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

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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 antileukemic 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 AML1 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 to the efficient delivery of mAbs to leukemia cells (3), and therapeutic effects have been observed in murine and human leukemias (3-5). mAbs that react with CD33, a Mr 67,000 glycoprotein expressed on early myeloid progenitor cells and myeloid leukemia cells, but not normal stem cells, have been used in Phase I trials for the treatment of AML (4, 6-8). An animal model could allow rapid therapeutic development of these immunotherapies, but at present, there are no useful models of human myeloid leukemia in which new mAb therapies can be evaluated. Current models are extremely limited: (a) A true mouse nonlymphoid leukemia, such as Friend or Rauscher leukemia (3, 9), is not useful, because the antigen targets of human leukemias are not expressed on mouse cells or in other small animals. (b) Xenografted nude mouse tumors have been proposed (10-13), but these models are not relevant as a model of leukemia, because the cells grow as a solid tumor or as ascites, and many of the biological and pharmacological issues are not addressed. (c) The growth of human cells from AML patients in the hematopoietic tissues of immunodeficient mice has been described (14, 15). These human leukemia cells were grown in irradiated SCID mice. This model mimics the human disease, but it is less reproducible because it uses fresh human cells as opposed to a cell line. Moreover, the mice are heavily immunocompromised, which renders their care and maintenance 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 sterilized atmosphere using 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) injected 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...
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). Mv7 (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 \times 10^7 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 \times 10^7 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 the probe specific for human chromosome 17 that does not hybridize to mouse 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 modela

<table>
<thead>
<tr>
<th>Weeks (no. of mice)</th>
<th>% of HL-60 cells by flow cytometryb</th>
<th>% of HL-60 cells by fluorescence microscopic assay</th>
<th>% of Human cells (FISH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ($n = 7$)</td>
<td>Bone marrow: 8 ± 4 10 ± 3 15 ± 5 90 ± 8 90 ± 11 7 ± 4 11 ± 3 16 ± 7 17 ± 7</td>
<td>0 0 0 39 ± 14</td>
<td>0 0 0</td>
</tr>
<tr>
<td>4 ($n = 7$)</td>
<td>Bone marrow: 17 ± 9 26 ± 4 28 ± 6 92 ± 6 91 ± 12 19 ± 9 28 ± 7 32 ± 9 30 ± 10</td>
<td>0 0 0 39 ± 14</td>
<td>0 0 0</td>
</tr>
<tr>
<td>6 ($n = 7$)</td>
<td>Bone marrow: 17 ± 9 26 ± 4 28 ± 6 92 ± 6 91 ± 12 19 ± 9 28 ± 7 32 ± 9 30 ± 10</td>
<td>0 0 0 39 ± 14</td>
<td>0 0 0</td>
</tr>
<tr>
<td>10 ($n = 6$)</td>
<td>Bone marrow: 17 ± 9 26 ± 4 28 ± 6 92 ± 6 91 ± 12 19 ± 9 28 ± 7 32 ± 9 30 ± 10</td>
<td>0 0 0 39 ± 14</td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

a 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.

b For CD13+ and CD33+ HL-60 cells.

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

d <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 vivo (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
weeks for the presence of leukemia. Eight of these mice had
without pretreatment with cyclophosphamide were assayed at 6
the possible mechanisms of immune rejection in these animals,
3A).

This finding suggests that actinonin could not

5 weeks, beginning on the same day as the initiation of
model. This schedule was identical to that of F23 described
above, and the experiment was done concurrently with the F23
experiments as a control. Actinonin was unable to inhibit HL-60
cells grown in the mice. The mice treated with actinonin were
not protected, and the mortality of the group was approximately
70%, which is similar to that seen in the untreated control
groups (Fig. 3A). This finding suggests that actinonin could not
protect mice from HL-60 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 $3 \times 10^7$ HL-60 cells i.v.
without pretreatment with cyclophosphamide were assayed at 6
weeks for the presence of leukemia. Eight of these mice had
CD33³ cells in the lungs, whereas two of the mice had no CD33³
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 pre-
treated 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 rel-
ative 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 CD33³ cells were found (Table 1). CD13
antigen down-regulation was similar to CD33 down-regulation,
as determined by flow cytometry using MY7-RDI (data not
shown). In this group of mice pretreated with cyclophospha-
damide, low levels of anti-HL-60 IgG still were produced (Fig. 5).
The levels peaked at 6 weeks, then fell, coincident with CD33
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 hema-
topoietic antigens has been found in vitro and in vivo (3–5, 33,
34).

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 anti-
gens on HL-60 cells, such as CD33, CD15, and CD13, all of

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 anti-
gens on HL-60 cells, such as CD33, CD15, and CD13, all of

Fig. 3 Survival curves of Swiss nude mice after transplantation of
HL-60 leukemia cells. HL-60 cells ($3 \times 10^7$) were transplanted into
Swiss nude mice as described in "Materials and Methods." After the
transplantation, the mice were treated with 200 μg mAb or 250 μg
actinonin i.p., three times a week for 5 weeks. A, treatment beginning at
the first day of the transplantation: no treatment (15 mice); actinonin (7
mice); TA99 (7 mice); F23 (7 mice); M195 (7 mice); and F23 plus
M195 (7 mice). The latter three treatments all resulted in 100% survival
and are, therefore, shown as overlapping lines. B, treatment beginning at
the 28th day after the transplantation: no treatment (7 mice); F23
treatment (7 mice).

![Graph A](attachment:image1.png)

![Graph B](attachment:image2.png)
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 steady loss of cell surface markers over a 8-10-week period rendered the use of immunophenotyping inadequate to study the model at later time points. The FISH results reflected more adequately the HL-60 cell distribution in the mice. FISH data were also consistent with colony formation assays at 10 weeks (Table 2).

The loss of markers over time appeared to be the result of immune modulation. Some human antigen down-regulation has been shown after transplantation of human peripheral blood lymphocytes into SCID mice (35). However, little attention to this phenomenon or its linkage to the mouse immune response has been paid previously. As a result, immunophenotypic analysis without DNA analysis or colony formation assays may lead to inaccurate conclusions in these AML models. Therefore, the fluorescence assays alone resulted in false-negative results at the later time points. Indirect flow cytometric assay, as described (16), also was attempted to increase the sensitivity of detection of the HL-60 cells. Washed single-cell suspensions from the mice were incubated with murine mAb against CD13 (F23) and CD33 (M195) and then FITC-conjugated F(ab')2 goat antibodies to mouse IgG, washed, and analyzed by fluorescence microscope. Control mice that had not received injections of HL-60 cells showed 30% or more positive cells 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 x 10^7 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 10^7 bone marrow and spleen mononuclear cells taken at 10 weeks in 10 ml 10% FCS/10% Serum Plus/RPMI 1640; live cells were counted after 3 weeks. Data are the means ± SE.

### Table 2

<table>
<thead>
<tr>
<th>Treatment (no. of mice)</th>
<th>% of Cells with human chromosome 17</th>
<th>HL-60 colonies/10^6 cells</th>
<th>Culture assay/10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bone marrow</td>
<td>Spleen</td>
<td>Peripheral blood lymphocytes</td>
</tr>
<tr>
<td>Control (none) (n = 7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>None (n = 6)</td>
<td>30 ± 12</td>
<td>35 ± 12</td>
<td>39 ± 14</td>
</tr>
<tr>
<td>TA99 (n = 3)</td>
<td>28 ± 11</td>
<td>31 ± 10</td>
<td>41 ± 20</td>
</tr>
<tr>
<td>F23 (n = 7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M195 (n = 7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F23 + M195 (n = 7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*HL-60 leukemia cells (3 x 10^7) 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, 10^6 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, 10^6 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 cyclobophosphamide rejected HL-60 cells (Fig. 4), suggesting that negative for cells in the lungs, whereas eight mice engrafted and were the lungs were assayed. Two mice rejected the HL-60 cells and were mide (CPA) previous cyclophosphamide treatment.

**Fig. 4** Relative murine anti-HL-60 IgG levels were influenced by previous cyclophosphamide treatment. A, HL-60 leukemia cells (3 × 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 × 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 × 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).

**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 × 10^7) 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 × 10^6) 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 μl mouse serum were incubated with 5 × 10^6 HL-60 cells as described in “Materials and Methods.” MPF was shown.

result of its immunological effects rather than of inhibition of APN.

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, ^213Bi-HuM195, ^90Y-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-
gional new drugs, may be tested rapidly under various conditions in this model before their proposal for human use.

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REFERENCES


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

Y Xu and D A Scheinberg


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