Expression of Antigens Coded in Murine Leukemia Viruses on Thymocytes of Allogeneic Donor Origin in AKR Mice following Syngeneic or Allogeneic Bone Marrow Transplantation

Thomas P. U. Wustrow and Robert A. Good

Memorial Sloan-Kettering Cancer Center, New York, New York 10021, and University of South Florida, All Children's Hospital, St. Petersburg, Florida 33701

ABSTRACT

Removal of T-lymphocytes from marrow inoculum with monoclonal antibody plus complement permitted establishment of long-lived allogeneic chimeras between C57BL/6 and AKR/J mice. Development of leukemia was prevented for 15 mo. Protection from leukemia occurred with both young (4 wk) and older (4 mo) recipients. AKR mice reconstituted with syngeneic marrow or control AKR mice all developed leukemia-lymphoma before 1 yr of age. During spontaneous lymphomagenesis in AKR mice, amplified expression of gag or env gene-coded virus antigens on the surface of thymocytes preceded leukemia development and evidence for amplification of other virus genes. These changes generally appeared before 6 mo. Similar viral gene expression and viral gene amplification occurred in the thymus and spleen cells of leukemia-resistant chimeric mice. Using monoclonal antibodies to M, 70,000 glycoprotein epitopes characteristic of ecotropic, xenotropic, or dualtropic viruses, antigens marking each virus form were found on thymocytes of allogeneic 4-wk and 4-mo chimeras as well as on the cells of AKR mice and of AKR mice reconstituted with syngeneic marrow. Flow cytometric analysis showed amplification of the virus genes in mice protected from leukemia-lymphoma by allogeneic bone marrow transplantation from leukemia-resistant mice. Allogeneic chimeras and syngeneically transplanted mice both showed evidence of accelerated viremia and of recombinant virus formation. The findings suggest that an event essential to leukemogenesis which occurs within the AKR lymphoid cells or their environment is lacking in the allogeneic chimeras. The nature of this influence of a resistance gene or genes introduced into AKR mice by allogeneic bone marrow transplantation deserves further study.

INTRODUCTION

Genetic factors influence resistance of mice to virus-induced leukemias (8, 22). The location in the genome of genes which exert influences on susceptibility and resistance to leukemia has been elucidated (18, 23, 38). Viruses are involved in the pathogenesis of leukemia in mice (10), but virus host relationships are complex because development of leukemia involves a family of viruses (41) and rapidly acting viruses derived via recombinant genetic events (1, 5, 9, 11, 21, 25, 30, 35, 40, 43). Murine leukemia viruses vary in capacity to induce leukemia-lymphoma in mice and in ability to replicate in mouse cells (41). Susceptibility to leukemia in genetically determined, and resistance to leukemia characterizes certain long-lived strains of mice, e.g., CBA/H and C57BL/6 (38).

To learn to inhibit recurrence of leukemia by marrow transplantation, we compared resistance to leukemia and expression of MuLV\(^4\) encoded antigens on thymocytes after lethal irradiation and marrow transplantation from allogeneic leukemia-resistant donors or syngeneic leukemia-susceptible donors. In earlier experiments, we showed that by bone marrow transplantation of mice matched at the MHC resistance could be introduced into highly susceptible recipients. Lethally irradiated AKR mice were transplanted with marrow from CBA/H, leukemia-resistant donors. Recipients lived up to 30 mo and did not develop leukemia. By contrast, AKR mice lethally irradiated and given bone marrow transplants at 2 mo of age from susceptible mice, e.g., C3H/Bl or AKR mice, developed the leukemia-lymphoma complex at the same age as unmanipulated AKR mice (9, 40, 43).

Herein we report producing long-lived allogeneic chimeras by transplanting marrow across MHC to lethally irradiated AKR recipients from leukemia-resistant C57BL/6J mice. Allogeneic chimeras whose hematopoietic and lymphoid cells are derived from stem cells of resistant donors did not develop leukemia. Their thymocytes expressed surface antigens in a pattern which indicates the foreign cells of these chimeras are infected with leukemogenic retrovirus and also with dual tropic recombinant viruses. Even though these chimeras showed accelerated virus production, they did not develop leukemia.

MATERIALS AND METHODS

Animals. C57BL/6J (H-2\(^b\)) and AKR/J (H-2\(^k\)) mice obtained from the Jackson Laboratory, Bar Harbor, ME, were used. Following lethal irradiation and syngeneic or allogeneic bone marrow transplantation, the animals were housed 1 to 5 mice per cage in laminar flow isolators. They were given sterilized laboratory chow and acidified drinking water ad libitum.

Bone Marrow Transplantation. AKR/J mice of both sexes were the irradiated recipients of bone marrow cells. One-mo-old mice were used as young (AAY, BAY) and 4-mo-old mice as older bone marrow (AAO, BAO) recipients. The mice were irradiated with 950 cGy in a plastic box

\(^4\) The abbreviations used are: MuLV, murine leukemia virus; MHC, major histocompatibility complex; FCS, fetal calf serum; HBSS, Hank's balanced salt solution; AAY, AKR/J mice lethally irradiated at the age of 4 wk and reconstituted with complement-treated AKR/J bone marrow from donors 4 wk of age; AAO, AKR/J mice lethally irradiated at the age of 4 mo and reconstituted with complement-treated AKR/J bone marrow from donors 4 wk of age; BAY, AKR/J mice lethally irradiated at the age of 4 wk and reconstituted with anti-Thy 1.2 plus complement-treated C57BL/6J bone marrow from donors 4 wk of age; BAO, AKR/J mice lethally irradiated at the age of 4 mo and reconstituted with anti-Thy 1.2 plus complement-treated C57BL/6J bone marrow from donors 4 wk of age; gp70, glycoprotein with a molecular weight of 70,000.
LEUKEMIA VIRUS ANTIGENS ON MARROW CHIMERA CELLS

without anesthesia using 125I-cesium as a γ-irradiation source at a dose rate of approximately 120 cGy/min. Two h after irradiation, recipients were given injections into the retroorbital plexus with 2 x 10^7 viable marrow cells in 0.2 ml of complete medium.

C57Bl/6J and AKR/J mice were donors at 4 to 5 wk of age. Donors were sacrificed by cervical dislocation, long bones (femur, tibia, and humerus) were removed surgically, and marrow was flushed from the cavities with cold RPMI-1640 medium using a 25-gauge needle. Approximately 2 x 10^7 marrow cells with viabilities of 88 to 97% were transplanted in lethally irradiated recipients (44).

Elimination of Thy 1.2-positive Cells. To eliminate Thy 1.2-positive cells from the allogeneic C57Bl/6J (Thy 1.2, H-2") marrow inoculum, a monoclonal hybridoma anti-Thy 1.2 serum (6/68), from U. Hammerling, Sloan-Kettering Institute, was used. Between 1:512,000 and 1:1,024,000 dilution of which complement gave 50% cytotoxicity for C57Bl/6J thymocytes with selected rabbit complement. One-m1 aliquots of marrow cells, 3 x 10^7 nucleated cells/ml, were dispensed into 12 x 75-mm plastic tubes and centrifuged at 100 x g for 10 min. Pellets were resuspended in 1 ml of diluted anti-Thy 1.2 serum in HBSS with calcium and magnesium or HBSS for controls, and they were incubated on ice for 30 min with occasional shaking. After washing, the cells were centrifuged and resuspended in 1 ml of complement at a dilution of 1:10 to 1:15 in HBSS. Cell suspensions were incubated in a shaking water bath at 37°C for 45 min. The cells were pooled, centrifuged, resuspended in complete medium, and adjusted to 1 x 10^6 viable nucleated cells/ml for injection into irradiated recipients. Since rabbit complement alone was not different than Thy 1.2 antisera + complement, controls used AKR marrow exposed to complement alone.

Antiserum. Mouse monoclonal antibody 19-E12 recognizes specifically Thy 1.1 alloantigen (1). Mouse monoclonal antibodies 16-B7 and 16-C1 recognize the gp70* and gp70" epitopes of AKR ecotropic MuLV gp70, respectively. The origin, specificity, and characterization of these antibodies have been described by Lostrom et al. (21), Nowinski et al. (26), Stone et al. (42), and O’Donnell et al. (31). Monospecific goat antisera prepared against gp70 (Lot 78 S-225) of Rauscher MuLV were founded by Dr. P. O’Donnell, Sloan-Kettering Institute. Antibody 35/56 recognizes an additional AKR ecotropic MuLV (gp70*) (27, 36). Hybridomas producing the 35/56 antibody were prepared by fusion of NSI/1 mouse myeloma cells with lymphocytes from W/Fu rats after immunization with purified AKR ecotropic MuLV. Monoclonal antibody-producing hybridomas were selected by testing culture supernatants for reactivity in fixed cell immunofluorescence assay on mouse SC-1 cells infected by AKR ecotropic MuLV isolate 69E5. Monoclonal antibody 35/56 belongs to the IgG2a subclass. Antibody against GSPLDS, a type-specific gp70 antigen of xenotropic MuLV, was raised as antiserum in mice with anti-Thy 1.2 (6/68) of the IgM class was used at a dilution of 1:500, and goat anti-gp70 (Rauscher) serum was measured at a gain of 4, and monoclonal antibodies 35/56, 16-C1, and 16-B7, at a gain of 8. By appropriate gating, dead cells or RBC which scatter light less intensively and which are scored in light scatter channels less than 80 (7) were excluded from fluorescence analysis. Forty thousand cells were counted in each run at flow rates of 1000 to 2000 cells/s.

Assessment of Chimerism. Each transplantation group comprised 36 animals: 10 were taken for fluorescence analysis; 4 for H-2 typing; 4 for macroscopic and microscopic analysis; and 18 for studies after long-time survival. All the transplantation experiments were done on consecutive days with analyses on one-half the mice of each group being carried out at each sitting.

Besides the testing of the Thy 1.1 and Thy 1.2 phenotypes in the fluorescence-activated cell sorter, spleen cells of the lethally irradiated mice reconstituted with autologous or allogeneic bone marrow were analyzed for susceptibility to cytolyse by specific alloantisera that recognize antigens of the H-2 complex. Anti-H-2b (BALB/c anti-BPB) and anti-H-2^d (E^a anti-EL-4) antisera were originally prepared by Boyse et al. and by F. W. Shen, Sloan-Kettering Institute, for H-2 typing (3). Briefly, 1 x 10^6 spleen cells/ml were incubated with the appropriate dilution of the specific alloantisera for 30 min on ice. After washing, the cells were incubated for 45 min at 37°C with selected rabbit complement. The percentage of dead cells was counted in a Neugebauer chamber after addition of 0.2% trypan blue dye. Cytotoxic index was determined according to the following formula.

\[
\text{Cytotoxic index} = \frac{\% \text{ of cells alive in complement control} - \% \text{ of cells alive in complement control}}{\% \text{ of cells alive in complement control}} \times 100
\]

Leukemia Incidence. The animals were followed for up to 14.0 mo and observed 3 times per week for evidence of leukemia. All dead animals and 4 representative surviving animals from each long-lived group were analyzed at 15 mo of age both macroscopically and microscopically for morphological changes attributable to graft-versus-host disease or leukemia.

RESULTS

In Table 1 are the H-2 characteristics of thymocytes from representative mice of each group reconstituted after lethal irradiation with marrow cells of AKR or C57Bl/6J donors in young or older AKR mice. Seven mo following bone marrow
LEUKEMIA VIRUS ANTIGENS ON MARROW CHIMERA CELLS

Table 1
Cytotoxic index of AKR thymocytes following syngeneic or allogeneic bone marrow transplantation

<table>
<thead>
<tr>
<th>Animals</th>
<th>Animal</th>
<th>H-2&lt;sup&gt;+&lt;/sup&gt;</th>
<th>H-2&lt;sup&gt;-&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAY</td>
<td>1</td>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>5.6</td>
<td>94.0</td>
</tr>
<tr>
<td>AAO</td>
<td>1</td>
<td>5</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>6.4</td>
<td>94.2</td>
</tr>
<tr>
<td>BAY</td>
<td>1</td>
<td>98</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>95</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>96</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>95.6</td>
<td>5.7</td>
</tr>
<tr>
<td>BAO</td>
<td>1</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>97</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>98</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>98</td>
<td>6</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>96.5</td>
<td>5.2</td>
</tr>
<tr>
<td>AKR mean</td>
<td></td>
<td>3.58</td>
<td>98.38</td>
</tr>
<tr>
<td>C57BL/6J mean</td>
<td>93.73</td>
<td>2.00</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Thymus weights of AKR, C57BL/6J, and chimeric mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Age of mice (mo)</th>
<th>Thymus wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR</td>
<td>2</td>
<td>61.6 ± 15.3*</td>
</tr>
<tr>
<td>AKR</td>
<td>6</td>
<td>36.4 ± 11.5</td>
</tr>
<tr>
<td>AKR</td>
<td>Leukemic</td>
<td>703.0 ± 117.5</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>2</td>
<td>36.1 ± 13.0</td>
</tr>
<tr>
<td>AAY</td>
<td>g&lt;sup&gt;+&lt;/sup&gt;</td>
<td>10.3 ± 5.9</td>
</tr>
<tr>
<td>AAO</td>
<td>12</td>
<td>30.0 ± 3.9</td>
</tr>
<tr>
<td>BAY</td>
<td>8</td>
<td>10.1 ± 7.2</td>
</tr>
<tr>
<td>BAO</td>
<td>12</td>
<td>10.1 ± 6.6</td>
</tr>
</tbody>
</table>

* Mean ± SD.
<sup>a</sup> Sacrificed for analysis of the surface phenotypes and viral antigens on the cells.

transplantation, when donors would have been 8 mo old and recipient mice either 8 or 12 mo old, the analyses of H-2 antigens on the thymus cells showed only H-2 antigens characteristic of the donors. No morphological evidence of graft-versus-host reaction was encountered in these chimeras. The mice transplanted with either AKR or C57BL/6J cells were raised in a laminar flow environment and had remained in apparent good health until sacrificed. Fully allogeneic bone marrow chimerism was regularly achieved after complete elimination of Thy 1.2-positive cells from the inoculum.

The chimeric state is fully allogeneic, stable, and sustained for at least 7 mo (Table 1). In Table 2 are recorded comparative thymic weights at various ages in AKR mice and at various intervals when sampled in the bone marrow-transplanted mice of AAY, AAO, BAY, and BAO groups. It will be seen from the table that, except for the AKR mice and mice receiving AKR marrow transplants, during periods when leukemia was present and developing, the size of the thymuses of mice of the several groups did not differ greatly from one another. In Table 3, observations analyze Thy-1 antigen, gp70 antigens, and other antigens encoded in MuLV present on thymocytes of AKR mice at various ages, C57BL/6J mice, leukemic AKR mice, and on thymocytes of lethally irradiated AKR mice following syngeneic or allogeneic bone marrow transplantation. Twenty-five thousand thymus cells from each mouse were the background counts for AKR mice for each antigen analyzed. Fluorochrome isothiocyanate was very low. The thymocytes of AKR and C57BL/6J mice showed the appropriate Thy 1.1 or Thy 1.2 antigens and low counts for alternative phenotype. Thymocytes of C57BL/6J mice express gp70 antigens in a middle range for thymocytes as did thymocytes from 2-mo-old AKR mice. In older nonleukemic or leukemic AKR mice, the gp70 antigen was detected on a higher percentage of thymus cells, and this disproportion increased after development of leukemia. By contrast, the proportions of cells expressing these antigens did not increase with aging in C57BL/6J mice.

Antibody 35/56, which recognizes a determinant coded in ecotropic and dualtropic MuLV, was not expressed significantly on C57BL/6J thymus cells but was identified on 28.85% of thymus cells of 2-mo-old AKR mice. The antigen recognized by the 35/56 antibody was present with increasing frequency and increasing intensity on thymus cells of AKR mice as they age. This antigen is present in high intensity on virtually all leukemic cells.

The 16-C1 antibody, which recognizes an antigen expressed by ecotropic and some dualtropic viruses was identified on 17.07% of C57BL/6J thymocytes, 30.66% of thymocytes of 2-mo-old AKR mice. The antigen recognized by the 35/56 antibody was present with increasing frequency and increasing intensity on thymus cells of AKR mice as they age. This antigen is present in high intensity on virtually all leukemic cells.

The 16-B7 antibody, which detects an antigen characteristic of the ecotropic gp70 molecule reacted little with C57BL/6J thymocytes (9.37%), a greater percentage of AKR thymus cells (24.12% at 2 mo), and on a predominance of thymus cells in leukemic AKR mice (86.70%).

G<sub>ep</sub> antibody, which detects gp70 MuLV-encoded antigens of xenotropic viruses was positive on a low proportion of thymus cells of C57BL/6J (8.14%) and of young 2-mo-old AKR mice. G<sub>ep</sub> antibody was, however, expressed in increasing proportions in older AKR mice (59.04%) and leukemic AKR mice (94.10%).

Comparison of the thymus cell phenotypes in unmanipulated mice and lethally irradiated reconstituted mice showed that none of the AKR mice reconstituted by transplantation with allogeneic C57BL/6J bone marrow cells developed morphological evidence of leukemia, and none showed graft-versus-host reaction clinically or at autopsy. In separate experiments (data not shown), however, a high proportion (80%) of AKR mice treated similarly but grafted with intact marrow cells from which T-lymphocytes had not been removed died of graft-versus-host disease. The irradiated AKR mice transplanted with allogeneic C57BL/6J marrow purged of Thy 1.2-positive postthymic cells had a Thy 1.2-positive thymic cell phenotype appropriate to C57BL/6J donors and no evidence of cells with the Thy 1.1 phenotype of the recipient among their thymocytes. AKR mice reconstituted with syngeneic marrow cells expressed Thy 1.1 and virtually no Thy 1.2 antigen on their thymocytes. These findings support obser-
LEUKEMIA VIRUS ANTIGENS ON MARROW CHIMERA CELLS

Table 3
Percentage of thymocytes with positive fluorescence staining for different antigens

<table>
<thead>
<tr>
<th>Mice</th>
<th>Recipient</th>
<th>Donor</th>
<th>Thy 1.1</th>
<th>Thy 1.2</th>
<th>gp70</th>
<th>16-Chloro</th>
<th>Gp85/80</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR</td>
<td>Control:&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td>1.95</td>
<td>1.87</td>
<td>1.91</td>
<td>1.68</td>
<td>1.24</td>
</tr>
<tr>
<td>AKR&lt;sub&gt;C57BL/6J&lt;/sub&gt;</td>
<td>2</td>
<td>3.58</td>
<td>98.38</td>
<td>47.68</td>
<td>2.82</td>
<td>17.07</td>
<td>9.37</td>
</tr>
<tr>
<td>AKR&lt;sub&gt;trans&lt;/sub&gt;</td>
<td>2</td>
<td>93.73</td>
<td>2.00</td>
<td>60.25</td>
<td>28.85</td>
<td>30.66</td>
<td>24.12</td>
</tr>
<tr>
<td>AKR&lt;sub&gt;trans&lt;/sub&gt;</td>
<td>7</td>
<td>94.03</td>
<td>20.53</td>
<td>94.81</td>
<td>75.69</td>
<td>72.62</td>
<td>ND</td>
</tr>
<tr>
<td>AKR&lt;sub&gt;trans&lt;/sub&gt;</td>
<td>10</td>
<td>98.51</td>
<td>28.38</td>
<td>98.50</td>
<td>98.50</td>
<td>98.50</td>
<td>86.70</td>
</tr>
<tr>
<td>AAY</td>
<td>8</td>
<td>8</td>
<td>97.15</td>
<td>6.21</td>
<td>56.10</td>
<td>19.77</td>
<td>17.17</td>
</tr>
<tr>
<td>AAO</td>
<td>12</td>
<td>9</td>
<td>90.98</td>
<td>18.74</td>
<td>99.51</td>
<td>86.40</td>
<td>85.65</td>
</tr>
<tr>
<td>BAY</td>
<td>8</td>
<td>8</td>
<td>97.90</td>
<td>70.15</td>
<td>50.12</td>
<td>56.46</td>
<td>41.68</td>
</tr>
<tr>
<td>BAO</td>
<td>12</td>
<td>9</td>
<td>96.82</td>
<td>97.83</td>
<td>96.56</td>
<td>93.46</td>
<td>86.66</td>
</tr>
</tbody>
</table>

<sup>a</sup> Background count of the percentage of cells in the absence of any fluorescein-tagged antibodies.

<sup>b</sup> AKR<sub>trans</sub>, AKR/J mice at the age of 2 mo; AKR<sub>trans</sub>, AKR/J mice at the age of 7 mo; ND, not determined; AKR<sub>trans</sub>, AKR macroscopically leukemic mice at the age of 9 to 10 mo.

Table 4
Microscopic examination of leukemia development at the time of death or until observation of 15 mo

The appearance of microscopically evident leukemia in untreated AKR/J mice and in AKR mice after syngeneic or allogeneic bone marrow transplantation is shown.

<table>
<thead>
<tr>
<th>Mice</th>
<th>No. of animals</th>
<th>%</th>
<th>Age of recipients (mo)</th>
<th>Age of donors (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR</td>
<td>54</td>
<td>100</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>AAY</td>
<td>18</td>
<td>100</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>AAO</td>
<td>18</td>
<td>100</td>
<td>3.3</td>
<td>9.3</td>
</tr>
<tr>
<td>BAY</td>
<td>18</td>
<td>0</td>
<td>No leukemia (&gt;15)</td>
<td>No leukemia (&gt;15)</td>
</tr>
<tr>
<td>BAO</td>
<td>18</td>
<td>0</td>
<td>No leukemia (&gt;19)</td>
<td>No leukemia (&gt;15)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Two mice died (2 of 18 mice in this group) from an apparent infection of the liver; both, however, had clear evidence of leukemia at autopsy.

vations from H-2 typing presented in Table 1 and show that the allogeneic chimera was complete. In striking contrast to the thymocytes of the C57BL/6J donor strain, the fully allogeneic BAY or BAO chimeric mice had large proportions of thymocytes with antigens on their cell surface that reflect eco-, dual-, or xenotropic murine leukemic viruses. Further, these antigens are expressed in a pattern similar to that seen with thymus cells of untreated AKR mice. The findings indicate that stem cells of C57BL/6J origin develop in the environment of AKR recipients and express MuLV antigens as they develop to thymocytes, C57BL/6J mice, leukemic AKR mice, and in lethally irradiated AKR mice after syngeneic or allogeneic bone marrow transplantation are shown.

The proportion of thymocytes which stained intensively for gp70 antigen increased with age in AKR mice and was high in the leukemic AKR mice. AAY and AAO mice had a staining frequency of very brightly staining cells observed that was found in the AKR mice have virus antigens at their surface and in an intensity at least comparable to controls, but leukemia does not develop in these cells. Chart 1 presents observations on expression of fluorescence intensity of the different antigens. It can be seen that, in the AKR mice, the Thy 1.1 antigen expression increases in intensity with age, and a rather striking increase of more intensively staining cells appears in the thymus when leukemia develops. In the allogeneic chimeras, BAY and BAO, the proportion of cells staining intensively for Thy 1.1 antigen remains very low and contrasts strikingly to the higher proportion of bright staining thymus cells in the AAO mice. The Thy 1.2 antigen expression was minimal or absent at all ages in AKR and in AKR mice irradiated and reconstituted at either a younger or an older age with cells from a syngeneic donor (AAY and AAO mice). In BAY and BAO mice, however, almost all thymocytes stained brightly for Thy 1.2 antigen.

The proportion of thymocytes which stained intensively for gp70 antigen increased with age in AKR mice and was high in the leukemic AKR mice. AAY and AAO mice had a staining pattern like that of unmanipulated nonleukemic mice. AAO and BAO mice developed a similar staining intensity for gp70 antigens, but BAO thymocytes tended to have even more of the brightly staining cells than did the AAO mice. For the antibodies 35/56, 16-C1, 16-B7, and 9<sub>ERALD</sub>, the animals showed an increased intensity of fluorescence after transplantation of bone marrow to older syngeneic or allogeneic recipients (AAO and BAO), compared with the instances where marrow was transplanted to younger animals (AAY and BAY), regardless of whether the cells were in a syngeneic or allogeneic environment. In none of these groups of mice was the extremely high frequency of very brightly staining cells observed that was found in mice with advanced leukemia. Strikingly, the fluorescence intensity with almost all of the antibodies against antigens coded in...
virus was very high in BAO mice, even though these mice did not develop evidence of leukemia. Further, mice from the same transplantation experiments survived up to 15 mo in both BAY and BAO groups after bone marrow transplantation without developing leukemia. AAY mice did not survive longer than 8 mo, and AAO, not longer than 9 mo after bone marrow transplantation. In addition, all mice of these groups developed the leukemia-lymphoma complex.
LEUKEMIA VIRUS ANTIGENS ON MARROW CHIMERA CELLS

DISCUSSION

Leukemia viruses that infect mouse cells are ecotropic. Those that infect preferentially cells of xenogeneic species are called xenotropic viruses. Hartley et al. (11) identified the dualtropic mink cell focus-forming MuLV which arises de novo in individual mice by recombinant events involving viral env gene sequences. Insights into virus-host interactions became possible through selective inbreeding of the AKR strain for high incidence of thymic leukemia-lymphoma complex (8, 22). A prerequisite for development of spontaneous leukemia is inheritance of genes which encode ecotropic MuLV (5, 24, 29, 38, 39).

The experiments reported herein, and prior studies (6, 9, 15, 20, 34, 43), show that lethal total-body irradiation followed by transplantation of bone marrow cells treated with anti-Thy-1 antibody plus complement permits establishment of long-lived fully allogeneic chimeric mice (33, 43). In several experiments where the mice were maintained in a conventional environment, fully allogeneic chimeras gradually sickened and died (31). These findings are similar to those of Rayfield and Brent (37). Such animals have involuted thymii and fibrotic spleens but do not show the other characteristic histopathology of graft-versus-host reaction (20). Such wasting AKR chimeric mice do not develop leukemia (20), but it is difficult to evaluate this influence because of gross deficiency of lymphoid cells in thymus and spleen. If, however, as in the present experiments, the fully allogeneic chimeras were maintained under conditions where they are not exposed to pathogens by keeping them in strict isolation using laminar flow methodology, very long-lived fully allogeneic chimeras may be established. In the present experiments, the thymus of the chimeric animals, although somewhat small, was found to contain abundant lymphoid cells, virtually all of which carried the H-2a antigen of the donor strain and lacked the H-2b antigen of the recipient strain. Correspondingly, the fully allogeneic chimeric state was reflected in analyses of the Thy-1 antigens on the thymus cells. Virtually all thymocytes of AKR mice transplanted with C57BL/6J marrow expressed the Thy 1.2 phenotype characteristic of C57BL/6J mice. These long-lived chimeras remained in good health until at least 15 mo of age. In the BAO group, however, the thymocytes expressed each of the gp70 epitopes on even a high percentage of lymphoid cells. However, neither the BAO nor BAY mice developed leukemia, even though the mice were followed to considerably older ages than the unmanipulated AKR mice or AKR mice transplanted with syngeneic AKR marrow cells, all of which developed leukemia prior to 11 mo of age. We can conclude that infection with ecotropic, dualtropic, and xenotropic forms of the MuLV occurs in the thymus cells derived from the resistant donor phenotype, but leukemia need not develop. It has been suggested from studies which link a regular temporal association of viral amplification and development of leukemia that viral amplification is involved in the process of leukemogenesis (13, 14). An important question which must be raised from present data is whether this association is as critical for development of the lymphoma-leukemia state as seemed from past analysis. Certainly other studies to quantify the viruses involved will be necessary before understanding of these issues can be achieved. But already in the experiments described, evidence that a high degree of amplification of all forms of MuLV occurs in BAO mice is at hand, yet these animals have resisted development of leukemia-lymphoma. This finding suggests that, if viral amplification is pathogenetically important, as it seems to be, in development of leukemia, it may be an event that, although necessary for development, is not sufficient. Perhaps it must be accompanied by an additional event which does not occur or is dampened in the allogeneic chimeras BAO and BAY. This hypothetical event occurring in the infected cells may be crucial to either transformation to the leukemic state or alternatively to expansion of a population of transformed cells. Thus, the cells of BAY and BAO mice appear to be infected with each of the forms of the MuLV that have been associated with leukemia but do not develop the pathology of the leukemia-lymphoma complex. These findings seem relevant to considerations of mechanisms of resistance to the influence of the MuLV. For example, the Fv-1a gene of the C57BL/6J mice is considered to confer resistance to infection with MuLV, while Fv-1b of the AKR mice seems to confer susceptibility to these viruses (16, 23, 28, 38, 39). In our studies where the cells bearing the Fv-1a gene are housed in a host environment that reflects the Fv-1b genetic influence, the C57BL/6J derived cells have been shown to be susceptible to virus infection but do not develop leukemia. In this context, it will be important to know whether the Fv-1a gene is expressed normally in the C57BL/6J derived cells in the allelo-
neic environment. Further studies will be needed to clarify this issue.

Ihle et al. (12) have previously stressed that a mechanism additional to the virus infection is essential if leukemia is to develop, and they have related this to cell-mediated immune responses to MuLV antigens. The present studies do not analyze this issue but are not incompatible with such a concept, and further experiments to test this possibility based on the chimeric preparations we have designed are clearly in order. The latent period following infection and development of leukemia might involve appearance of a cellular immune reaction which did not occur in leukemia-resistant CBA/N mice that became infected with the virus but did not develop leukemia (17). As an example, such immunologically based cellular events could be involved in development of leukemia by providing growth factors essential to the amplification of the malignant cell population. Additional studies, perhaps more direct than those used herein, to define better and with more parameters the virus replication which we have observed seem very much in order and should be carried out. Furthermore, it is still not known whether general viremia by ecotropic MuLV is essential to leukemogenesis in AKR mice or whether only a specific cell type must be infected, e.g., a bone marrow-derived stem cell, which differentiates later to prothymocytes. Furthermore, a question still to be answered is why recombinant viruses are not observed prior to 6 mo of age, whereas viremia by ecotropic MuLV is established as early as 1 mo of age. Future experiments must address the question of whether antigen amplification has any significance in leukemogenesis other than representing a reflection of infection of thymocytes by recombinant viruses. It is still unknown whether the recombinant env genes simply facilitate recognition of thymocyte receptors or whether they mediate in some other way infection of target cells. Furthermore, very little is known about the interaction of MuLV with the thymocyte subpopulations. Flow cytometry used in present studies and cell sorting which is already highly developed (45) coupled with allogeneic bone marrow transplantation may prove to be incisive tools to dissect these issues further. Such approaches could increase our understanding of leukemogenesis by MuLV and perhaps improve our perspectives on tumor biology in general. Furthermore, the hypothesis proposing that transformation is attributable to expression of certain host cellular genetic sequences under the direction of a viral promoter in the large terminal repeat (2, 44) region, or the specification proposing that transformation is attributable to expression of certain host cellular genetic sequences under the direction of a viral promoter in the large terminal repeat (2, 44) region, or the premature termination of the thymus development might be answered by using the model of accelerated infection of O'Donnell et al. (32) in which intrathymic injection of cloned isolates of dualtropic mink cell focus-forming MuLV might allow synchronization of the events of leukemogenesis in AKR mice. Such experiments could be especially revealing if coupled with allogeneic marrow transplantation as described herein. Attempts to detect activation of cellular transforming genes during leukemogenesis and relating these genes to influences of resistance genes might then be realistic.

REFERENCES

LEUKEMIA VIRUS ANTIGENS ON MARROW CHIMERA CELLS

31. O'Donnell, P. V., and Nowinski, R. C. Serological analysis of antigenic deter-
minants on the env gene products of AKR dualtropic (MCF) murine leukemia
32. O'Donnell, P. V., Nowinski, R. C., and Stockert, E. Amplified expression of
murine leukemia virus (MuLV)-coded antigens on thymocytes and leukemia
cells of AKR mice after infection by dualtropic (MCF) MuLV. Virology, 119:
33. Onoe, K., Fernandes, G., and Good, R. A. Humoral and cell-mediated immune
responses in fully allogeneic bone marrow chimeras in mice. J. Exp. Med.,
34. Onoe, K., Fernandes, G., and Good, R. A. Humoral and cell-mediated immune
responses in fully allogeneic bone marrow chimera in mice. J. Exp. Med., 151:
W. A. Novel leukemogenic retroviruses isolated from cell line derived from
36. Pinter, A., Honnen, W. J., Tung, J. S., O'Donnell, P. V., and Hammerling, U.
Structural domain of endogenous murine leukemia virus gp70s containing
specific antigenic determinants defined by monoclonal antibodies. Virology,
37. Rayfield, L. S., and Brent, L. Tolerance, immunocompetence, and secondary
disease in fully allogeneic radiation chimeras. Transplantation. (Baltimore), 36:
183–189, 1983.
38. Rowe, W. P., and Hartley, J. W. Genes affecting mink cell focus-inducing
(MCF) murine leukemia virus infection and spontaneous lymphoma in AKR F1
39. Rowe, W. P. Genetic factors in the natural history of murine leukemia virus
41. Stephenson, J. R. Molecular Biology of RNA Tumor Viruses. New York:
mouse antibodies as probes for polymorphism in murine leukemia viruses.
43. Tanaka, T., Obata, Y., Fernandes, G., Onoe, K., Stockert, E., and Good, R. A.
Prevention of leukemia in lethally irradiated AKR mice by CBA/H marrow
44. Tsichlis, P. N., and Coffin, J. M. Role of C region in relative growth rates of
endogenous and exogenous avian oncoviruses. Cold Spring Harbor Symp.
analysis by flow cytometry to define the site of appearance of leukemia in AKR

CANCER RESEARCH VOL. 45 DECEMBER 1985

6435
Expression of Antigens Coded in Murine Leukemia Viruses on Thymocytes of Allogeneic Donor Origin in AKR Mice following Syngeneic or Allogeneic Bone Marrow Transplantation

Thomas P. U. Wustrow and Robert A. Good


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/45/12_Part_1/6428

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.