Elimination of Human Leukemia by Monoclonal Antibodies in an Athymic Nude Mouse Leukemia Model

Yang Xu and David A. Scheinberg
Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT
A human acute myeloid leukemia model has been developed by i.v. transplantation of HL-60 myeloid leukemia cells into Swiss nude mice pretreated with cyclophosphamide. HL-60 cells disseminated into hematopoietic tissues as determined by flow cytometric analysis, fluorescence microscopy, fluorescence in situ hybridization analysis, and colony formation assay. Passive immunotherapy using murine anti-CD13 (F23) or anti-CD33 (M195) mAbs was able to eliminate completely the HL-60 cells in the mice, as determined by fluorescence in situ hybridization analysis, colony formation assay, and culture of mouse blood and tissue cells in vitro. Although F23 is able to inhibit completely CD13/aminopeptidase N enzymatic activity, actinonin, another potent inhibitor of CD13/aminopeptidase N, was not active as an antileukemogenic agent. HL-60 cell surface antigens, including CD13 (aminopeptidase N) and CD33 (p67), down-regulated over time, and murine anti-HL-60 antibody was generated while the cells grew in the mice. This response was suppressed by cyclophosphamide. These data suggest that leukemia cell elimination was antibody mediated.

INTRODUCTION
Human AML remains a largely incurable disease that is resistant to conventional therapies (1). Immunotherapies, in particular, mAb therapies, are under investigation as alternatives or adjuncts to chemotherapy (2). Pharmacokinetics are favorable; has low morbidity and mortality; is inexpensive; a pharmacological standpoint; has appropriate antigen targets; is manageable difficult and expensive. Therefore, we developed an alternative human leukemia model in cyclophosphamide-treated athymic nude mice. Although there are also limitations to this model, it adequately mimics disseminated human leukemia from a pharmacological standpoint; has appropriate antigen targets; is reproducible; has low morbidity and mortality; is inexpensive; and requires little technical expertise. Immunotherapy with mAbs was evaluated in this model.

MATERIALS AND METHODS
Animals. Six-week-old female outbred Swiss nu/nu mice were purchased from Sloan-Kettering Institute (New York, NY). All bedding material was sterilized before use; the cages were covered with an air filter and maintained in isolation cabinets. Animal handling and experiments were performed in a laminar flow hood. Swiss nude mice, 6–8 weeks of age, required i.p. injections with 3 mg cyclophosphamide/mouse 3 days before i.v. injection of HL-60 cells. The cyclophosphamide dose was based on preliminary experiments that showed that HL-60 cells did not engraft well in mice without previous injection of cyclophosphamide: 16% of Swiss nude mice (4/25 mice) without pretreatment of cyclophosphamide had no palpable tumor nodules at 8 weeks after local s.c. injection, whereas all (10/10) nude mice developed local tumors at 4 weeks with pretreatment with 3 mg cyclophosphamide. Similar results were observed in mice that received i.v. injections by assaying CD13+ and CD33+ cells in the lungs, where leukemia cells were initially trapped (16). This suggested that the nude mice retain limited ability to prevent HL-60 xenografting.

HL-60 Human Leukemia Cell Line. The HL-60 cell line is a well-characterized human acute myeloid leukemia derived from peripheral blood blasts (17). The HL-60 cells expressed both CD13+ and CD33+, two human myeloid markers (6, 18, 19). The numbers of CD13 and CD33 antigens/cell were 20,000 (20) and 10,000 (21), respectively. The HL-60 cell line was maintained routinely in our laboratory. Cells were

Received 2/2/95; revised 5/22/95; accepted 6/26/95.

1 This work was supported by NIH Grant RO1 CA55349 and by the Markey Charitable Trust. D.A.S. is a Lucille P. Markey Scholar.
2 To whom requests for reprints should be addressed, at Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.
3 The abbreviations used are: AML, acute myeloid leukemia; FISH, fluorescence in situ hybridization; MPF, mean peak fluorescence; SCID, severe combined immunodeficient; APN, aminopeptidase N; HuM195, humanized M195.
cultured in 10% FCS/10% Serum Plus/RPMI 1640 and 1% penicillin-streptomycin at 37°C in a humidified atmosphere of 5% CO2/air. Cell viability was always higher than 95%, and cells were free of Mycoplasma contamination.

**mAbs.** mAbs F23 (IgG2a), reactive with CD13 antigen, and M195 (IgG2a) and HuM195 (human IgG1), reactive with CD33 antigen, were prepared at Sloan-Kettering Institute (22). MY7 (IgG1), reactive with CD13 antigen, and MY9 (IgG2b), reactive with CD33 antigen, were purchased from Coulter Electronics.

**Xenotransplantation.** A 0.2-ml aliquot containing 3 × 10⁷ HL-60 cells from suspension culture was transplanted i.v. into the tail veins of nude mice. Mice were killed after transplantation at the times indicated. For tissue studies, pieces of organs were minced, and intact single cells were isolated on a Ficoll-Hypaque density gradient or after passage through a 70-μm nylon filter (Spectrum). Preliminary work showed that HL-60 cells could grow in nude mice after i.p. injection. At 7–8 weeks after injection of 3 × 10⁷ HL-60 cells without pretreatment with cyclophosphamide, CD33⁺ cells could be found outside of the peritoneal cavity. We found 31% of the cells in the liver, 15% of the cells in the lung, 9% in the spleen, and 3% in the bone marrow. However, one third of the mice died at 5 weeks. The poor leukemia distribution and the rapid deaths made this i.p. model less useful for studying therapy, and it was not pursued.

**Flow Cytometry Analysis and Fluorescence Microscopic Assay.** Flow cytometry were done as described previously (22). For direct immunofluorescence, 1,000,000 cells were incubated in a 0.1-ml final volume with the fluorescein- or rhodamine-conjugated mAb for 60 min on ice, washed twice, and fixed with 1% paraformaldehyde before analysis. Cells were analyzed on an EPICS flow cytometer (Coulter) or evaluated by fluorescence microscopy. HL-60 cells were preincubated with 2% heat-inactivated rabbit serum/10% FCS/RPMI 1640 at room temperature for 15 min to reduce nonspecific binding. Thirty μl of test nude mouse serum were incubated with 5 × 10⁷ HL-60 cells on ice for 60 min, then washed, and 50 μl goat antibodies to mouse IgG-FITC (50 μg/ml) were added for 30 min. Washed cells were analyzed by flow cytometry as described above. Serum from untreated, untransplanted Swiss nude mice was used as negative control. The MPF of cells was determined relative to the background MPF of cells stained with isotype-matched control mAb or normal nude mouse serum, in which a value of 1.0 reflects MPF equivalent to background. Under these conditions, the MPF represents a quantitative value for the maximum number of bound murine Ig on the surface of the leukemia cells.

**FISH Analysis.** FISH analysis was performed using standard techniques (23–25) with some modifications and is described in brief below. Single tissue cells (10⁷) were cultured in 10% FCS/10% serum/RPMI 1640 for 24 h and treated with 10 ng/ml Colcemid for the final 4 h. At this condition, all HL-60 cells were arrested in the metaphase or interphase stage, as determined by our laboratory. After hypotonic treatment in 0.075 M KCl and fixation in 3:1 fixative (three parts absolute methyl alcohol:one part glacial acetic acid), slides were incubated in 2× standard SSC/0.5% NP40 at 37°C for 30 min; dehydrated successively in 70, 80, and 95% ethanol at room temperature for 2 min each; and then dried in air. The chromosomes were denatured by incubation at 70°C for 2 min in 70% formamide/2X SSC (pH 7.0) and dehydrated as described above. FITC-conjugated direct-labeled specific human chromosome 17 α-satellite DNA probe D17Z1 (Oncor; Ref. 26) was denatured by incubation at 70°C for 5 min and cooled on ice. For hybridization, 30 μl of the probe (1 ng/μl) were used under a 22 × 50-mm coverslip. The coverslip was sealed with rubber cement, and the slides were incubated at 37°C for 30 min in a prewarmed humidified chamber. After hybridization and coverslip removal, excess probe was removed by incubation in 0.5× SSC at 72°C for 5 min, followed by two changes of 2 min each of PBS plus 0.1% Triton X-100. The chromosomes were counterstained by incubation for 2 min in 0.3 μg/ml propidium iodide and immediately photographed with a Zeiss microscope. At least 200 cells were scored for each slide.

**RESULTS**

**Detection of CD13⁺ and CD33⁺ HL-60 Cells in Mice by Flow Cytometry and Fluorescence Microscopy.** The model was initiated by injection of 3 × 10⁷ HL-60 cells via the tail vein. Four weeks after the transplant, the bone marrow, spleen, peripheral blood, lung, and lymph node contained 8–90% leukemia cells (Fig. 1 and Table 1). The number of cells was determined by the percentage of CD13⁺ and CD33⁺ cells; neither of these antigens is expressed on mouse cells. Mice that received no injections showed no positive cells and were used as negative controls. After 6 weeks, the proportion of HL-60 cells rose to 17% in bone marrow, 26% in the spleen, 28% in the blood, 92% in the lung, and 91% in the lymph node (Table 1). The high percentage of HL-60 cells seen in the lungs is similar to previous observations in the SCID mouse and may be caused by leukemia cells trapped in the lungs initially (16). The percentage of HL-60 cells in mouse bone marrow, spleen, and peripheral blood also was evaluated by fluorescence microscopy. Both results were similar (Table 1), suggesting that either of these two assays may be used.

The percentage of human leukemia cells in peripheral blood of the mice also was confirmed by a human α-satellite probe specific for human chromosome 17 that does not hybridize with mouse DNA (Refs. 16, 24, 26; Table 1). At 10 weeks, HL-60 cells were present in the blood and bone marrow (Fig. 2), as well as in the liver, lung, kidney, and spleen (data not shown), as determined by FISH using a human chromosome 17 α-satellite probe, D17Z1. These HL-60 cells were also two to three times larger than were the mouse cells (Fig. 2) and could be readily detected morphologically. In addition, the D17Z1 probe did not hybridize with mouse cells from blood, liver, kidney, and spleen (data not shown), confirming that the probe is specific for human cells. Therefore, the widespread distribution of HL-60 leukemia cells in Swiss nude mice into multiple organs, as well as hematopoietic tissue, makes it a useful model for evaluating immunotherapy and other therapeutic approaches.
Fig. 1 Flow cytometry analysis of the blood and tissues from Swiss nude mice that received human HL-60 leukemia cells after 4 weeks. Single-cell suspensions from peripheral blood leukocytes (PBL), bone marrow (BM), spleen (SPL), lungs (LU), and lymph nodes (LN) of representative mice, which were pretreated with cyclophosphamide and into which were transplanted $3 \times 10^7$ HL-60 cells, were incubated with MY9-FITC, and were assayed as described in Materials and Methods. Dotted line, control mice; solid line, mice that received HL-60 cells. HL-60 cells from tissue culture are shown as a comparison (dotted line, cells plus IgG2b-FITC; solid line, cells plus MY9-FITC).

Table 1 Flow cytometric, fluorescence microscopic, and FISH analysis of Swiss nude mice transplanted with HL-60 leukemia cells as a human AML model

<table>
<thead>
<tr>
<th>Weeks (no. of mice)</th>
<th>Control (n = 7)</th>
<th>4 (n = 7)</th>
<th>6 (n = 7)</th>
<th>10 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>8 ± 4</td>
<td>10 ± 3</td>
<td>17 ± 9</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Spleen</td>
<td>15 ± 5</td>
<td>15 ± 5</td>
<td>26 ± 4</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>90 ± 8</td>
<td>92 ± 6</td>
<td>91 ± 12</td>
<td>91 ± 12</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>90 ± 11</td>
<td>90 ± 11</td>
<td>91 ± 12</td>
<td>91 ± 12</td>
</tr>
<tr>
<td>Lungs</td>
<td>7 ± 4</td>
<td>7 ± 4</td>
<td>19 ± 9</td>
<td>19 ± 9</td>
</tr>
<tr>
<td>Lymph node</td>
<td>0</td>
<td>0</td>
<td>28 ± 7</td>
<td>28 ± 7</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>0</td>
<td>0</td>
<td>32 ± 9</td>
<td>32 ± 9</td>
</tr>
<tr>
<td>Spleen</td>
<td>0</td>
<td>0</td>
<td>17 ± 7</td>
<td>17 ± 7</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>0</td>
<td>0</td>
<td>30 ± 10</td>
<td>30 ± 10</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>0</td>
<td>0</td>
<td>39 ± 14</td>
<td>39 ± 14</td>
</tr>
</tbody>
</table>

The Swiss nude mice bred in our defined-flora colony (Memorial Sloan-Kettering Institute) were given injections i.p. of 3 mg cyclophosphamide and, 3 days later, were given $3 \times 10^7$ HL-60 cells via tail vein injection. At the times indicated, the bone marrow, spleen, peripheral blood leukocytes, lungs, and lymph node were analyzed by flow cytometry for CD13⁺ (MY7-RD1) and CD33⁺ (MY9-FITC) cells. The control murine organs served as negative control. For fluorescence microscopic assay, cells were stained with MY9-FITC or MY7-RD1. Percentages of human cells were determined by human chromosome 17 probe. At least 200 cells were scored for each slide. The results are the means ± SE.

* For CD13⁺ and CD33⁺ HL-60 cells.
* Control mice were not given injections of HL-60 cells.
* ---, <1%.
Immunotherapy in the AML Model. Immunotherapy of the HL-60 leukemia using myeloid-specific mAb was tested in the model to determine its usefulness as a model for mAb-based therapies. The test animals were treated with 200 μg mAb i.p., three times a week for 5 weeks, beginning on the same day as the initiation of the model. On a weight basis, the doses used here are not unlike doses of mAb in human trials.

Approximately 60% of the untreated control animals were dead at 10 weeks after the HL-60 cell injection (Fig. 3A). The mice treated with F23, M195, and F23 plus M195 were all alive at 10 weeks, whereas the mortality of the groups treated by control antibody IgG2a (TA99) was similar to the untreated control groups. This suggests that F23 and M195 can specifically protect mice from HL-60 leukemia. In the groups treated with F23 or M195 for 5 weeks and tested at 10 weeks, there were no detectable human cells, as determined by FISH analysis (Table 2). In the untreated and TA99-treated groups, 28–41% human cells were found in bone marrow, spleen, and peripheral blood (Table 2). In addition, a lymph node from the untreated group at 10 weeks showed nearly 100% human chromosome 17-positive cells, as determined by FISH (data not shown).

The elimination of cells by use of an antibody infused at this early time point may reflect prevention of engraftment as well as killing. Therefore, additional mice were allowed to fully engraft before treatment began. F23 was able to protect mice from death 4 weeks after HL-60 leukemia cells had been transplanted (Fig. 3B). This suggested that passive immunotherapy could eliminate AML engrafted previously, because leukemia dissemination was clearly seen by 4 weeks (Table 1).

**Effects of Actinonin on Metastasis of Leukemia Cells in Vivo.** F23 is a potent inhibitor of CD13/APN (EC 3.4.11.2) in vitro (20, 27, 28). CD13/APN inhibitors have been examined in clinical studies and could inhibit various tumor metastasis in vitro (29–31). Anti-CD13/APN mAb could also inhibit the invasion of human metastatic tumor cells into reconstituted basement membrane (32). Therefore, the antileukemic action of F23 might be attributable to its potent effect on CD13/APN rather than to its immunological actions. Therefore, we tested whether the most potent inhibitor of CD13/APN, actinonin, could inhibit HL-60 leukemia cell dissemination in vivo in mice. Swiss nude mice were treated with 250 μg (an estimated initial serum concentration of 0.3 mM) actinonin i.p., three times a week for
cells in the lungs. To assess whether an immune response by the 8 weeks for the presence of leukemia. Eight of these mice had CD33 cells in the lungs, whereas two of the mice had no CD33 cells in the lungs. This finding suggests that actinonin could not protect mice from HL60 leukemia.

**Mouse Anti-HL-60 IgG Levels.** In an attempt to explain the possible mechanisms of immune rejection in these animals, 10 mice that received injections of 3 × 10⁷ HL-60 cells i.v. without pretreatment with cyclophosphamide were assayed at 6 weeks for the presence of leukemia. Eight of these mice had CD33⁺ cells in the lungs, whereas two of the mice had no CD33⁺ cells in the lungs. To assess whether an immune response by the nude mouse might contribute to the rejection of the HL-60 cells, the levels of mouse anti-HL-60 IgG in these two sets of mice were compared. IgG anti-HL-60 levels in the leukemia-negative mice were approximately three times higher than those in mice that did not reject the leukemia cells (Fig. 4A). This result suggested that a fraction of nude mice that had not been pretreated with cyclophosphamide has an ability to reject HL-60 cells and that specific Ig may contribute to this effect. After pretreatment with cyclophosphamide, the lungs from all seven mice were positive for CD33-staining cells. In addition, the anti-HL-60 IgG levels in these mice were approximately 8-fold lower than those in the mice that had not been pretreated with cyclophosphamide, which had rejected the HL-60 cells (Fig. 4A). These data suggested that cyclophosphamide reduced the level of immune response to HL-60 cells, in this instance, as measured by IgG levels; therefore, this response appeared to be important in the rejection of HL-60 cells.

Similar results were obtained from mice that received s.c. injections of HL-60 (Fig. 4B). Of the Swiss nude mice, 16% (4/25) that were not pretreated with cyclophosphamide had no palpable tumor nodules at 8 weeks after local s.c. injection, whereas all (10/10) nude mice developed local tumors at 4 weeks when pretreated with 3 mg cyclophosphamide. The relative anti-HL-60 levels at 6 weeks in these groups of mice showed the same distribution as had been described with mice that received i.v. injections. Mice that rejected the HL-60 cells had the highest levels (27.6), whereas mice with tumor growth and mice treated with cyclophosphamide had levels of 11.7 and 4.2, respectively (Fig. 4B).

**Modulation of Cell Surface Antigen Targets on HL-60 Cells in Vivo.** Analysis of MPF of human leukemia cells in the mice showed a time-dependent decrease in the expression of the cell surface markers CD13 and CD33. At 10 weeks after the injection, CD33 antigen on HL-60 cells was nearly undetectable (Fig. 5) and no CD33⁺ cells were found (Table 1). CD13 antigen down-regulation was similar to CD33 down-regulation, as determined by flow cytometry using MY7-RD1 (data not shown). In this group of mice pretreated with cyclophosphamide, low levels of anti-HL-60 IgG still were produced (Fig. 5). The levels peaked at 6 weeks, then fell, coincident with CD33 and CD13 surface antigen down-regulation. The loss of CD13 and CD33 expression may be attributed to development of a polyclonal murine anti-HL-60 immune response (see above), which bound to and modulated the antigens from the surface. Other antigens on the HL-60 cell (CD29 and CD71) also down-regulated (data not shown). Rapid modulation of human hematopoietic antigens has been found in vitro and in vivo (3–5, 33, 34).

**DISCUSSION**

We describe a leukemia model in nude mice using HL-60 cells injected i.v. after cyclophosphamide pretreatment. Cells engrafted and spread rapidly throughout the body. Although this model is neither appropriate nor proposed as a biological model of leukemia cell growth in vivo, it is appropriate for the study of antibody-based immunotherapy with mAbs reactive with antigens on HL-60 cells, such as CD33, CD15, and CD13, all of

![Survival curves of Swiss nude mice after transplantation of HL-60 leukemia cells.](image-url)
which are under investigation in human clinical trials. We used this model to study immunotherapy by use of passively administered mAbs. The model is not appropriate for study of T-cell-mediated events nor for long-term therapy because of the down-regulation of target antigens.

Although leukemia cells were detected readily by flow cytometry at 4 and 6 weeks in these mice, surprisingly, the nude mice contained no detectable CD13⁺ or CD33⁺ cells in bone marrow, spleen, and peripheral blood at 10 weeks (Table 1). However, FISH analysis of these organs at 10 weeks showed that they still contained human chromosome 17⁺ cells (Table 2). The loss of markers over time appeared to be the result of prevention of engraftment by the mAb. To further determine whether there were viable residual HL-60 cells in these mice, we incubated the cultures from bone marrow and spleens of the mice with surface-bound mouse IgG in the spleen and bone marrow (data not shown). This suggested that there were many cells with surface-bound mouse IgG in the spleen and bone marrow. Therefore, the secondary antibodies bound nonspecifically to various mouse cells. As a result, indirect assay cannot be used easily for detection of human cells.

Immunotherapy of the HL-60 leukemia using myeloid-specific mAb F23 and M195 was able to completely eliminate 3 × 10⁷ HL-60 cells and prevent death in this model (Fig. 3A and Table 2). We further confirmed the ability of antibody-mediated antileukemic effects in additional mice in which complete engraftment of HL-60 cells (4 weeks) had occurred. At this later time point, the antileukemic effects could not be attributed to prevention of engraftment by the mAb. To further determine whether there were viable residual HL-60 cells in these mice, we cultured 10⁶ bone marrow and spleen cells taken at 10 weeks in 10 ml liquid medium containing 10% FCS.

After 3 weeks of culture, there were approximately 10⁷ cells in the cultures from bone marrow and spleens of the untreated and TA99-treated mice, but no cells in the mice that did not receive injections of HL-60 or in the F23-, M195-, or F23 plus M195-treated groups (Table 2). The cells that grew out of the bone marrow and spleens in the untreated or TA99-treated groups were 100% CD13⁺, CD33⁺ and human chromosome 17⁺ (data not shown). These various ex vivo assays were therefore, consistent with FISH analysis, suggesting that HL-60 cells were completely eliminated from the mice after treatment with F23 or M195.

The mechanism by which leukemia cells were eliminated by F23 or M195 is unclear. F23 is capable of completely inhibiting CD13/APN enzymatic function in vitro (20, 27). CD13/APN has been implicated in tumor metastasis (32). Therefore, we tested whether another potent APN inhibitor (actinonin), which binds to the same zinc motif of CD13/APN to which mAb F23 binds (20, 27, 28), could inhibit HL-60 leukemia cell growth in vivo in mice. Actinonin, at a concentration of approximately 0.3 mM, administered three times a week, was unable to inhibit HL-60 cell growth in the mice. We observed that the death rate in the actinonin-treated group was similar to that in the untreated control group. This suggested that the elimination of HL-60 cells in mice by F23 was more likely a

---

**Table 2** FISH analysis, colony formation, and culture assays of Swiss nude mice 10 weeks after transplantation with the HL-60 leukemia cell line with or without mAb treatment

<table>
<thead>
<tr>
<th>Treatment (no. of mice)</th>
<th>% of Cells with human chromosome 17</th>
<th>HL-60 colonies/10⁶ cells</th>
<th>Culture assay/10⁵ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/none (n = 7)</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>None (n = 6)</td>
<td>30 ± 12 35 ± 12 39 ± 14</td>
<td>TNNTC' TNNTC TNNTC</td>
<td>&gt;10⁷ &gt;10⁷</td>
</tr>
<tr>
<td>TA99 (n = 3)</td>
<td>28 ± 11 31 ± 10 41 ± 20</td>
<td>TNNTC TNNTC TNNTC</td>
<td>&gt;10⁷ &gt;10⁷</td>
</tr>
<tr>
<td>F23 (n = 7)</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>M195 (n = 7)</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>F23 + M195 (n = 7)</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

* HL-60 leukemia cells (3 × 10⁷) were transplanted into Swiss nude mice as described in Table 1. Immunotherapy was described in Fig. 2. At 10 weeks, the bone marrow, spleen, and peripheral blood leukocytes were analyzed by FISH for human chromosome 17⁺ cells; colony formation assay; and ex vivo culture in liquid phase. For colony formation assay, 10⁶ mononuclear cells were plated in 0.6% agar containing 10% FCS/10% serum plus/RPMI 1640; colonies were scored after 2 weeks. For culture assay, 10⁶ bone marrow or spleen mononuclear cells were cultured in 10 ml 10% FCS/10% Serum Plus/RPMI 1640; live cells were counted after 3 weeks. Data are the means ± SE.

* Control mice were not given injections of HL-60 cells.

* TNTC, too numerous to count (>3000 colonies).
with cyclophosphamide were killed, and CD1Y and CD33 HL-60 cells in cyclophosphamide rejected HL-60 cells (Fig. 4), suggesting that APN.

\[ \text{Relative murine anti-HL-60 IgG levels were influenced by previous cyclophosphamide treatment A. HL-60 leukemia cells (3} \times 10^{7} \text{) were transplanted i.v. into Swiss nude mice as described in "Materials and Methods." At the times indicated, the enlarged lymph nodes and mouse sera were collected. Single-cell suspensions (5} \times 10^{7} \text{) were stained with MY9-FITC on ice for 60 min, washed, and analyzed by flow cytometry using the EPICS Profile II (Coulter). Normal Swiss nude mouse cells were stained with MY9-FITC as negative control. For anti-HL-60 IgG assays, 30 \mu l mouse serum were incubated with 5} \times 10^{7} \text{HL-60 cells as described in "Materials and Methods." MPF was shown.} \]

Fig. 4. Relative murine anti-HL-60 IgG levels were influenced by previous cyclophosphamide treatment A. HL-60 leukemia cells (3 \times 10^7) were transplanted i.v. into Swiss nude mice as described in "Materials and Methods." Except with or without previous cyclophosphamide (CPA) treatment. At 6 weeks, 10 mice that did not require cyclophosphamide were killed, and CD13" and CD33" HL-60 cells in the lungs were assayed. Two mice rejected the HL-60 cells and were negative for cells in the lungs, whereas eight mice engrafted and were positive for cells in the lungs. The sera from all 10 mice were incubated with 5 \times 10^7 HL-60 cells followed by goat antibodies to mouse IgG-FITC as described in "Materials and Methods." A group of seven mice that were pretreated with cyclophosphamide and a group of mice that did not receive HL-60 are shown for comparison. B. Twenty-five mice received 3 \times 10^7 HL-60 cells s.c. without previous cyclophosphamide treatment. Four mice had no palpable tumor nodules at 6 or 8 weeks. Ten mice pretreated with 3 mg cyclophosphamide developed local tumors at 4 weeks. All sera were collected at 6 weeks and assayed as described in A. Bars, SD of the mean. A comparison of the data from each group by Student's t test showed significant differences between groups (P < 0.05).

\[ \text{Fig. 5. HL-60 cell surface antigen down-regulation and relative murine anti-HL-60 IgG levels in the mice after transplantation of HL-60 cells with pretreatment with cyclophosphamide and without mAb treatment. HL-60 leukemia cells (3} \times 10^{7} \text{) were transplanted into Swiss nude mice as described in "Materials and Methods." At the times indicated, the enlarged lymph nodes and mouse sera were collected. Single-cell suspensions (5} \times 10^{7} \text{) were stained with MY9-FITC on ice for 60 min, washed, and analyzed by flow cytometry using the EPICS Profile II (Coulter). Normal Swiss nude mouse cells were stained with MY9-FITC as negative control. For anti-HL-60 IgG assays, 30 \mu l mouse serum were incubated with 5} \times 10^{7} \text{HL-60 cells as described in "Materials and Methods." MPF was shown.} \]

A fraction of the nude mice that was pretreated with cyclophosphamide rejected HL-60 cells (Fig. 4), suggesting that these untreated mice retained a partial immune response that was adequate to occasionally reject HL-60 cells. Because these mice lack functional T lymphocytes, the elimination may be caused by a humoral response, NK cells, or macrophage-based opsonization. Even in SCID mice, NK and lymphokine-activated killer cell activity exist (36, 37). Cyclophosphamide further depressed the immune response so that mice were unable to reject the HL-60 cells. A comparison of the mice that did not reject HL-60 cells with and without pretreatment with cyclophosphamide, for levels of specific polyclonal anti-HL-60 antibody, showed that the specific immunoglobulin response decreased at least 50% after the treatment (Fig. 4). This finding suggested that cyclophosphamide can reduce directly the ability of mice to generate a specific response to HL-60. Reductions of other arms of the immune response are also likely. F23 or M195 may eliminate the HL-60 cells in the mice as a result of specific IgG-mediated tumor suppression via neutrophil-, macrophage-, or NK-mediated antibody-dependent cell-mediated cytotoxicity or via organization. A role for the participation of a low-level polyclonal immune response in the elimination of HL-60 cells in conjunction with the passive infusion cannot be excluded, however. On the basis of these model data, a trial using high doses of HuM195 has been initiated at Memorial Hospital (New York, NY). In addition, this model will allow the efficient evaluation of a series of constructs of HuM195 that have been prepared. These include 131I-HuM195, $^{213}$Bi-HuM195, $^{90}$Y-HuM195, HuM195-gelonin, and HuM195 in combination with cytokines, such as interleukin 2. Moreover, combinations of mAb that are now in human use alone (such as anti-CD33, anti-CD15, and anti-CD13), but that cannot be infused together feasibly in humans because of restrictions in the development of investi-
gential new drugs, may be tested rapidly under various conditions in this model before their proposal for human use.

ACKNOWLEDGMENTS

We thank Dr. Joseph R. Bertino for thoughtful suggestions and critical review of the manuscript.

REFERENCES


Elimination of human leukemia by monoclonal antibodies in an athymic nude mouse leukemia model.

Y Xu and D A Scheinberg


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/1/10/1179

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.