in relapse with an acute leukemia that was characterized by the t(4;11) chromosomal abnormality. The cell line and the patient's fresh leukemic cells both had the t(4;11)(q21;q23) and an isochromosome for the long arm of No. 7. Morphologically, all cells were lymphoid in appearance. Ultrastructurally and cytochemically, approximately 30% of the cells possessed myeloid features. The cells were strongly positive for terminal deoxynucleotidyl transferase. They were HLA-DR positive and expressed surface antigens characteristic for B lineage cells, including those detected by anti-B4, BA-1, BA-2, and PI153). Immunoglobulin gene analysis revealed rearrangements of the heavy chain and kappa chain genes.

In the past decade, increasing numbers of human leukemia cell lines that express the immunologic, cytogenetic, and enzymatic characteristics of various forms of leukemia have been described. Analyses of these cell lines have contributed to the identification of clinically significant subsets of acute lymphoblastic leukemia (ALL) and chronic myelogenous leukemia in blast crisis. Moreover, careful analysis of the composite phenotypes of leukemic cells and their normal counterparts has provided important insights into leukocyte differentiation. Significantly, the vast majority of human leukemic cell lines show clear-cut evidence of commitment to lymphoid or myeloid lineages. Thus, those derived from lymphoid leukemias express the E rosette receptor or other surface antigens characteristic of T cells or they express the common ALL antigen (CALLA), cytoplasmic immunoglobulin (Clg), or surface immunoglobulin (Slg), which characterize distinct stages of B cell differentiation.

Most cell lines derived from nonlymphoid leukemias stain with cytochemical reagents normally positive in granulocytes or monocytes.

In this article, we report the establishment and characterization of a cell line, RS4:11, derived from a patient with acute leukemia and distinguished by the t(4;11) chromosomal rearrangement. RS4:11 cells possess rearranged immunoglobulin (Ig) heavy and light chain genes, a characteristic believed to be restricted to B lineage cells and apparent early in B cell differentiation. RS4:11 cells were treated with the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) because such agents have been shown to induce differentiation of leukemic cells and cell lines in a manner dictated by the genetic potential of the target cells. Surprisingly, TPA treatment of RS4:11 cells induced a monocyte-like phenotype. We interpret the data to suggest that RS4:11 cells represent a somewhat unrestricted state of differentiation with the capacity to express both B cell and myelomonocytic-associated antigens. To our knowledge, RS4:11 is the first cell line established from t(4;11)-associated acute leukemia.

**MATERIALS AND METHODS**

**Establishment of Cell Line**

Cell line RS4:11 was established from frozen bone marrow obtained from a 32-year-old woman in first relapse with t(4;11) acute leukemia. The clinical features of this case have been reported previously (case 4 and case 3, respectively). Relapse bone marrow contained 98.5% malignant cells. The bone marrow karyotype at relapse revealed the t(4;11), which was present at diagnosis, and an isochromosome for the long arm of No. 7, i(7q). Relapse leukemic cells lacked immunologic markers for T and B cells (E-E, Slg-E, Clg-E). were CALLA-E, and were negative for myeloperoxidase, chloroacetate esterase, and Sudan black. They were BA-1-, BA-2-, HLA-DR-, and were strongly positive for terminal deoxynucleotidyl transferase (TdT).

Leukemic cells were isolated from bone marrow by Ficoll-Hypaque density centrifugation, washed with RPMI 1640 containing 5% fetal calf serum (FCS), and frozen for three months in 10% dimethyl sulfoxide (DMSO). Thawed cells were washed and inoculated in 25-cm² tissue culture flasks (Falcon, Oxnard, Calif) in alpha-modified minimal essential medium (MEM) (KC Biologicals, Lenexa, Ky) containing 10% heat-inactivated FCS (HyClone, Sterile

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Systems, Logan, Utah), 100 IU penicillin/mL, and 100 μg streptomycin/mL. The initial cell concentration was 10^6/mL. Cultures were incubated at 37 °C under hypoxic conditions (5% O₂, 5% CO₂, balance N₂). After one week, cells began to proliferate slowly. Thereafter, fresh medium was added semiweekly to maintain cells at 10^6/mL. After one month, cultures were transferred from the hypoxic environment to a 5% CO₂, 95% ambient air, humidified atmosphere.

Cytogenetic Studies

Chromosome analysis was performed two months after establishment of the cell line. Cultures were fed, cultured for 48 hours, and 10^3 cells were processed. Metaphase chromosome preparations were obtained using a direct harvest method that has been previously described. G-banding was performed using the Wright’s technique of Sanchez et al. A total of 50 mitoses was analyzed, ten of which were photographed on high-contrast S0115 film. Photokaryotypes were prepared in the standard manner.

Cell Morphology and Cytochemistry

Cyto centrifuge preparations were stained with Wright’s stain. Cytochemical stains were performed by Dr Robert McKenna and Ella Spanjers (University of Minnesota Hospitals) and included myeloperoxidase, Sudan black B, alpha-naphthyl nonspecific esterase, acid phosphatase, and naphthol AS-D chloroacetate esterase.

Immunologic Marker Studies

Receptors for sheep erythrocytes (E) and for complement (C3) were detected using rosette techniques that have been previously described. E-rosette receptors were detected at 4 °C. C3 receptors were detected using ox erythrocytes coated with rabbit IgM and AKR mouse complement. Receptors for the Fc portion of IgG were detected by an immunofluorescence technique using heat-aggregated IgG. Additional surface markers were detected by direct or indirect immunofluorescence under noncapping conditions with FITC-conjugated goat anti-rabbit serum (both kindly supplied by F.J. Bollum, Uniformed Services University of the Health Sciences, Bethesda, Md).

Determination of Ig Gene Configurations

High molecular weight DNA was extracted from RS4; 11 cells, digested to completion with BamHI or EcoRI restriction endonuclease, size-fractionated by agarose gel electrophoresis, and assayed by Southern blot analysis using DNA probes of the immunoglobulin genes shown to be capable of recognizing germline and rearranged alleles. This methodology has been previously described in detail, and the DNA probes utilized in this study are presented in Fig 4.

Total RNA was extracted from RS4; 11 by resuspending the cells in 4 mol/L guanidine thiocyanate, disrupting them with a polytron homogenizer (Brinkman Instruments, Westbury, NY), and centrifuging the RNA through a 5.7 mol/L CsCl gradient to form a pellet. The total RNA from RS4; 11, together with control sources of RNA from μ, κ, and λ-producing B cell lines, were electrophoresed through a formaldehyde gel, transferred to nitrocellulose paper, and then hybridized with the probes shown in Fig 4.

Ultrastructural Studies

Electron microscopy was performed using methods previously described. Ultrastructural localization of peroxidase was performed according to the methods of Graham and Karnovsky and Breton-Gorius et al. Cells were fixed for one-half hour at 4 °C and then incubated at room temperature in a buffered diaminobenzidine/hydrogen peroxide solution for one hour. Control cells were incubated in a solution lacking the hydrogen peroxide.

Ultrastructural localization of nonspecific esterase (NSE) was performed using a modification of the method of Payne et al. Cells were fixed for one hour in 0.1 mol/L phosphate-buffered 2.5% glutaraldehyde with 2% paraformaldehyde added (pH 7.4), and then stored in phosphate buffer for at least four hours at 4 °C. After rinsing in buffer, cells were incubated at room temperature for two hours in a solution containing 2-naphthylthiol acetate as the substrate and Fast Blue BB salt as the coupling agent, followed by postfixation in 1% osmium tetroxide for four hours at 36 °C. Control specimens were incubated with elimination of either the 2-naphthylthiol acetate or the osmium tetroxide.

Induction Studies

The following agents were studied for possible effect on proliferation and differentiation: TPA, retinoic acid, and 5-azacytidine (5AC). TPA was initially diluted in acetone at 100 μg/mL and then diluted in growth medium to the final concentration. The TPA was a gift of Dr T. LeBien (University of Minnesota). Retinoic acid (Sigma Chemical Co, St Louis) was diluted in ethanol before final dilution in culture medium. Medium containing an equivalent amount of acetone or ethanol, respectively, served as controls for these experiments. The 5AC was prepared fresh and diluted in growth medium to the appropriate molar concentration. In some experiments, cells were preincubated for 24 hours with 1 mmol/L thymidine (Sigma) before addition of 1 mmol/L 5AC. Pretreatment with thymidine has been shown to increase the intracellular concentration of 5AC. Growth medium served as the control. All manipulations with retinoic acid and 5AC were performed in subdued light.

For induction experiments, RS4; 11 cells were taken from exponential growth, washed twice, and resuspended at 10^6/mL in growth medium containing the test agents. Cells were cultured in 25-cm² or
75-cm² tissue culture flasks (Falcon), depending on the size of the experiment. All cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. Cultures were examined daily for cell adherence. Aliquots were taken daily for cell counting, cytochemical staining, and immunologic marker analysis. For the latter, we used a panel of representative MoAb reactive with B lineage, T lineage, and myelomonocytic cells. Cell viability was determined by trypan blue exclusion. Phagocytosis was determined by incubating cells with latex particles at 37°C for 30 minutes, washing four times, and examining microscopically both viable cells using a hemacytometer and Wright-Giemsa-stained cytocentrifuge preparations.

Isolation of Clones

RS4;11 clones were obtained by taking advantage of the ability of the cell line to form colonies in semisolid methylcellulose medium cultured under hypoxic conditions.26 A frozen aliquot of early passage (three weeks) cells was thawed, grown two days in liquid culture, and then plated in methylcellulose. After 14 days, the cell line formed colonies of 20 to 100 cells, with a cloning efficiency of 10% to 20%. The presence of 2-mercaptoethanol (5 × 10⁻² mol/L) in the culture medium increased the cloning efficiency to 25% to 35%. After 21 days, colonies of several hundred cells were individually plucked from culture wells containing one to three colonies. These colonies were expanded in liquid culture medium for subsequent analysis.

Detection of Epstein-Barr Virus (EBV) and Mycoplasma

Detection of the EBV genome by nucleic acid hybridization,27 was kindly performed by Dr K. McClain (University of Minnesota). The anticomplement immunofluorescence test for Epstein-Barr nuclear antigen was generously performed, according to previously described techniques,28,29 by Dr Gary Pearson (Mayo Clinic, Rochester, Minn). Mycoplasma contamination was assayed by Meloy Laboratories (Springfield, Va), using agar culture and fluorescent DNA staining techniques.

RESULTS

Establishment

The RS4;11 cell line was established under hypoxic conditions (5% O₂). After one month, it grew equally well under hypoxic and standard O₂ concentrations (18%), so thereafter, it was cultured under standard conditions. The cell line grew as a single cell suspension with a doubling time of 60 to 70 hours. RS4;11 was not contaminated with mycoplasma and was found to be negative for Epstein-Barr nuclear antigen and for EBV genome.

Morphology and Cytochemistry

The cell line resembled the donor’s leukemic cells. The cells displayed size heterogeneity (Fig 1) and contained moderately basophilic cytoplasm, with a pale perinuclear region present in most cells. The blasts were characterized by irregularly shaped, often indented, nuclei that had moderately condensed chromatin and one to two small nucleoli. Mitotic figures were frequently seen.

Cytochemical stains for peroxidase and Sudan black

B were negative. About half the blasts manifested perinuclear foci of weak NSE activity; this activity was inhibitable by fluoride. Rare cells were very weakly positive for chloroacetate esterase, exhibiting punctate focal activity. Moderate to strong scattered granular acid phosphatase activity was detected in all of the leukemic blasts. This pattern of cytochemical staining is consistent with B precursor ALL.

Cytogenetics

Karyotype analyses of RS4;11 were performed two months after culture initiation. Fifty metaphases were analyzed, and all had the same karyotype: 46,XX, i(7q),t(4;11)(q21,q23). Abnormalities included an isochromosome for the long arm of No. 7 and a translocation of most of the long arm of one No. 4 to the distal long arm of one No. 11 (Fig 2). These same abnormalities were found in the patient’s bone marrow, which was obtained at the time of relapse.

Immunologic Markers

Results of immunologic marker studies are shown in Table 1. Cultured cells were tested on several occasions during a 12-month period. The vast majority of cells strongly expressed the B lineage-associated antigens BA-1, BA-2, PI153/3, and B4. CALLA, the B1 antigen, and SIg were undetectable. Rare cells (<1%) exhibiting weak perinuclear staining for Clg were detected repeatedly. Clg staining was blocked by preincubation with purified human IgM, indicating that it was not artifactual. Blasts manifested strong TdT activity. Interestingly, the percentage of TdT-positive cells increased from 87% at initiation, to greater than 99% after 12 months of culture. The percentages of cells expressing HLA-DR and BA-1 also increased during culture. At initiation, the proportions of HLA-DR and BA-1 cells were 11% and 20%, respectively; after prolonged culture, greater than 90% of the cells were positive for these two markers. Cultured cells expressed, albeit weakly, the precursor cell antigens RFB-1 and OKT10. The myeloid antigen
identified by 1G10 was strongly expressed on the majority of cells. In contrast, other myeloid-specific MoAb failed to bind. TA-1 reacted with a minority of cells, but was so weakly reactive that it could be detected by cytofluorimetry only and not by microscopy. Antigenic markers characteristic of T lineage cells were also undetectable. The cells exhibited no receptors for sheep erythrocytes, complement, or IgG. The absence of Fc-IgG receptors from the cell line indicates that the detected binding of MoAb was specific.

Analysis of RS4;11 Clones

To determine if the observed phenotypic heterogeneity was due to mixed cell types, RS4;11 clones were isolated and analyzed. Twenty-four colonies were indi-

Table 1. RS4:11—Immunologic Characteristics

<table>
<thead>
<tr>
<th>Marker</th>
<th>RS4;11*</th>
<th>Normal Hematopoietic Cellular Distribution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA-1</td>
<td>20–92</td>
<td>Pre-B cells, B cells, granulocytes</td>
<td>16</td>
</tr>
<tr>
<td>BA-2</td>
<td>94–98</td>
<td>Pre-B cells, thymocytes, platelets, activated T cells</td>
<td>17</td>
</tr>
<tr>
<td>PI153/3</td>
<td>91–94</td>
<td>Pre-B cells, B cells</td>
<td>30</td>
</tr>
<tr>
<td>BA-3 (anti-CALLA)</td>
<td>0</td>
<td>Pre-B cells, thymocytes, granulocytes</td>
<td>18, 31</td>
</tr>
<tr>
<td>B1</td>
<td>0</td>
<td>Pre-B cells, B cells</td>
<td>32</td>
</tr>
<tr>
<td>B4</td>
<td>99</td>
<td>Pre-B cells, B cells</td>
<td>33</td>
</tr>
<tr>
<td>S1g</td>
<td>0</td>
<td>B cells</td>
<td>2</td>
</tr>
<tr>
<td>Clg</td>
<td>0.3–1.0</td>
<td>Pre-B cells, plasma cells</td>
<td>2</td>
</tr>
<tr>
<td>anti-TdT</td>
<td>87–99</td>
<td>Lymphoid progenitor cells</td>
<td>34</td>
</tr>
<tr>
<td>anti-HLA-DR</td>
<td>11–92</td>
<td>B lineage cells, monocytes, immature myeloid cells, activated T cells</td>
<td>35</td>
</tr>
<tr>
<td>RFB-1</td>
<td>50–90</td>
<td>Early stem cells</td>
<td>36</td>
</tr>
<tr>
<td>OKT10</td>
<td>59–93</td>
<td>Early stem cells, activated lymphocytes</td>
<td>37</td>
</tr>
<tr>
<td>1G10</td>
<td>82–96</td>
<td>Granulocytic cells, CFU-GM, some monocytes</td>
<td>38</td>
</tr>
<tr>
<td>SF1</td>
<td>0</td>
<td>Monocytes, nucleated erythroid cells</td>
<td>38</td>
</tr>
<tr>
<td>anti-MPA</td>
<td>0</td>
<td>Monocytes, platelets, megakaryocytes</td>
<td>39</td>
</tr>
<tr>
<td>MCS2</td>
<td>0–1</td>
<td>Mononuclear cells</td>
<td>40</td>
</tr>
<tr>
<td>OKM1</td>
<td>0</td>
<td>Monocytes, granulocytes, null cells</td>
<td>41, 42</td>
</tr>
<tr>
<td>TA-1</td>
<td>0–36</td>
<td>Monocytes, T cells</td>
<td>19</td>
</tr>
<tr>
<td>35-1</td>
<td>0</td>
<td>T lineage cells</td>
<td>43</td>
</tr>
<tr>
<td>T101</td>
<td>0–4</td>
<td>T lineage cells</td>
<td>44</td>
</tr>
<tr>
<td>OKT3</td>
<td>0</td>
<td>Mature T cells</td>
<td>45</td>
</tr>
<tr>
<td>3A-1</td>
<td>0–2</td>
<td>T lineage cells</td>
<td>46</td>
</tr>
<tr>
<td>Leu-3</td>
<td>0</td>
<td>Helper/inducer T cells</td>
<td>47</td>
</tr>
<tr>
<td>OKT8</td>
<td>0</td>
<td>Suppressor/cytotoxic T cells</td>
<td>45</td>
</tr>
<tr>
<td>OKT6</td>
<td>0</td>
<td>Thymocytes</td>
<td>45</td>
</tr>
<tr>
<td>SE9 (anti-transferrin receptor)</td>
<td>64–95</td>
<td>Proliferating cells, including stem cells and activated lymphocytes</td>
<td>48</td>
</tr>
<tr>
<td>E rosettes</td>
<td>0</td>
<td>T lineage cells</td>
<td>14</td>
</tr>
<tr>
<td>IgG Fc receptor</td>
<td>0</td>
<td>Pre-B cells, B cells, monocytes</td>
<td>15</td>
</tr>
<tr>
<td>C3 receptor</td>
<td>0</td>
<td>Pre-B cells, B cells, monocytes</td>
<td>14</td>
</tr>
</tbody>
</table>

*Range of the percentage of positive cells observed during a 12-month period.
individually isolated from methylcellulose medium, and 16 of these were successfully expanded in liquid culture. Twelve were phenotyped using a panel of ten representative lineage-associated MoAb. The clones tested included those grown with and without 2-mercaptoethanol. MoAb reactivity of all clones resembled that of the parental cell line, although some interclonal variability in the frequency and intensity of antigen expression was apparent. This variability was similar to that seen for the parental line in repeated typings over the 12-month period.

**Electron Microscopy**

The blast population was heterogeneous both in cell size and cytology (Fig 3A). The majority of blasts were 8 to 10 μ in diameter, although a few cells were as large as 17 μ. Many blasts exhibited cell surface irregularity characterized by pseudopod formation. Nuclear irregularity was prominent. In an initial study performed three months after culture initiation, a subpopulation (about 30%) of large and small blasts was detected that contained large granules resembling immature basophil/mast cell granules. Certain rare cells within this subpopulation manifested peroxidase positivity in the mast cell/basophil-like granules (Fig 3B). All remaining blasts were peroxidase negative. An additional minor subpopulation of blasts (less than 5%) was noted that had a monocytic appearance. These cells were characterized by scattered small electron-dense granules, an indented nucleus, and small bundles of microfilaments; these small granules were often peroxidase positive (Fig 3C).

Ultrastructural studies performed five months later detected no basophil/mast cell granules. The subpopulation of monocytoid cells remained; however, the small granules within these cells no longer manifested peroxidase positivity. Staining for NSE at this time revealed activity in rare cells, which was localized to the perinuclear region.

**Ig Gene Analysis**

The BamHI-digested genomic DNA of RS4;11 demonstrated a rearranged heavy chain gene allele when probed with a human J allele region fragment (Fig 4). The k-gene configuration was that of one rearranged allele, while the other Ck-gene region was deleted from the cell line (Fig 4). Deletion of Ck-gene regions has been noted previously in some B cell precursor leukemias.5 The λ-genes were retained in their germline form as compared to a control source of DNA from placenta (Fig 4). No hybridization to k-genes of germline size was detected.

Despite the presence of rearranged immunoglobulin genes, no surface immunoglobulin and essentially no cytoplasmic immunoglobulin, except in rare cells, was detectable. This prompted an examination of the transcriptional activity of the rearranged immunoglobulin genes in RS4;11. As displayed in Fig 4, no meaningful amount of μ, κ, or λ mRNA was present within RS4;11 as compared to μ-, κ-, λ-producing B cell lines. Thus, despite the uniform, clonal rearrangement of μ- and κ-genes within each cell of RS4;11, the vast majority of these cells were not actively transcribing these genes. It is possible that rare cells within this population generate transcripts and account for the weak perinuclear cytoplasmic μ-chain seen in <1% of cells. Overall, however, RS4;11 represents a stage of B cell precursor differentiation with rearranged Ig genes, but no remarkable transcriptional activity.

**Induction Studies**

To analyze the effect of TPA on the differentiative capacity of RS4;11, cells were cultured with various concentrations (0.01 to 10.00 ng/mL) of TPA for five days and tested daily for reactivity with our test panel...
of MoAb. A dose of 1 ng/mL was found to be optimal. The glycoprotein (gp) 170,95 antigen detected by TA-1 was strongly expressed by the vast majority of cells following TPA treatment at this dose (Fig 5). Greater than 80% of viable cells expressed gp170,95 after four days in culture. Thereafter, the percentage of positive cells began to slowly decline (Fig 6). Cytofluorimetric analysis detected a small minority of RS4;11 cells that expressed very low levels of gp170,95 in the absence of TPA treatment; this level did not change during the experimental period. Under the influence of TPA, RS4;11 cells were also induced to express the myelomonocytic markers detected by OKM1 and MCS2 (Fig 5). As shown in Fig 6, these markers were induced on an average of 19% and 28% of treated cells, respectively, after three days in culture. In the absence of TPA, RS4;11 showed no reactivity with OKM1 or MCS2. Dose–response experiments demonstrated that a concentration of 10 ng/mL TPA increased to 48% the percentage of cells reactive with MCS2; however, cell viability was reduced commensurately. The percentage of cells reactive with TA-1 did not exceed 90%, even with this higher concentration of TPA. Coincident with the expression of OKM1 and MCS2 markers, the proportion of cells expressing the 1G10 antigen declined significantly (from 85% to 39%) during days 1 through 3 (Fig 6); thereafter, the percentage of positive cells increased to near pretreatment levels. TPA treatment did not reduce the expression of TdT, BA-1, BA-2, or HLA-DR; throughout these experiments, greater than 90% of untreated and treated cells were positive for these markers. TPA did not induce expression of
CALLA, C1g, B1, nor the antigens detected by 5F1 or 35.1. In all experiments, the fluorescence controls (TPA-treated cells + IgG2a myeloma + FITC-conjugated goat anti-mouse IgG) were negative.

The morphology of untreated and TPA-treated cells were examined by Wright-Giemsa staining. In general, TPA-treated cells resembled the untreated cells, although they were larger, often vacuolated, and their cell surfaces had a ruffled appearance.

Other lineage-associated characteristics that were examined for a TPA effect are summarized in Table 2. During the five-day cultures, 20% to 30% of TPA-treated cells adhered weakly to the bottom of the tissue culture flasks. These cells could be dislodged by gentle pipetting. In contrast, rare (<1%) cells in untreated cultures adhered to the flasks. Untreated RS4;11 cells showed little phagocytic function; approximately 6% of cells ingested small numbers (two to three) of latex beads. After exposure to TPA, 42% of cells became phagocytic, with each cell ingesting large numbers of latex beads (usually more than ten beads per cell). TPA did not induce Fc or C3 receptors on RS4;11 cells. Chloroacetate esterase activity remained negative.

By electron microscopy, the majority of TPA-treated cells was characterized by cytoplasmic projections, nuclear irregularity, lipid inclusions within the cytoplasm, small dense granules, and phagolysosomes. Ultrastructural studies revealed rare untreated cells with NSE positivity in the perinuclear region. In contrast, 75% of treated cells displayed the strong cytoplasmic NSE positivity characteristic of monocytoplaid cells (Fig 7). Both treated and untreated cells were negative for peroxidase.

The presence of TPA altered the rate of cell growth, an effect that was apparent by day 2. A concentration

Fig 6. Kinetics of myelomonocytic antigen expression induced by TPA. RS4;11 cultures were initiated at 10^5/mL in medium with TPA (1 ng/mL) and without TPA. Aliquots of cells from untreated and treated cultures were removed daily and assayed microscopically for antigen expression using indirect immunofluorescence. Values shown are means ± SE of five separate experiments. Antigen expression on control cells did not change during culture and are the same as day 0 values for TPA-treated cultures.

Fig 7. Electron micrograph showing ultrastructural localization of NSE in RS4;11 cells cultured with TPA (10 ng/mL) and without TPA. (A) Untreated cell shows typical undifferentiated lymphoid morphology and exhibits no NSE positivity (original magnification ×16,000). (B) TPA-treated cell shows strong cytoplasmic NSE positivity, indented nucleus, and cytoplasmic projections (original magnification ×13,000).

| Table 2. Induction of Differentiation by TPA |
|------------------|------------------|
|                  | Control (%)      | TPA (%)         |
| Adherence        | 0.0              | 20–30           |
| Fc receptors     | 1.0              | 1.0             |
| C3 receptors     | 0.0              | 0.0             |
| Phagocytosis     | 5.9 ± 2.9        | 41.8 ± 15.8     |
| Nonspecific esterase | ±               | ++              |
| Chloroacetate esterase | –               | –               |
of 0.1 ng/mL slightly inhibited cell proliferation (based on absolute cell numbers), but did not affect cell viability. Higher concentrations inhibited cell proliferation markedly and caused a dose-dependent decline in cell viability. These results are summarized in Fig 8. The high viability and inhibition of proliferation exclude the possibility that the TPA-induced phenotype resulted from the selective expansion of more differentiated cells within the RS4;11 population.

In other experiments, a wide range of concentrations of retinoic acid and 5-azacytidine were studied for their differentiative effect on RS4;11. Neither agent altered the pattern of reactivity of RS4;11 cells with our test panel of MoAb. Cells preincubated with 1 mmol/L thymidine for 24 hours and then exposed to 1 μmol/L SAC expressed weak reactivity with TA-1 and MCS2; however, fewer than 10% of cells reacted with these antibodies.

DISCUSSION

The RS4;11 cell line was established from bone marrow of a patient with t(4;11)-associated acute leukemia. Morphological, immunologic (summarized in Table 1), and cytochemical characteristics of RS4;11 cells were found to be consistent with ALL. The cells are strongly positive for TdT. An in-depth analysis of RS4;11 revealed characteristics of both lymphoid and myeloid lineages.

The cells are rearranged for immunoglobulin heavy and κ-chain genes, providing strong evidence for a commitment to B cell lineage. Although occasional heavy chain gene rearrangements have been noted in T cells and myeloid cells, light chain gene rearrangements have been restricted to the B cell lineage.4-6 The expression of B4 is additional support for B lineage classification, since within the hematopoietic system, this antigen is expressed very early in normal B cell ontogeny and is restricted to B lineage cells.39 Reactivity with BA-1, BA-2, and PI153/3 is consistent with B lineage classification because these MoAbs react with normal pre-B and B cells as well as with the vast majority of non-T ALL, although their binding cannot be considered to be definitive for lymphoid leukemias.49

In addition to these lymphoid characteristics, RS4;11 cells bind 1G10, a MoAb that reacts with granulocytic cells, some monocytes,38 and CFU-GM precursor cells.30 Some RS4;11 cells weakly express the gpl70,95/TA-1 antigen found on monocyctic precursors30 and peripheral blood monocytes.19 The ultrastructural detection of basophil/mast cell granules and peroxidase activity in a minor population of RS4;11 cells is supportive evidence of myeloid commitment. Similar basophil/mast cell granules have been detected in some cases of lymphoid blast crisis of chronic myelogenous leukemia and in Philadelphia-positive ALL.11 The disappearance of this more differentiated subpopulation of RS4;11 suggests that these cells were at proliferative disadvantage or that the in vitro conditions could not support their phenotypic expression.

The monocyte-like phenotype of RS4;11 induced after TPA treatment is persuasive evidence for the myelomonocytic nature of RS4;11. Several laboratories have reported that TPA can induce human myeloid and lymphoid leukemic cells to more differentiated phenotypes that are primarily dictated by the differentiative potential of the target cells.3,7-9 In response to TPA (0.5 to 10.0 ng/mL), RS4;11 cells became reactive with TA-1, OKM1, and MCS2, became phagocytic, and showed greatly enhanced NSE activity in a pattern characteristic of monocyctic cells. A subpopulation of treated cells became adherent, but this response resembled the weak adherence of certain TPA-treated lymphoid lines31 more closely than the strong adherence displayed by treated myeloid lines, such as HL-60 and KG-1.3,51 Ultrastructurally, treated cells exhibited a monocytoid morphology. Our results are consistent with those of Nagaska et al,53 who reported that TPA induced a monocyte phenotype in cells from two cases of t(4;11) acute leukemia.

The expression of lymphoid and myeloid characteristics by RS4;11 cannot be explained by the presence of mixed cell types within the cell line. Three lines of evidence argue against such an explanation. (1) Immunoglobulin gene analysis indicated that RS4;11 cells had rearranged one κ-allele and deleted the other.
Contaminating myeloid cells with germline \( \kappa \)-genes would have been detected if they comprised greater than 5% of the total population.\(^4\) (2) Fifty metaphases of RS4;11 were analyzed for karyotype and all had the t(4;11) and i(7q). (3) Twelve clones of RS4;11 showed the same phenotype as the parental population when analyzed for immunologic markers. Rather, these results suggest that at the level of an individual cell, RS4;11 exhibits characteristics of both early lymphoid and early myeloid cells. Whether this in turn reflects the potential to differentiate along both lineages is as yet unknown. Neither 5AC nor retinoic acid induced further B lineage differentiation. It may be that the immunoglobulin genes are ineffectively rearranged within RS4;11 cells and thereby prevent B cell differentiation beyond the precursor stage.

The biphenotypic character of RS4;11 makes the precise lineage affiliation and cellular origin of the cell line difficult to ascertain. A similar problem surrounds hairy cell leukemia (HCL).\(^5\) Although morphologically different than RS4;11, HCL cells resemble RS4;11 in that they are rearranged for heavy and light chain immunoglobulin genes and, in response to TPA, exhibit a monocytic phenotype.\(^6\) Unlike RS4;11, HCL cells often transcribe and translate their rearranged immunoglobulin genes and surprisingly exhibit the Tac receptor, which was thought to be restricted to activated T cells and certain T cell malignancies.\(^5\) Interestingly, three cases of childhood acute leukemia have recently been reported in which some blasts expressed dual lymphoid (CALLA+, HLA-DR+, TdT+) and myeloid (myeloperoxidase and Sudan black activity) markers.\(^6\) The rapid conversion with chemotherapy of cases of non-T cell ALL to myelomonocytic leukemia\(^7\) and from T lymphoblastic leukemia to promyelocytic leukemia\(^8\) has also been reported. These leukemias indicate that the apparent plasticity of genome expression by RS4;11 is not an artifact of culture nor is it unique. In fact, the human erythroleukemia cell line HEL develops a macrophage phenotype in response to TPA (\(10^{-8} \) to \(10^{-4}\) mol/L).\(^9\) Moreover, our results are highly reminiscent of those reported for the murine pre-B cell line, ABLS 8-1. This cell line has rearranged immunoglobulin heavy and light genes and, in response to 5AC, develops such monocyte/macrophage characteristics as phagocytosis, NSE activity, and expression of the MAC-1 antigen and Fc receptors.\(^10\)

An important question that arises is whether or not the dual “lymphoid-myelomonocytic” phenotype and potential of these leukemias represents an equivalent normal cell. If so, this could be a bipotential cell or perhaps a progenitor cell recently committed to either the lymphoid or myeloid pathway. Perhaps certain cells with lymphoid characteristics can be induced to become myelomonocytic, especially if they have non-productive immunoglobulin genes. Alternatively, these characteristics may result from a transformation-associated aberrant event.

The apparent myelomonocytic nature of RS4;11 is noteworthy in light of the numerous reports that chromosome No. 11 band q23 is also commonly involved in acute monocytic leukemia and acute myelomonocytic leukemia.\(^11\) Of some interest is the recent mapping to chromosome No. 11 of a myeloid-associated antigen, 3-fucosyl-N-acetyl-lactosamine (FAL), which was detected by antibodies related to Ig10.\(^2\) Our findings support the correlation between oncogenesis and translocation involving this chromosomal segment and provide additional evidence that the genes involved in normal myelomonocytic differentiation are located within this chromosomal region. Modern molecular biologic techniques may permit the isolation of the genes controlling these processes from RS4;11 cells.

To our knowledge, RS4;11 is the first cell line having the t(4;11). Several studies have indicated that this translocation, involving the long arm of chromosomes Nos. 4 and 11, identifies a subtype of acute leukemia that is characterized by consistent clinical and laboratory features.\(^12\) The RS4;11 cell line should contribute to our understanding of this subtype of acute leukemia. Moreover, RS4;11 should prove useful in analysis of the early stages of leukocyte differentiation as well as in the evaluation of the role of chromosomal translocation in leukemogenesis.

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