Hematogones: A Multiparameter Analysis of Bone Marrow Precursor Cells

By Teri A. Longacre, Kathy Foucar, Sylvia Crago, I-Ming Chen, Barbara Griffith, Lynn Dressler, Thomas S. McConnell, Marilyn Duncan, and John Gribble

Morphologically distinct lymphoid cells with homogeneous, condensed chromatin and scant cytoplasm can be observed in large numbers in the bone marrow of children with a variety of hematologic and nonhematologic disorders. In some patients, these cells may account for >50% of the bone marrow cells, creating a picture that can be confused with acute lymphoblastic leukemia (ALL) or metastatic tumor. Although originally called hematogones (HG), a variety of other names have been proposed for these unique cells. The clinical significance of expanded HGs has not been resolved, and the biologic features of these cells are incompletely described. In this study, we correlate the clinical, morphologic, cytotoxic, flow cytometric, molecular, and cytogenetic properties of bone marrow samples from 12 children with substantial numbers of HGs (range 8% to 55% of bone marrow cells). Diagnoses in these patients included anemia, four; neutropenia, one; anemia and neutropenia, one; idiopathic thrombocytopenic purpura, two; retinoblastoma, one; Ewing’s sarcoma, one; and germ cell tumor, one. Flow cytometric analyses of bone marrow cells demonstrated a spectrum extending from early B-cell precursors (CD10+, CD19+, TdT+, HLA-DR+) to mature surface immunoglobulin-bearing B cells in these patients, corroborating our morphologic impression of HGs, intermediate forms, and mature lymphocytes. DNA content was normal, and no clonal abnormality was identified by either cytogenetic or immunoglobulin and T-cell receptor (TCR) gene rearrangement studies. Follow-up ranged from 3 months to 3 years. None of the patients has developed acute leukemia or bone marrow involvement by solid tumor. The possible role of HGs in immune recovery and hematopoiesis is presented.

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MATERIALS AND METHODS

Morphologic and cytochemical studies. Samples of bone marrow aspirates were obtained, after informed consent, from 12 children undergoing evaluation either for staging of malignant solid tumors or for establishment of diagnosis of a variety of nonmalignant hematologic disorders (Table 1).

Bone marrow smears and cytospin centrifuge preparations were stained with Wright’s stain and periodic acid-Schiff (PAS). Based on a count of 200 cells, the percentage of HGs and total lymphoid cells were calculated by two of the investigators (T.L. and K.F.).

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Blood, Vol 73, No 2 (February), 1989: pp 543-552
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Hemoglobin (g/dL)</th>
<th>Leukocytes ($\times 10^9$/L)</th>
<th>Lymphocytes ($\times 10^9$/L)</th>
<th>Platelets ($\times 10^9$/L)</th>
<th>Indication for Bone Marrow</th>
<th>Bone Marrow</th>
<th>Bone Marrow Biopsy</th>
<th>Diagnosis</th>
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<td>32</td>
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<td>559</td>
<td>Staging for germ cell tumor</td>
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<td>17</td>
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<td>14 mo/M</td>
<td>10.3</td>
<td>11.7†</td>
<td>4.2</td>
<td>418</td>
<td>Staging for retino-blastoma</td>
<td>72</td>
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<td>21</td>
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<tr>
<td>5</td>
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<td>425</td>
<td>Anemia</td>
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<td>&lt;1</td>
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<td>6</td>
<td>2 yr/M</td>
<td>6.4</td>
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<td>7.6</td>
<td>389</td>
<td>Anemia, neutropenia</td>
<td>45</td>
<td>4</td>
<td>21</td>
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Table 1. Clinical and Laboratory Findings in 12 Patients With Increased Bone Marrow HGs
<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>WBC</th>
<th>RBC</th>
<th>Hgb</th>
<th>Platelets</th>
<th>Diagnosis</th>
<th>Leukocytes</th>
<th>RBC</th>
<th>MCV</th>
<th>Platelets</th>
<th>Leukocytes</th>
<th>RBC</th>
<th>MCV</th>
<th>Platelets</th>
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<td>7</td>
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<td>253</td>
<td>Staging for Ewing's sarcoma</td>
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<td>6</td>
<td>6</td>
<td>15</td>
<td>&lt;1</td>
<td>Two small lymphoid aggregates (one with clusters of epithelioid macrophages)</td>
<td></td>
<td></td>
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<td>8</td>
<td>3 mo/M</td>
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<td>10.1</td>
<td>330</td>
<td>Leukocytosis and anemia</td>
<td>55</td>
<td>4</td>
<td>19</td>
<td>11</td>
<td>ND</td>
<td>Bone marrow lymphocytosis</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>3 mo/M</td>
<td>8.1</td>
<td>6.3</td>
<td>3.9</td>
<td>700</td>
<td>Anemia</td>
<td>45</td>
<td>10.8</td>
<td>39</td>
<td>55</td>
<td>0</td>
<td>Marked diffuse lymphocytosis; nuclear contours round; minimal mitotic activity</td>
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<tr>
<td>10</td>
<td>4 mo/M</td>
<td>11.7</td>
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<td>7.1</td>
<td>563</td>
<td>Staging for retinoblastoma</td>
<td>60</td>
<td>5</td>
<td>14</td>
<td>23</td>
<td>&lt;1</td>
<td>No metastatic tumor</td>
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<td></td>
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<tr>
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<td>18 mo/F</td>
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<td>Thrombocytopenia</td>
<td>70</td>
<td>2</td>
<td>12</td>
<td>18</td>
<td>0</td>
<td>Idiopathic thrombocytopenic purpura</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3 yr/F</td>
<td>12.6</td>
<td>7.3</td>
<td>2.0</td>
<td>31</td>
<td>Thrombocytopenia</td>
<td>80</td>
<td>1</td>
<td>9</td>
<td>23</td>
<td>0</td>
<td>Idiopathic thrombocytopenic purpura</td>
<td></td>
<td></td>
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</tbody>
</table>

ND, not done.
*BMLs, Bone marrow lymphocytes not including HGs.
†Rare HG (<0.1%) in blood.
Bone marrow clot and biopsy sections were prepared using standard techniques, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E).

**Immunophenotyping and flow cytometric analysis.** After standard cell separation techniques were applied to bone marrow aspirations, the following purified murine monoclonal antibodies (MoAbs) were used: anti–HLA-Dr, antitransferrin receptor, anti–CD10 (CALLA), -CD34 (My10), -CD16 (Leu11a), -CD7 (Leu9), -CD38 (Leu17), -CD3 (Leu4), -CD4 (Leu3a), -CD1 (Leu6), -CD8 (Leu2a), -CD2 (Leu5b), -κ, -λ, -IgG, -IgM, -IgD (Becton Dickinson Monoclonal Center, Mountain View, CA); and anti–CD20 (B1), -CD19 (B4), -CD33 (My9), -CD13 (My7) (Coulter Immunology, Hialeah FL). For single-color analysis, these reagents were used in indirect immunofluorescence procedures with FITC-conjugated goat anti-mouse immunoglobulin as the second-step reagent (Tago Diagnostics, Burlingame, CA). Control preparations consisted of unstained cells, cells stained with the labeled second-step reagent alone, and cells stained with an irrelevant antibody of the same isotype as the test antibodies. All incubations with antibody were at 4°C, followed by two washes in Hank’s balanced salt solution (HBSS). For two-color immunofluorescence (IF) staining of CD10 v CD19 and CD19 v CD38, the cells were incubated with unlabeled CD19 followed by FITC-conjugated goat anti-mouse immunoglobulin, washed, and then incubated with phycoerythrin-conjugated CD10 or CD38. Propidium iodide (final concentration, 1 µg/mL) was added to each sample to minimize interference by nonspecific staining of dead cells.

Samples were analyzed either fresh or after fixation with 1% paraformaldehyde on a FACSscan flow cytometer (Becton Dickinson) using the 488-nm line of an argon laser at 15-mW power. Single-parameter (and dual-parameter) histograms and scattergrams were analyzed for percentages of positive cells using the FACSscan research data analysis software (Becton Dickinson).

**Cytoplasmic μ and TdT immunofluorescence.** Cytoplasmic μ and TdT analyses were performed on fixed cytospin slides by immunostaining with mouse anti-human IgM (Becton Dickinson) and rabbit anti-call TdT (Supertechs). Slides were washed in HBSS and coverslipped for viewing under an IF microscope.

**DNA content and cell cycle analysis.** After Ficoll-Hypaque separation, cellular DNA content was determined by a modification of the method of Kristan.31 Immediately prior to flow analysis, nuclei were passed through a 27-gauge needle and filtered through 37-µm mesh. Nuclei were analyzed on an EPICS 753 flow cytometer (Coulter EPICS Division, Hialeah, FL) using the 488-nm line of an argon laser at 250-mW power. Forward-angle light scatter, peak red fluorescence, and integrated red fluorescence were collected on a photomultiplier tube 1 (PMT 1) using the following filter setup: 488 dichroic, 459 to 502/488 blocking filter, 550 dichroic, 635 band-pass, and 6,730 long-pass filter. A bit map was set on integrated v peak red fluorescence to minimize doublets. Integrated red fluorescence histograms were obtained from 25,000 nuclei per sample. The histograms were analyzed for percentage of S phase with the PARA-1/PARA-2 (Coulter Electronics) modeling system as previously described.31

**Cytogenetic studies.** Cytogenetic studies of heparinized bone marrow clots were performed using standard techniques for direct and 24-hour analyses. Chromosomes were Giemsa-banded after trypsinization.

**Molecular studies.** DNA was extracted from cryopreserved cells in a selected number of patients, digested to completion with the restriction endonucleases HindIII, BamHI, and EcoRI, and size-fractionated through a 0.8% agarose gel. After transfer to nitrocellulose paper, the filters were hybridized with the following nick-translated, 32P-labeled human DNA probes: JH (immunoglobulin heavy chain, J region), 6 kilobase (kb) BamHI-HindIII genomic fragment (courtesy of Dr Philip Leder, Harvard Medical School, Boston); Jκ (immunoglobulin κ light chain, J region), 1.8-kb SacI genomic fragment (courtesy of Dr Philip Leder); Cκ (immunoglobulin light chain, constant region), 0.8-kb EcoRI genomic fragment (courtesy of Dr Stanley Korsemeyer, Washington University, St Louis); T-cell β receptor (joining and constant regions), 0.75-kb PstI-Aval fragment (courtesy of Dr Tak W. Mak, Ontario Cancer Center, Toronto); and T-cell γ receptor (variable, joining, and constant regions), 1.4-kb EcoRI fragment (courtesy of Dr J. G. Seidman, Harvard Medical School, Boston). After washes at the appropriate stringency, hybridization was detected by autoradiography using Kodak X-OMAT AR x-ray film exposed at ~70°C from two to ten days.

**RESULTS**

The patients entered into this study consisted of 12 children (ages 3 months to 9 years, median 16 months) who underwent bone marrow examination to establish the diagnosis or to stage for the following disease processes: neutropenia (two), anemia (three red cell aplasia, one suspected transient bone marrow injury, one undetermined etiology, possibly viral), idiopathic thrombocytopenic purpura (two), retinoblastoma (two), Ewing’s sarcoma (one), and germ cell tumor (one). One of these patients presented with both anemia and neutropenia. All patients included in the study had substantial expansions of bone marrow HGs. The relevant clinical features and laboratory findings are summarized in Table 1. Two of the patients were referred to our institution with initial diagnoses of possible ALL, and one patient was believed to have metastatic retinoblastoma. In all but one case (patient 2), bone marrow cells were obtained prior to therapy.

The percentage of bone marrow HGs in these patients ranged from 8% to 55% (median 25%). On Wright’s-stained bone marrow smears, these cells were characterized by scant cytoplasm and dense nuclei with smooth homogeneous chromatin and round or notched contours (Figs 1 and 2). Although most HGs were small (10 to 12 µm), they could be distinguished from lymphocytes by their unique nuclear characteristics. However, intermediate forms with features of both HGs and mature lymphocytes were evident. Nucleoli were generally absent; however, occasional comparatively larger HGs (17 to 20 µm) had a slightly more reticulated chromatin pattern with small, inconspicuous nucleoli. In contrast to the smaller, more characteristic HGs, these larger HGs often contained a thin rim of homogenous blue cytoplasm, without granules, inclusions, or vacuoles (Figs 1 and 2). Cytotochemical analysis in four of the patients showed a small percentage (up to 4%) of HGs with moderate to large granular cytoplasmic PAS staining; in all other cases, these cells were PAS negative. The percentage of mature bone marrow lymphocytes ranged from 6% to 39% (median 20%). Blasts were not increased and were distinguished from HGs by their finely dispersed chromatin and easily visible nucleoli (Fig 2).

A diffuse lymphocytosis was present on trephine and clotted aspirate H & E-stained sections in 10 (83%) of the patients. In half of the cases, this diffuse infiltrate was associated with small, loosely arranged lymphoid aggregates, composed of cytologically mature lymphocytes and occa-
Fig 1. Photomicrographs of Wright's-stained smears from pediatric patients with expanded bone marrow HGs illustrate a spectrum of morphology of HGs and other lymphoid cells (a,b). Although HGs may vary in size, the small HG with scant cytoplasm usually predominates (b). Chromatin is smooth and homogenous and nucleoli are indistinct (c). HGs often exhibit distinct nuclear clefts (d).

HG cells of this morphology were identified in the bone marrow aspirates from 12 of the 14 patients studied. Although the HGs were small, they were readily recognizable by light microscopy, containing a single round nucleus with a smooth contour, smooth homogeneous chromatin, and indistinct or absent nucleoli (Fig 1a,b,c). Some HGs exhibited distinct nuclear clefts (Fig 1d).

HG cells may have some scattered organelles, including small cytoplasmic vesicles and mitochondria, and a small condensed nuclear envelope was observed (Fig 1a,b). Although HGs may vary in size, the small HG with scant cytoplasm usually predominates (Fig 1b). Chromatin is smooth and homogenous and nucleoli are indistinct (Fig 1c). HGs often exhibit distinct nuclear clefts (Fig 1d).

The described morphology is characteristic of HGs, which are characterized by their unique morphological features. In this study, HGs were identified in the bone marrow aspirates from 12 of the 14 patients studied. Although the HGs were small, they were readily recognizable by light microscopy, containing a single round nucleus with a smooth contour, smooth homogeneous chromatin, and indistinct or absent nucleoli (Fig 1a,b,c). Some HGs exhibited distinct nuclear clefts (Fig 1d).

These lymphoid aggregates generally had indistinct borders, without germinal center formation or mitotic activity. Although some nuclear irregularity was appreciated, marked nuclear convolutions, such as occur in ALL, were not present.

The absolute peripheral blood lymphocyte count ranged from 0.9 to 10.1 x 10⁹/L in the 12 patients. Rare HG-like cells were identified in the blood of only one patient. In all other patients, HGs were absent from the peripheral blood.

Surface immunophenotypic studies of the bone marrow mononuclear fraction showed a uniform pattern of increased expression of the B-cell antigens CD19 and CD20 and the nonlineage-specific antigens CD10 and HLA-Dr (Fig 3). With the exception of CD20, the histograms of these antigens' FITC fluorescence suggested the presence in most marrow samples of several populations of cells, with variation from weak to strong antigen intensity (Fig 4).

Two-color–correlated expression of CD19 (B4) and CD10 (CALLA) on five bone marrows with expanded HGs confirmed that CD19 was expressed on 68% to 75% of the CD10-bearing cells and that most cells expressing CD19 coexpressed CD10 (Fig 5). Most of the cases also exhibited increased expression of CD38 and, to a lesser extent, CD34 (Fig 3). Since CD38 is expressed on proliferating hematopoietic cells, we also examined two-color–correlated expression of CD19 and CD38 on six bone marrow samples. Although only 23% to 54% of the CD38-bearing cells also expressed CD19, 75% to 100% of the CD19-bearing cells coexpressed CD38 (Fig 6). All cases also contained an admixture of phenotypically mature B cells, defined by cell surface expression of immunoglobulin κ or λ light chains (26% to 56%) (Fig 3). The percentages of CD3-bearing (or CD2-bearing) cells were variable in the individual bone marrow samples (11% to 51%), as were the relative proportions of inducer-helper (CD4) and cytotoxic-suppressor (CD8) T cell subsets (1:1 to 4.5:1). Six of the cases contained...
a significant percentage of cells expressing the immature T-cell antigen, CD1 (4% to 29%).

To characterize the marrows of these children further, cytocentrifuge slides prepared after Ficoll-Hypaque density centrifugation were examined for morphology, nuclear TdT, and cytoplasmic μ expression. Strong cytoplasmic μ expression was present in 10% to 75% (median 50%) of the total lymphoid cell population (HGs plus lymphocytes). By extrapolation from bone marrow differential counts, these marrows contained 7.0% to 36.5% (median 22%) cytoplasmic μ-positive cells. Likewise, 15% to 75% (median 40%) of the total lymphoid cell population expressed intranuclear TdT, representing 8.0% to 37.5% (median 20%) of all bone marrow cells. On light microscopic illumination, most of the TdT positive cells exhibited the morphologic characteristics associated with HGs, whereas the cytoplasmic μ-positive cells constituted a morphologically heterogenous population consisting of both HGs and more mature-appearing lymphocytes.

The proliferative index of the Ficoll-Hypaque-separated bone marrow cells from seven patients was assessed by determining the percentage of cells in the S phase of the cell cycle.

**Fig 3.** Phenotypic analysis of bone marrow mononuclear cell suspensions from 12 pediatric patients with expanded HGs. Bar indicates median value.

**Fig 4.** Fluorescence histograms of CD10, CD19, CD38, and HLA-Dr in bone marrow mononuclear cell fraction of pediatric patient (patient 2) with 24% HGs and 22% mature lymphocytes. Several subpopulations of cells have weak and strong surface antigen expression.

**Fig 5.** Two-color-correlated expression of CD19 and CD10 on bone marrow mononuclear cells (patient 2). Virtually all CD19-bearing cells coexpressed CD10, whereas 72% of CD10-positive cells coexpressed CD19.
cycle (Table 2). Although the number of normal children we have studied is low, there appeared to be no significant difference in the number of proliferating cells in pediatric bone marrows with and without increased HGs. In all but one of the patients (patient 8), the DNA content was diploid. In this patient, a very wide single peak with a left shoulder was observed in the diploid region which could not be resolved as a unique population.

Cytogenetic studies were also performed on bone marrow specimens from patients 1, 2, 6, and 8 (Table 2). In all four patients, including the patient with questionable ploidy status by DNA content studies, karyotype was normal. However, bone marrow from two of the patients (patients 2 and 6) contained a substantially higher percentage (12% and 15%, respectively) of random aneuploidy on direct metaphase spreads than the 5% upper limit of normal for this cytogenetic laboratory. A follow-up karyotype in one of these patients (patient 2), performed 14 months later, showed normal karyotype with no random aneuploidy. No rearrangements of either the T-cell β and γ or B-cell immunoglobulin heavy and light chain loci were detected in any of the three patients studied.

Follow-up of the 12 patients ranged from 3 months to 3 years. A summary of their clinical course and treatment is shown in Table 3. All patients are alive and most (ten of 12) have normalized their blood picture. One of the patients with red cell aplasia (patient 2) has had four bone marrow studies, all of which showed an admixture of HGs (10% to 24%) and bone marrow lymphocytes (30% to 45%), without evidence of restoration of the erythroid hematopoietic compartment. None of the other patients has required repeat bone marrow examination, and no patient in this series has developed acute leukemia or bone marrow involvement by solid tumor.

**DISCUSSION**

Morphologically distinct lymphoid cells, originally termed HGs, are present in the bone marrow of normal children, and

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**Table 2. DNA Content, Cell Cycle Analysis, and Karyotype of Bone Marrows With Increased HGs**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>HGs (%)</th>
<th>DNA Content</th>
<th>S Phase (%)</th>
<th>Karyotype</th>
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<td>2n</td>
<td>13.28</td>
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<td>2</td>
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<td>8.59</td>
<td>46,XX</td>
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<td>3</td>
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<td>11.14</td>
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<td>6</td>
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<td>—∗</td>
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<td>ND</td>
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<td>11</td>
<td>18</td>
<td>2n</td>
<td>9.73</td>
<td>ND</td>
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<td>12</td>
<td>23</td>
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ND, not done.

∗Wide single peak with left shoulder in diploid region.

**Table 3. Status and Follow-up of Patients With Increased Bone Marrow HGs**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Bone Marrow Diagnosis</th>
<th>Treatment</th>
<th>Clinical Course</th>
<th>Follow-up (mo)</th>
<th>Evolution to ALL</th>
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<td>1</td>
<td>Suspected immune neutropenia</td>
<td>No treatment</td>
<td>Asymptomatic, still neutropenic</td>
<td>13</td>
<td>No</td>
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<tr>
<td>2</td>
<td>red cell aplasia</td>
<td>IV γ-globulin and prednisone</td>
<td>Transfusion dependent</td>
<td>13</td>
<td>No</td>
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<tr>
<td>3</td>
<td>No metastatic tumor</td>
<td>Surgery and chemotherapy</td>
<td>Alive and well, off treatment</td>
<td>14</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>No metastatic tumor</td>
<td>Surgery</td>
<td>Alive and well, off treatment</td>
<td>30</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>red cell aplasia</td>
<td>No treatment</td>
<td>Complete recovery</td>
<td>12</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Suspected transient bone marrow injury with resultant cytopenias</td>
<td>No treatment</td>
<td>Complete recovery</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>No metastatic tumor</td>
<td>Chemotherapy</td>
<td>Alive and well, off treatment</td>
<td>26</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Bone marrow lymphocytosis</td>
<td>No treatment</td>
<td>Complete recovery</td>
<td>14</td>
<td>No</td>
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<tr>
<td>9</td>
<td>red cell aplasia</td>
<td>Prednisone</td>
<td>Complete recovery, off treatment</td>
<td>11</td>
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<tr>
<td>10</td>
<td>No metastatic tumor</td>
<td>Chemotherapy</td>
<td>Alive and well, off treatment</td>
<td>6</td>
<td>No</td>
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<tr>
<td>11</td>
<td>Idiopathic thrombocytopenic purpura</td>
<td>IV γ-globulin and prednisone</td>
<td>Complete recovery</td>
<td>6</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>Idiopathic thrombocytopenic purpura</td>
<td>No treatment</td>
<td>Slow recovery</td>
<td>6</td>
<td>No</td>
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</table>

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their number markedly decreases with age. In some children, the increase in HGs can be pronounced, resulting in confusion with ALL. HGs may also be misinterpreted as metastatic tumor or malignant lymphoma. Because of these potential diagnostic hazards, an awareness of the clinical settings in which increased numbers of bone marrow HGs can occur is critical to the appropriate care and treatment of these pediatric patients. In addition, hematologic and morphologic criteria can be used to distinguish HG from leukemic blasts. One important distinguishing characteristic in our patients was the virtual absence of HGs from the peripheral blood. In bone marrow smears, HGs lacked nucleoli and were therefore distinct from typical blasts. Although rare HGs contained moderate granular PAS-positive cytoplasmic material, these cells did not exhibit the block-positive pattern characteristic of ALL. Other investigators have shown that HGs are nonreactive with Sudan black B, myeloperoxidase, and nonspecific esterase stains.

In section material, HGs from our patients' marrow samples were uniform in size, not mitotically active, and did not exhibit the nuclear convolutions characteristic of many leukemias.

Flow cytometric immunophenotypic analysis of bone marrows with increased HGs often revealed increased expression of CD19, CD20, CD10, TdT, HLA-DR, and cytoplasmic μ, similar to ALL. However, the pattern of FITC fluorescence for these antigens frequently exhibited more than one peak of fluorescence intensity, suggesting a spectrum of stages of differentiation. Correlation of the pattern of fluorescence on cytospin smears with morphology usually revealed several cell types including (a) TdT cytoplasmic μ cells, (b) TdT cytoplasmic μ' cells, and (c) TdT cytoplasmic μ'' cells. These flow cytometric and cytospin immunofluorescence data suggest that bone marrows with increased HGs contain an immunophenotypic spectrum of cells ranging from very immature B-cell precursors to mature, surface immunoglobulin (slg)-bearing lymphocytes, corroborating our morphologic impression of HGs, intermediate forms, and mature lymphocytes. Because TdT expression is not unique to B cells, a small subset of HGs may be T-cell precursors.

In our study, recognition that HGs were not leukemic infiltrates was further facilitated by DNA content analysis, karyotyping studies, and molecular analyses. Bone marrows with expanded HG populations exhibited a normal diploid DNA content, showed no clonal karyotypic abnormalities, and lacked evidence of clonal rearrangements of the immunoglobulin and T cell receptor (TCR) genes. Random aneuploidy in significant excess of that usually found in direct metaphase bone marrow preparations was observed in two of the patients. Although this random aneuploidy is seldom mentioned in current cytogenetic literature, in the past it has been proposed that this type of nonclonal cytogenetic abnormality may be secondary to bone marrow injury caused by viral, chemical, or radiation exposure.

The spontaneous recovery of hematopoietic function observed in one of the patients and the resolution of random aneuploidy in the other patient on subsequent karyotype studies, indicates that such a transient, toxic bone marrow insult may have occurred in these patients.

Although many studies have been made of lymphoid cells in the bone marrow of children, they are difficult to assimilate because of the variety of terms they use and because of their heterogeneity. However, from the information contained in many of these studies, we suspect that the cells being evaluated are HGs. In the 1960s and early 1970s, several researchers described the phenomenon of bone marrow lymphocytosis following cessation of chemotherapy for ALL and debated whether this lymphocytosis represented early relapse or immune recovery. Bone marrow lymphocytosis was also observed in other hematologic disorders, but subsequent immunologic investigations were predominantly directed toward children recovering from antileukemic chemotherapy. The earliest of these immunologic studies established that these bone marrow lymphoid cells were pre-B or more mature B cells, and later, CALLA and/or TdT expression was attributed to some of these cells. Since this "rebound" in bone marrow lymphoid cells appeared to occur without extrinsic antigenic stimulation and was subsequently followed by increased numbers of IgG-bearing and IgM-bearing peripheral blood lymphocytes, Borella et al and other investigators attributed elevated pre-B cells in these patients to a prolonged chemotherapy-induced dysfunction in lymphopoiesis and immune response. Other investigators proposed that these cells were not necessarily linked to prior chemotherapy, but were apparent in any actively regenerating or proliferating marrow. Currently, sophisticated flow cytometric multicolor analyses with cell gating are being used to study bone marrow lymphoid cells. From these investigations, several distinct stages of B-cell maturation have been described, and we propose that the earliest of these hypothetical B-cell precursors are morphologically HGs, whereas the cells at later stages of differentiation correspond morphologically to more mature lymphocytes.

Although the expansion of HGs in pediatric patients appears to represent a benign, age-dependent immune response phenomenon, the association of large numbers of these cells with a variety of cytopenias raises the possibility of an interaction between HGs and the rest of the hematopoietic system. T cells have been convincingly implicated in the pathogenesis of some cases of neutropenia and aplastic anemia, and these cells elaborate a variety of lymphokines, including a multitude of B-cell growth factors. Several lines of evidence now indicate that activated B cells also produce biologically active molecules that may participate in the regulation of cellular differentiation and gene expression. In addition, studies within the murine system suggest that B cells secrete molecules capable of directly or indirectly stimulating hematopoietic precursors in vivo. Thus, in at least some cases, HGs may have a specific hematopoietic restorative or regenerative function. An alternative, but not mutually exclusive, possibility is that HGs are a heterogeneous population and that a proportion of these cells represent precursor cells of lineages other than the B lymphoid cell lineage. Several investigators have described erythroid precursors as being very lymphoid in appearance, and the progenitor of erythroid cells may reside in a small heterogeneous null Fc receptor-negative subpopulation of lymphoid cells.

Although HGs are morphologically and immunologically
immature, these cells should not be called lymphoblasts. Careful attention to the clinical setting, histopathology, cytochemistry, and if necessary, the total immunophenotypic profile, karyotype, and molecular features of the bone marrow cells in any given patient should enable the physician to distinguish HGs from neoplastic cells. Since HGs appear to be most prominent in the bone marrow of children with immune cytopenias and solid tumors, the etiology and biologic function of increased HGs in these patients is of considerable clinical interest. The temporal sequence we have observed in several of our patients of numerous bone marrow HGs immediately prior to recovery of cytopenia(s) suggests that in at least some cases, these cells may play a critical role in regulation of hematopoiesis as well as in immune function.

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REFERENCES

5. Muehleck SD, McKenna RW, Gale PF, Bruning RD: Terminal deoxynucleotidyl transferase (TdT)-positive cells in bone marrow in the absence of hematologic malignancy. Am J Clin Pathol 79:277, 1983
Hematogones: a multiparameter analysis of bone marrow precursor cells

TA Longacre, K Foucar, S Crago, IM Chen, B Griffith, L Dressler, TS McConnell, M Duncan and J Gribble