Normal Human Pluripotential and Committed Hematopoietic Progenitors Do Not Express the p24 Antigen Detected by Monoclonal Antibody BA-2: Implications for Immunotherapy of Lymphocytic Leukemia

By Robert C. Ash, Jan Jansen, John H. Kersey, Tucker W. LeBien, and Esmail D. Zanjani

Analysis of surface antigenic determinants of hematopoietic progenitor cells has relevance both to basic biologic study of cell differentiation and to potential clinical application in the diagnosis and treatment of hematologic neoplasia. The production and characterization of monoclonal antibody BA-2 by immunization with a pre-B-ALL cell line has been reported previously. In this study we utilized complement-dependent cytotoxicity and rosette-separation with antibody indirectly coupled to ox RBC to determine if the antigen (p24) recognized by the antibody BA-2 is represented on human pluripotential (CFU-GEMM) or committed hematopoietic progenitors (CFU-GM, BFU-E). BA-2 showed no reactivity with normal hematopoietic progenitors by either method. In contrast, BA-2 exhibited potent complement-mediated cytotoxicity for selected ALL-derived cell lines. These results show that normal human hematopoietic progenitors do not express antigenic sites represented on ALL cells that are recognized by BA-2 and suggest that this monoclonal antibody may serve as a potent and specific agent for treatment of lymphocytic leukemia, perhaps most useful in ex vivo marrow conditioning for autologous bone marrow transplantation.

The production of monoclonal antibodies against surface antigenic structures of neoplastic and normal lymphoid cells has facilitated the study of questions concerning gene expression in the processes of differentiation and consideration of potential clinical application of such antibodies in the classification and treatment of hematologic neoplasia. The recognition that certain immunologically defined cellular phenotypes may reflect "maturation arrest" of T and B lymphocyte differentiation and may correlate with the clinical features of some neoplasms has underscored the importance of understanding the developmental expression of these cell surface structures. Moreover, recent demonstrations of the efficacy of monoclonal antibodies directed against neoplasia-associated antigens in the treatment of several malignancies in animal studies have also raised the possibility of use of these antibodies for immunotherapy of human neoplasia.

The production of monoclonal antibody BA-2 by immunization with a pre-B-ALL cell line has been previously reported. This antibody binds to a 24,000-dalton cell surface polypeptide (p24) in approximately 70% of non-T, non-B acute lymphocytic leukemias (ALL) and a small population of normal bone marrow lymphoid progenitors. Both in order to better define the expression of the p24 antigen during normal lymphohematopoietic differentiation and because possible therapeutic use of any monoclonal antibody requires prior demonstration of nontoxicity to relevant normal tissues (such as normal marrow hematopoietic precursors), we have explored the interactions of monoclonal antibody BA-2 with committed granulocyte-macrophage and erythroid progenitors as well as the human pluripotential hemopoietic progenitors, the CFU-GEMM.

Materials and Methods

Marrow Samples

Hematologically normal paid volunteer donors and normal donors for bone marrow transplantation served as control subjects. The age of these 12 normal subjects ranged from 18 to 45. After informed consent was obtained, 3-5-ml bone marrow aspirates were drawn into heparinized syringes. Samples were diluted three-fold in RPMI 1640 containing 10% fetal calf serum (RPMI-10% FCS), layered over Ficoll-Hypaque gradients (density 1.077), and centrifuged 25-30 min at 400 g. Interface mononuclear cells were collected, washed 3 times, and resuspended in RPMI-10% FCS.

Monoclonal Antibodies

BA-2 is an antibody of the IgG3 subclass produced by immunization of BALB/c female mice with the NALM-6-M1 cell line. BA-2 ascitic fluid was collected, allowed to clot, and stored at −70°C, and...
used in this study at appropriate dilutions after passage through a 0.45-μm Nalgene filter. The IgG concentration of BA-2 ascitic fluid lots used in this study was approximately 1–5 mg/ml. Monoclonal antibody W6/32, an IgG2a antibody recognizing HLA-A,B,C and framework structures, was purchased from Sera Lab (through Accurate Chemical and Scientific Corporation, Westbury, N.Y.). Other antibodies used as controls in cytotoxicity experiments or for immunofluorescence included the monoclonal antibodies 9.6, which recognizes the sheep erythrocyte receptor, and OKT-3, reacting with the T-cell antigenic structure p19; these were gifts from Drs. J. A. Hansen (Seattle, Wash.) and G. Goldstein (Raritan, N.J.), respectively. Monoclonal antibody BA-1, reactive with cells of human B-cell lineage, and monoclonal antibody Tab, which binds to platelet-megakaryocyte-specific glycoprotein IIb-IIIa (the latter a gift from Dr. R. McEver, San Antonio) were also used.

Cytotoxicity Assays

Bone marrow mononuclear cells at 20–24 × 10⁶/ml in RPMI-10% FCS were incubated for 30 min at 4°C with equal volumes of appropriately diluted antibody, followed by the addition of rabbit complement (10% final concentration) and incubation, with continuous gentle agitation, at 37°C for 60 min. Complement used was obtained from Pelfreez Biologicals (Rogers, Arks.) from lots pre-screened in this laboratory for maximum specific cytotoxicity with a panel of monoclonal antibodies (including BA-2, W6/32, 9.6, and OKT-3) and minimal nonspecific toxicity against human hematopoietic progenitors; this two-step incubation was used to maximize cytotoxicity. In some preliminary experiments, as indicated in Results, a one-step cytotoxicity incubation (i.e., with cells, antibody, and complement added simultaneously and incubated for 60 min at 37°C) was utilized: specific cell lysis was minimally reduced with this procedure.

Rosette Separation

In order to confirm results of complement-dependent cytotoxicity studies, an alternate method of obtaining BA-2-depleted and BA-2-enriched marrow cell populations was adopted from methods previously published.21 One part of a solution of Staphylococcal protein A (Pharmacia Fine Chemicals, Piscataway, N.J.) at 0.5 mg/ml in 0.9% NaCl was mixed with 10 parts of 2.5 × 10⁻⁴ M CrCl₃, in 0.9% NaCl and 1 part packed washed ox RBCs. The mixture was incubated 1 hr at 30°C, and the Staph-protein A-coated ox RBCs (SPA-oxRBCs) thereafter washed 3 times and resuspended in RPMI-10% FCS to a concentration of 10⁷/ml. Marrow mononuclear cells at 20–24 × 10⁶/ml were incubated with a 1:50 dilution of BA-2 at 4°C for 30 min, then washed twice in cold media and resuspended to 10 × 10⁶/ml in RPMI-10% FCS. The SPA-oxRBC suspension was then added in equal volumes to the marrow cell suspension (SPA-oxRBC:marrow cell ratio approximately 100:1), mixed, centrifuged at 150 g for 10 min, and incubated for 20 min at room temperature. The pellet was then gently resuspended, an aliquot taken for counting of rosetting cells, and the remainder subjected to ficoll-hypaque gradient centrifugation (25–30 min, 400 g, 15°C). Interface and pellet cell fractions were then collected, contaminating RBCs lysed from pellet and interface with Tris-buffered 0.83% NH₄Cl, and the cell fractions washed and appropriately diluted.

Immunofluorescence Analyses

Indirect immunofluorescence was used to assess binding of monoclonal antibodies to marrow cell populations before and after complement-dependent cytotoxicity or rosetting experiments. Desired monoclonal or control asctic fluid was mixed with target cells (at 10–25 × 10⁶/ml) and incubated for 30 min at 4°C. After washing, cells were stained with fluorescein-isothiocyanate-conjugated goat anti-mouse Ig (FITC-GAMG). FITC-GAMG was obtained either from Meloy Laboratories (Springville, Va.) or Cappel Laboratories (Cochraville, Pa.) and, when possible, Fab( ) and F(ab’ )₂ conjugates were used. The second incubation was continued for 30 min at 4°C, followed by washing, mounting, and examination with a Zeiss fluorescent microscope equipped with Pleom epi-illumination. At least 200 cells were counted per slide. All antibodies were used at concentrations sufficient to achieve saturation of binding sites as determined by immunofluorescence intensity on the fluorescence-activated cell sorter (FACS).

Culture Assay for CFU-GEMM, BFU-E, and CFU-GM

A modification of the methylcellulose assay described by Fauser and Messner was used. Concentrations of 0.25–2 × 10⁶ mononuclear marrow cells were cultured in the presence of 1 IU erythropoietin (Ep) in quadruplicate 1-ml aliquots in 35-mm culture dishes containing 0.9% methylcellulose, 5% PHA-LCM (media conditioned by human blood leukocytes in the presence of phytohemagglutinin), 30% fetal calf serum, Iscove's modified Dulbecco's medium, and 5 × 10⁻⁴ M 2-mercaptoethanol; Ep was added at the start of culture. Plates were incubated at 37°C in an atmosphere of 5% CO₂ and high humidity, and colonies enumerated by direct observation in situ at 40–200× magnification on day 15. Hemoglobinized colonies, easily recognizable by their red color, consist of those with "pure" erythroid elements (BFU-E derived) and "mixed" cellular elements (CFU-GEMM derived) in which an identifiable periphery of nonhemoglobinized small and large cells can be seen. Granulocyte-macrophage colonies (CFU-GM derived) can also be recognized at this time as morphologically distinct colonies with a flatter arrangement of nonhemoglobinized cells. The morphological heterogeneity and presence of specific cell types within individual mixed colonies have been demonstrated by Wright-Giemsa staining and the use of other selective histochemical stains. The megakaryocyte content of these mixed colonies has also been confirmed by immunofluorescence analysis with monoclonal antibody Tab, which recognizes a functionally specific glycoprotein complex (IIb-IIIa) of megakaryocyte-platelet lineage.22 Colonies were scored as "mixed" only when they appeared to be discrete single colonies in nonconfluent areas of plates.

Culture Assay for CFU-E

The plasma clot system was used as previously described. Marrow samples were cultured at 2–6 × 10⁶ cells/ml in quadruplicate 0.1-ml aliquots containing 1.5 IU Ep/ml, and benzidine-positive colonies scored on day 7.

Microculture Assay

In some preliminary experiments evaluating the separation steps (indicated separately in Results), CFU-GM alone were cultured according to a microwell modification of the method of Issov et al. Briefly, 0.2 ml of cells (at 5 × 10⁶/ml), 0.2 ml leukocyte conditioned medium, 0.2 ml FCS, and 0.4 ml of 2% methylcellulose were mixed, and 6 replicates of 0.1 ml pipetted into the wells of a flat-bottomed microtiter plate (Falcon, Oxnard, Calif.) Colonies were scored after 10 days of incubation at 37°C in an atmosphere of 5% CO₂ and high humidity. In the W6/32 rosetting experiment, BFU-E and CFU-E were also cultured in this system in the presence of 1.5 IU Ep/ml and assayed after appropriate fixation and staining.
Statistical Considerations

All culture data presented in this paper represent the mean of quadruplicate cultures at individual data points. p Values were calculated using the Student’s t test.

RESULTS

Figure 1 shows results of cytotoxicity experiments with ALL-derived leukemic cell lines demonstrating that incubations with BA-2 and complement, under conditions effective for cytolysis of BA-2* cells from normal marrow (Table 1), are highly effective in accomplishing cytolysis of ALL cells. The efficiency of cytolysis varied between target cell lines, presumably dependent in part on the density of the p24 antigen on the respective cell lines.

The binding of monoclonal antibody BA-2 to normal marrow mononuclear cells was evaluated by indirect immunofluorescence and complement-dependent cytolysis. Approximately 4%-5% (4.5 ± 0.8, n = 9) of marrow mononuclear cells were BA-2 positive by immunofluorescence for adult (age 18-45, median 27) marrow samples. The BA-2* cells were small round mononuclear cells, and stained brightly to dilutions of at least 1:10,000 of ascites. The number of BA-2* marrow mononuclear cells enumerated by immunofluorescence showed good agreement with that determined by specific cell lysis using complement-dependent cytolysis (Table 1).

When marrow mononuclear cells were incubated with BA-2 and complement under conditions effective for accomplishing optimal cytolysis of BA-2* cells, no significant effect on the number of either pluripotent (CFU-GEMM derived) or committed hematopoietic precursors was observed (Table 2). Table 2 shows detailed data or a representative experiment, demonstrating the absence of significant inhibition of colony formation by BA-2 and complement, compared to both media and complement-incubated controls. The efficacy of this system for abrogating clonogenic

### Table 1. Reactivity of Monoclonal Antibody BA-2 With Normal Marrow Mononuclear Cells*

<table>
<thead>
<tr>
<th>Antibody Concentration</th>
<th>Percent (–) Immunofluorescence</th>
<th>Percent (+) C′ Cytotoxicity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1000</td>
<td>3.5 ± 0.7</td>
<td>6 ± 13</td>
</tr>
<tr>
<td>1:200</td>
<td>4 ± 0.5</td>
<td>8 ± 6</td>
</tr>
<tr>
<td>1:50</td>
<td>5 ± 1.7</td>
<td>5 ± 6</td>
</tr>
<tr>
<td>1:25</td>
<td>4.5 ± 1.3</td>
<td>5 ± 15</td>
</tr>
</tbody>
</table>

*Combined data of determinations with 9 adult marrow samples. †As percent of complement control, one-step cytotoxicity incubation.

Specific cell lysis determined by enumeration of total viable (Trypan-blue excluding) cells remaining after 60-min incubation with antibody and complement.

### Table 2. Effect of Monoclonal Antibody BA-2 on Normal Marrow Hematopoietic Progenitors*

<table>
<thead>
<tr>
<th>Incubation Additives†</th>
<th>CFU-GEMM</th>
<th>BFU-E</th>
<th>CFU-E</th>
<th>CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media alone</td>
<td>13.5 ± 2</td>
<td>37 ± 1.5</td>
<td>187 ± 7</td>
<td>33 ± 1.5</td>
</tr>
<tr>
<td>Media + C′ (1:5)†</td>
<td>12.1 ± 2.7</td>
<td>34.5 ± 3</td>
<td>165 ± 15</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>Media + C′ (1:10)</td>
<td>12.7 ± 1.5</td>
<td>38.3 ± 4</td>
<td>178 ± 12</td>
<td>31 ± 3.5</td>
</tr>
<tr>
<td>Media + C′ (1:10) + W6/32 (1:50)†</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Media + C′ (1:10) + W6/32 (1:500)</td>
<td>&lt;0.5</td>
<td>1.5 ± 2.3</td>
<td>3.5 ± 2.1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Media + W6/32 (1:500) (no C′)</td>
<td>13.1 ± 2.1</td>
<td>35 ± 2.1</td>
<td>172 ± 22</td>
<td>36 ± 2.3</td>
</tr>
<tr>
<td>Media + C′ (1:10) + BA-2 (1:25)</td>
<td>12.5 ± 1.5</td>
<td>39 ± 4</td>
<td>169 ± 11</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>Media + C′ (1:10) + BA-2 (1:50)</td>
<td>14.3 ± 2.9</td>
<td>41 ± 4.5</td>
<td>177 ± 21</td>
<td>33.5 ± 3.1</td>
</tr>
<tr>
<td>Media + C′ (1:10) + BA-2 (1:100)</td>
<td>13.7 ± 1.8</td>
<td>33 ± 2.9</td>
<td>185 ± 17</td>
<td>37 ± 1.7</td>
</tr>
<tr>
<td>Media + C′ (1:10) + BA-2 (1:200)</td>
<td>15.1 ± 3</td>
<td>37.5 ± 3.1</td>
<td>159 ± 23</td>
<td>34 ± 2.5</td>
</tr>
<tr>
<td>Media + C′ (1:10) + BA-2 (1:400)</td>
<td>11.9 ± 2.3</td>
<td>38 ± 1.8</td>
<td>172 ± 15</td>
<td>33.7 ± 3</td>
</tr>
<tr>
<td>Media + C′ (1:10) + BA-2 (1:800)</td>
<td>14 ± 2.5</td>
<td>36.5 ± 5</td>
<td>179 ± 8</td>
<td>31.3 ± 1.9</td>
</tr>
</tbody>
</table>

*Data of a representative experiment with a normal adult donor.
†Two-step cytotoxicity incubation, as described in Materials and Methods. Dilutions listed refer to final concentrations of antibody and/or complement present during 60-min incubation.
viability (and/or accomplishing cytolysis) of hematopoietic progenitors in the presence of an antibody that identifies antigens expressed on their cell surfaces is demonstrated by the nearly total absence of colony formation by marrow after incubation with the anti-HLA antibody W6/32 and complement; this antibody–complement treatment served as a positive control in each experiment with BA-2. In companion experiments, the specificity of cytolysis was further demonstrated by the reduction of viable T cells bearing the respective antigens after incubation with two other murine IgG class monoclonals, OKT-3 and 9.6 (data not shown). In 5 separate experiments similarly performed, the number of hematopoietic colonies of each class remaining after BA-2 and complement incubation was unaffected over a wide concentration of antibody (mean percentage inhibition of CFU-GEMM, BFU-E, CFU-E, and CFU-GM derived colony formation, determined with respect to complement-incubated control, was zero at BA-2 dilutions of 1:20–1:1000). This contrasted with the consistent inhibition of colony formation observed with W6/32 and complement (mean percentage inhibition of 99% for CFU-GEMM, 98% for BFU-E, 99% for CFU/E, and 97% for CFU-GM derived colony formation at W6/32 dilution of 1:500).

In order to further confirm the apparent absence of binding of BA-2 to hematopoietic progenitors, rosette separation with BA-2 indirectly bound to ox RBCs was utilized to obtain BA-2-depleted and BA-2-enriched marrow cell populations; these populations were then subjected to hematopoietic cell culture. Table 3 shows the results of a representative experiment with rosette separation. The interface cell population was highly depleted of BA-2+ cells compared to the prerosetting marrow, with <0.5% residual BA-2+ cells detected in a 200-cell count of interface cells postdepletion. As shown in Table 4, the BA-2-depleted interface cell population contained the bulk of assayable progenitor cells, compared to the BA-2-enriched pellet. The pellet cell population contained a number of cells nonspecifically trapped in the rosetting and/or sedimentation procedure, so that BA-2+ cells constituted only 20.5% ± 4.6% of BA-2-enriched pelleted cell fractions in 5 similar experiments. The specificity of the enrichment for BA-2+ cells was, however, demonstrated by immunofluorescence analysis showing random distribution of other T (OKT-3, 9.6) and B (BA-1) antigen-bearing cells between interface and pellet compared to the significant (p < 0.001) concentration of BA-2+ cells in the pellet. Moreover, the appearance of small numbers of hematopoietic progenitor cells in the pellet cell fractions following BA-2 rosette separation was similar to the numbers of progenitors in the pellet following sham rosetting with SPA-oRBC in the absence of antibody. The results of 5 experiments in which all four progenitors (CFU-GEMM, BFU-E, CFU-E, and CFU-GM) were assayed after BA-2 SPA-oRBC rosetting were confirmed by 6 additional experiments in which CFU-GM alone were cultured in the microwell assay; all experiments demonstrated the bulk of hematopoietic progenitors to be in the BA-2-depleted interface cell fractions.

An additional experiment was performed, using the

| Table 3. Effect of BA-2 Rosette Separation on Normal Marrow Hematopoietic Progenitors* |
|------------------------------------------|---------------------------------|----------------|
|                                          | Interface                      | Pellet |
|                                         | %BA-2+                         | Colonies/10^5 Cells |
| BFU-E                                   | 8 ± 2                          | <1 |
| CFU-GM                                  | 26 ± 7                         | <3 |
| CFU-E                                   | 18 ± 4                         | <3 |
|                                         | 74 ± 6                         | 12 ± 6 |  
* SPA-oRBC rosetting, representative experiment.  
† Prerosetting marrow was 4.5% BA-2+.

| Table 4. Effect of W6/32 Rosette Separation on Normal Marrow Hematopoietic Progenitors* |
|------------------------------------------|---------------------------------|----------------|
|                                          | Unseparated                     | Interface     | Pellet |
| Percent cells* by immunofluorescence     | W6/32                          | >90           | >90 |
|                                         | BA-2                            | 5             | 0    |
| Percent rosetting cells                  | W6/32                          | 45            | 39   |
|                                         | BA-2                            | 15            | 37   |
| Percent of total cells recovered after rosette separation | W6/32 | 6.9 ± 1.1 | 8.6 ± 3.5 |
|                                         | BA-2                            | 19.8 ± 4.0    | 1.3 ± 4.0 |
| Colonies/10^5 cells*                     | W6/32                          | 16.1 ± 3.5    | 4.0 ± 1.0 |
|                                         | BA-2                            | 33.8 ± 5.0    | 4.0 ± 1.0 |
|                                         |                                 | 60.0 ± 10.6   | 20.3 ± 6.3 |
|                                          |                                 | 74.8 ± 14.0   | 3.1 ± 1.0 |
* Experiment cultured in microassay. CFU-GEMM numbers are too low for detection in this system.
microculture assay, as a positive control for the rosetting experiments. Because BA-2' lymphoid cells might theoretically be inherently more rosettable than hematopoietic progenitor cells, we performed W6/32 rosetting to determine whether hematopoietic progenitors could indeed be positively selected by antibody-directed rosetting with an antibody that recognized antigens known to be expressed on such progenitor cells (Table 4). The overall efficiency of rosetting was less with W6/32 than with BA-2 (i.e., while >90% of marrow mononuclear cells were W6/32' by immunofluorescence, only some 45% of these cells rosetted). However, those progenitors assayed were distributed between pellet and interface fractions after W6/32 rosetting in numbers roughly equivalent to the proportion of cells that rosetted. This was in marked contrast with the results obtained with BA-2 rosetting, in which essentially all BA-2' cells are removed from the interface population, while the large majority of progenitors remain in the BA-2' interface cell population. These results demonstrate that hematopoietic progenitors can be positively selected by rosetting with an antibody (such as W6/32) against antigens that they express, and strengthen our conclusion that hematopoietic progenitors do not express the p24 antigen.

DISCUSSION

Previous studies with monoclonal antibody BA-2 have demonstrated that this antibody binds a cell surface antigen (p24) expressed at an early stage of normal lymphoid cell development; indeed greater than 50% of Td'T normal marrow cells expressed the p24 antigen.13,28 The demonstration that BA-2 binds strongly to most non-T, non-B ALL and most pre-B-ALL suggests that BA-2 primarily defines a human lymphoid progenitor/leukemia associated antigen,13,24 with these leukemic cells presumably displaying structures indicative of “arrested development” at early stages of B-cell differentiation. During early developmental stages, the entire lymphohematopoietic system shares a common cellular origin,30,31 and therefore it is of biologic importance to define the relationship of such early lymphoid developmental markers to early hemopoietic differentiation events. The availability of in vitro clonal assays for committed hemopoietic progenitors30,32,33 and the recent development of a system for identification of a human pluripotential hemopoietic progenitor14-16 now facilitate studies of this kind.

Our studies demonstrate clearly that, while BA-2 binds to a small population of lymphoid progenitor cells within normal marrow (approximately 4%-5% of adult marrow mononuclear cells and a greater percentage in pediatric marrows28), it does not appear to bind antigenic sites present on either the human pluripotential hemopoietic progenitor (the CFU-GEMM) or committed hemopoietic progenitors (BFU-E, CFU-E, and CFU-GM). Because hemopoietic progenitor cells constitute very small minority populations within marrow and are not morphologically distinguishable from other small lymphoid cells, indirect methods such as those utilized here must be applied to this analysis. The use of antibody and complement-dependent cytotoxicity has been widely applied to similar studies,34 and our data with BA-2 showed no evidence of toxicity to hemopoietic progenitors in conditions suitable for effecting cytotoxicity with an antibody (W6/32) against the HLA-A,B,C locus presumably present on such cells. There are recognized disadvantages of complement cytotoxicity,34 however, including the theoretical concern that progenitor cells may have antigens inaccessible to antibody-complement interactions or be resistant to lysis because of low surface antigen density. Moreover, failure of cytotoxicity may reflect inefficient complement fixation rather than absence of surface antigens. Therefore, we took pains to explore BA-2 binding by another method independent of complement-cytotoxicity. The experiments with BA-2 SPA-oRBC rosetting therefore further support our conclusion that BA-2 does not recognize antigenic sites present on normal marrow hemopoietic progenitors.

The antigen p24, the molecular structure which is identified by BA-2, has now been studied immunochromically in some detail. Its failure to bind to Ricinus communis, Lens culinaris, and concanavalin-A affinity columns and the absence of any molecular weight change after treatment with glycosidases or tunicamycin suggest that N-asparagine linked oligosaccharide chains are not present on the antigen.35 Its lack of labeling with a lipophilic nitrene reagent or 32P suggests that, like the common ALL antigen (CALLA), p24 is a nonphilic cell surface protein.35 Although direct comparisons have not been carried out, other recently described monoclonal antibodies36,37 most likely also recognize this structure. Published data on cell binding specificities and molecular weight determinations of the antigens immunoprecipitated by these antibodies36,38 are similar to those observed with BA-213,28 (T. W. LeBien et al., unpublished data). The published report of nonreactivity of monoclonal Du-ALL-1 with CFU-GM37 is also in agreement with our results with BA-2 in this study. It should also be noted that, while BA-2/p24 expression can conveniently be thought of as primarily defining a primitive lymphoid cell/lymphoid leukemia cell phenotype, p24 is expressed variably on some activated lymphocytes, human platelets, neuroblastoma, and some epithelial-
deranged neoplasms\textsuperscript{13} (T. W. LeBien et al., unpublished data).

The demonstration in this study of absence of p24 expression on hematopoietic progenitors is in contrast to reports concerning certain other antigenic structures associated with lymphoid differentiation and/or lymphoid neoplasia.\textsuperscript{31,39,42} The pattern of reactivity of BA-2 differs significantly from that of anti-HLA-DR (Ia) antibodies, which have been reported to react with CFU-GM,\textsuperscript{39} BFU-E,\textsuperscript{40} and CFU-GEMM.\textsuperscript{41} A recently described monoclonal antibody (RFB-1) produced against a T-lymphoblastic leukemia and reactive with T-lineage cells was found to be reactive with CFU-GM.\textsuperscript{42} These examples suggest that developmental markers associated with both B- and T-cell differentiation/neoplasias may also be expressed on early hematopoietic progenitors, in contrast to the relative selectivity for lymphoid progenitors demonstrated by p24/BA-2.

In addition to the basic biologic implications of these studies in defining the developmental expression of p24, the demonstration that BA-2 recognizes an antigenic determinant on neoplastic cells that is not found on normal hematopoietic progenitors suggests that this monoclonal antibody may be clinically useful as a selective immunotherapeutic agent for treatment of lymphocytic leukemia. The very different results of experiments with ALL-derived cell lines and normal marrow clearly demonstrate that, in the presence of an appropriate complement source, BA-2 can be potently cytotoxic to leukemic cells at concentrations that are nontoxic to normal marrow hematopoietic progenitors. Whether the antibody is used with complement incubation or coupled to a toxic effector molecule like ricin,\textsuperscript{9,43} its potential for selective immunotherapy of BA-2 neoplasia seems manifest, and is especially attractive as an agent for ex vivo marrow conditioning in autologous bone marrow transplantation.

The expression of p24 on some nonlymphoid cells may also be important in considering clinical application of such monoclonals as BA-2. BA-2 might be useful, for instance, for marrow treatment in autologous marrow transplant for neuroblastoma as well as for ALL. Also of potential clinical importance is the expression of p24 on human platelets and determination of the point in megakaryocyte–platelet differentiation at which p24 expression occurs. Inasmuch as we have observed usual numbers of megakaryocytes within CFU-GEMM-derived mixed colonies after BA-2 and complement treatment, it seems unlikely that early events in megakaryocytopenesis are affected by removal of BA-2 positive cells. The question of whether p24 is expressed at the level of the committed megakaryocyte progenitor cell (CFU-Mk) might well be important for hematopoietic reconstitution after monoclonal antibody treatment. Additional studies, using recently developed techniques for assay of human megakaryocyte progenitors,\textsuperscript{12} have been undertaken to examine this question.

ACKNOWLEDGMENT
We would like to thank Maria McGinnis and Daniel R. Boue for excellent technical assistance, and Bernetta Kambetz for help with preparation of the manuscript.

REFERENCES


15. Fauser AA, Messner HA: Identification of megakaryocytes,
macrophages, and eosinophils in colonies of human bone marrow containing granulocytes and erythroblasts. Blood 53:1023, 1979


38. Jones N, Borowitz MJ, Megzger RS: Characterization and distribution of a 24,000-dalton antigen defined by a monoclonal (Du-All-l) elicited to common acute lymphoblastic leukemia (cALL) cells. Leuk Res (in press)


Normal human pluripotential and committed hematopoietic progenitors do not express the p24 antigen detected by monoclonal antibody BA-2: implications for immunotherapy of lymphocytic leukemia

RC Ash, J Jansen, JH Kersey, TW LeBien and ED Zanjani