Induction of Leukemia Regression in Mice by Immunotherapeutic Transfer of T-Lymphocytes

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ABSTRACT

The erythroleukemia induced in susceptible mice by Friend virus (FV) is a progressive, lethal disease. A variant strain of Friend virus (regressing FV) produces a histopathologically identical leukemia except that the disease spontaneously regresses in 50% of leukemic mice. Normal T-cell and macrophage function are required for regression to occur and in animals that are going to regress, specifically reactive T-cells are found in the spleen. Passive transfer of sensitized T-cells from regressing FV immunized or regressed mice caused regression of the conventional lethal leukemia induced by FV. To expand the effector cell populations, characterize them and improve their therapeutic efficacy, sensitized T-cells were cultured \textit{in vitro}. The T-cells, isolated from regressed or immunized mice, were grown and expanded \textit{in vitro} with interleukin 2 and antigen (mitomycin C treated regressing FV-infected cell lines). The T-cells demonstrated high levels of \textit{in vitro} cytotoxicity against FV antigens but exhibited no blastogenic response to the same antigens. When fully FV-induced leukemic mice (14 days post virus inoculation; spleen weight, >0.75 g) were given one injection of $5 \times 10^6$ in \textit{vitro} cultured T-cells and no other treatment the mice experienced permanent regressions of their disease. From T-cell cultures depleted of specific cell populations with monoclonal antibodies, helper Lyt-1$^+$ cells were shown to be responsible for permanent regressions (cures), whereas cytotoxic Lyt-2$^+$ cells caused temporary leukemia remissions. This model thus provides an experimental system of highly effective passive cellular immunotherapy against an autochthonous, fully developed leukemia, requiring no adjunctive treatment for activity.

INTRODUCTION

The potential for use of T-cells in cancer therapy has received considerable attention (1, 2). This immunotherapeutic approach has the advantages of high immunological specificity with a minimal likelihood of significant toxicity and is based on the effector arm of the immune system most often implicated in resistance against the disease.

Immunotherapy with T-cells requires the ability to consistently develop large numbers of reactive cells that are capable of eliminating the tumor \textit{in vivo} as well as understanding of T-cell functions and interactions. The identification of a T-cell growth factor, IL-2,\textsuperscript{2} has made it possible to grow and expand tumor specific T-cells \textit{in vitro} (3, 4). IL-2-developed T-cell cultures have been used in immunotherapeutic trials \textit{in vivo} in several model experimental systems (5-7). The therapeutic efficacy of such cultured T-cells is, however, sometimes short-lived, has not been consistently effective against established tumors, and is often dependent on pre- or concurrent treatment of recipients with radiation or cytotoxic drugs.

In a model system developed in our laboratory, acute leukemia in mice induced by a variant strain of Friend virus (RFV) undergoes predictable, immunologically mediated spontaneous regression (8-10). Normal T-cell (11, 12) and macrophage (13, 14) functions are essential for regression to occur and in leukemic animals that are going to regress, specifically reactive T-cells are found in the spleen. To determine the potential for immunotherapeutic use of T-cells in causing leukemia regression, T-cells were cultured from regressed or immunized mice and were used to treat animals with the progressive lethal form of the disease induced by conventional strains of virus. We found that permanent regressions can be induced in leukemic animals by the transfer of cultured T-cells that are specifically reactive with virus-leukemia cell antigens. This immunotherapy was effective even in fully leukemic animals and required no concurrent or prior adjunctive treatment of the animals. This system thus provides a model for the highly efficacious use of T-cells in the immunotherapy of autochthonous cancer and the potential for developing an understanding of the mechanism of their effects.

MATERIALS AND METHODS

Mice. These experiments were carried out in the NIH/PLCR inbred mouse line. Inbred NIH/PLCR mice were originally obtained through the courtesy of the Veterinary Resources Branch, Division of Research Sciences, NIH and subsequently bred by brother/sister matings in our laboratory. Mice were checked in a coded blind fashion biweekly by spleen palpation for leukemia development and regression. We have previously shown that spleen weight determined by palpation is an accurate indicator for leukemic status (8).

Virus and Cells. The RFV strain of Friend virus was originally isolated in our laboratory and is maintained by serial passage of cell-free virus stocks prepared from spleens of leukemic mice (20% wt/vol in PBS) as previously described (15) and stored in sealed ampules at $-70^\circ$C. Mice were inoculated i.p. with 0.5 ml of PBS containing approximately 100 times the dose of virus that produces leukemia in 50% of inoculated mice in 25 days. The FV-A strain was obtained from Dr. C. Friend and is maintained in our laboratory by serial passage of cell-free homogenates as described above; the CFV strain of Friend virus is an N-tropic virus originally obtained from Dr. C. Friend, and induces an identical, progressive, and lethal erythroleukemia.

Fibroblasts infected with the RFV or FV complex were prepared as previously described (10, 16) using cell lines derived in our laboratory from the NIH/PLCR mouse strain or the BALB/c/3T3 cell line. The cell line NIH/RF/23 was routinely used as stimulator in T-cell culture procedures. These cells are NIH/PLCR fibroblasts that are infected with...
RFV and express gp70 and other viral proteins on their surface (10). We also utilized BALB/c/3T3s that are infected with the MuLV component of FV, termed BALB/c/MuLV.

**Antisera.** Mouse monoclonal anti-Thy-1.2, anti-Lyt-1.2, and anti-Lyt-2.2 antibodies were obtained from Becton, Dickinson and Co., Oxnard, CA. Goat anti-mouse immunoglobulin was purchased from Antibodies Inc., Davis, CA. Rabbit anti-macrophage serum was prepared and characterized in our laboratory as described previously (13). Low toxicity rabbit complement was purchased from Cederlane Laboratories, Westbury, NY. Guinea pig complement was purchased from Colorado Serum Co., Denver, Co. Lyophilized complement was reconstituted with sterile distilled water and stored in sealed ampules at -70°C until used.

**Immunization of Animals.** NIH/PLCR mice were immunized with the NIH/RFB 23 cell line (17). Cells were UV irradiated under a GE 15T8 bulb at 100 ergs/s/cm² for 13 min. The cells were washed 3 times and 5 x 10⁶ cells in 0.5 ml of PBS were inoculated s.c. in the following sites: nape of neck; both axillae; and both inguinal areas (0.1 ml/site). Mice received 6 weekly injections, then rested for at least 2 wk. The animals were then boosted by i.p. injection with 5 x 10⁶ cells 3-4 days prior to removal of T-cells for in vitro culturing.

**Production and Testing of IL-2 Preparations.** The gibbon lymphoid cell line MLA 144, which was obtained from Dr. R. H. Neubauer, Frederick Cancer Research Facility, Frederick, MD, was routinely utilized for interleukin 2 production (18). Briefly cells were seeded at 4 x 10⁶ cells/ml in RPMI 1640 plus 10% fetal calf serum and conditioned media were harvested after 3 days' incubation when the cell density was 1-2 x 10⁹/ml. Supernatants were filtered and stored at -20°C. The activity of IL-2 preparations was tested in the IL-2 assay of Gillis et al. (19) using either cultured T-cells or the IL-2-dependent cell line, CTLL-2, which was obtained from Dr. K. J. Pennline, Georgetown University, Washington, DC.

**T-Cell Culture and Passive Transfer Assay.** Spleens from immunized or regenerated animals were teased and the suspensions adjusted to 5 x 10⁶ nucleated cells/ml. A 5-ml aliquot of these responder cells was incubated with 5 ml of mitomycin C-treated (250 µg/ml) stimulator cells at 1.25 x 10⁶ cells/ml and 5 ml of IL-2. The cultures were fed weekly with fresh IL-2 and mitomycin C-treated stimulator cells. Cultures of T-cells containing antigen and IL-2 were plated at an initial concentration of 5 x 10⁶ cells/ml and were split weekly when cells reached a density of 5 x 10⁷ cells/ml. T-cell growth in the presence of IL-2 was also determined as described previously (14).

Groups of test animals (at least 15 animals/group) were inoculated i.p. with 0.5 ml of PBS containing approximately 100 times the dose of virus that produces leukemia in 50% of inoculated mice in 25 days. We routinely used the CFV and FV-A strains of Friend virus which induce a uniformly progressive and lethal erythroleukemia. At 14 days post virus, leukemic mice were given one injection i.p. of 5 x 10⁶ cultured T-cells, unless otherwise indicated. Animals were palpated at least twice weekly for leukemic status. Disease status was confirmed by spleen weight, virus assays, and histopathology (8, 20).

**Chromium Release Cytotoxicity Assay.** Target cells were labeled with ⁵¹Cr (250 µCi/10⁷ cells) in RPMI 1640 plus 10% fetal calf serum, washed, and plated in U-bottom microtiter plate wells. Effector cells were added at either a 100:1 or 20:1 effector:target cell ratio. The plates were centrifuged at 350 x g for 10 min. After incubation at 37°C for 4 h, the plates were centrifuged again, 100 µl of the supernatant was removed, and released radioactivity was determined.

**Blastogenesis Assay.** As described by Enjuanes et al. (21), T-cells (5 x 10⁶ cells/well) and mitomycin C-treated stimulator cells (2.5 x 10⁹ well) in RPMI 1640 plus 2% fetal calf serum were plated in the microtiter plates. After incubation for 2-4 h at 37°C, 0.5 µCi of [³²]H-thymidine (specific activity, 2.0 Ci/mmol; New England Nuclear, Boston, MA) was added to each well. After 6-10 h the cultures were harvested onto glass fiber filter strips using a PHD Cell Harvester, Cambridge Technology, Cambridge, MA, and counted in a liquid scintillation counter.

**Interferon Assay.** We utilized the standard vesicular stomatitis virus assay (22) for quantitation of interferon present in conditioned media from T-cell cultures. Briefly 0.5 ml of the test interferon preparation was placed on confluent monolayers in L-cells in 24-well Costar plates. After 24 h incubation, a predetermined dilution of vesicular stomatitis virus was added. After 1 h adsorption, the medium was removed and nutrient agar, 0.4 ml/well, was added. After 48 h incubation, the plates were stained with neutral red and plaques were counted. Standards of mouse α-, β-interferon (specific activity, 4.4 x 10⁶ units/mg protein; ENZO Biochem, Inc., New York, NY) and human γ-interferon (specific activity, 1 x 10⁶ units/mg protein; Meloy Laboratories, Springfield, VA) were utilized as controls. Interferon was characterized as γ-interferon by its instability at pH 2 for 4 h, as compared with a known standard of fibroblast interferon.

**Antibody-mediated Suppression of CFU-C Colony Formation.** Suppression of CFU-C colony formation by virus-specific antisera was determined as described previously (14).

**RESULTS**

**Passive Transfer of T-Cell-enriched Lymphocytes.** Based on the findings that intact T-cell function is necessary for regression to occur and that regenerated or immunized animals express potent T-cell-mediated immunity against virus and leukemia cell antigens (11, 12), we determined whether FV leukemic mice could be made to regress by the transfer of T-cell-enriched lymphocytes isolated from the spleens of immunized or regenerated mice. At 14 days post virus inoculation, leukemic mice were treated with 1 x 10⁷ nylon wool-enriched lymphocytes isolated from immunized mice. Approximately 80% of these lymphocytes were lysed by monoclonal anti-Thy-1.2 plus complement. By 14 days after virus, all of the recipient animals had become extensively leukemic with spleen weights >750 mg, heptomegaly, and viremia. Animals were palpated biweekly to determine their leukemic status. As shown in Chart 1, a large proportion of the leukemic animals experienced disease regression as a result of treatment with T-cells from immune mice. In most of the animals, the disease recurred between 28 and 35 days after regression, although in about one-third, the disease was permanently eliminated. At 98 days postvirus, between 10 and 20% of the animals were still leukemic, the remaining 80% of the animals had achieved complete regression.

**Antibody-mediated Suppression of CFU-C Colony Formation.** Suppression of CFU-C colony formation by virus-specific antisera was determined as described previously (14).

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and 20% of the animals treated with immune cells in individual experiments died of leukemia. No regressions were observed in the control untreated animals. By day 98 postvirus, 40 to 70% of the animals in this group had died, with the other animals remaining chronically leukemic and viremic. Leukemic animals treated with enriched T-cells from normal mice experienced a smaller proportion of only temporary regressions. FV-A leukemic mice treated with enriched T-cells from spontaneously regressed mice (RFV-infected animals) experienced the same high proportion of regressions and significant number of permanent cures as did animals treated with immune cells (data not shown).

In Vitro Culture of T-Cells from Regressed or Immunized Mice. To determine whether the reactive T-cells from immunized or regressed mice could be expanded and characterized in vitro for immunotherapeutic use, we utilized established T-cell culturing techniques (3, 4). As shown in Table 1 and in accord with the results of others (23), the spleen cells isolated directly from immunized or regressed mice were not cytotoxic to the immunogen or virus-infected cells.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>In vitro activity of spleen cells from immune and RFV-regressed animals</th>
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<tbody>
<tr>
<td>Effector cells</td>
<td>Target cells</td>
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<tr>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>Immune spleen cells</td>
<td>NIH/RFB23</td>
</tr>
<tr>
<td></td>
<td>NIH/3T3</td>
</tr>
<tr>
<td></td>
<td>BALB/c/MuLV</td>
</tr>
<tr>
<td></td>
<td>BALB/c/3T3</td>
</tr>
<tr>
<td>RFV-regressed spleen cells</td>
<td>NIH/RFB23</td>
</tr>
<tr>
<td></td>
<td>NIH/3T3</td>
</tr>
<tr>
<td></td>
<td>BALB/c/MuLV</td>
</tr>
<tr>
<td></td>
<td>BALB/c/3T3</td>
</tr>
<tr>
<td>Normal spleen cells</td>
<td>NIH/RFB23</td>
</tr>
<tr>
<td></td>
<td>NIH/3T3</td>
</tr>
<tr>
<td></td>
<td>BALB/c/MuLV</td>
</tr>
<tr>
<td></td>
<td>BALB/c/3T3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Plated at 100:1, effector:target cell ratio.
<sup>b</sup> Mean ± SD of 4 replicate samples calculated as

\[
\frac{\text{Experimental cpm - spontaneous cpm}}{\text{Maximum cpm - spontaneous cpm}} \times 100
\]

In Vivo Activity of Cultured T-Cells. To test whether cytotoxic T-cells can cause regression of chronic lethal FV (nonregressing conventional virus)-induced erythroleukemia, frankly FV leukemic NIH/PLCR mice were given one injection of 5 × 10⁶ syngeneic cultured T-cells at 14 days postvirus. At the time that treatment was initiated, these animals had fully developed disseminated leukemia (spleen weight >0.75 g, hepatomegaly, viremia). As shown in Chart 2, animals treated with CTL/RFB experienced a significant percentage of regressions and in most of these animals the disease did not recur. Animals given normal spleen cells experienced fewer and only temporary remissions. The same immunotherapeutic effect was obtained in seven independent experiments with freshly derived 14-day CTL/RFB cultures. Treatment of leukemic mice with CTL/MuLV, T-cells cultured against normal fibroblast cell lines, or normal T-cells cultured in IL-2 alone did not result in a significant number of regressions (data not shown).

To determine if a greater or more sustained effect could be
achieved with further treatment, fully leukemic animals were given two injections of \(5 \times 10^6\) CTL at 14 and 21 days postvirus. As shown in Chart 3, in a significant percentage of leukemic mice given two inoculations of either CTL/RFB or normal spleen cells the disease had regressed by day 35 postvirus. By 100 days postvirus, only in animals given CTL/RFB did the disease remain regressed; in animals given normal spleen cells the leukemia had mostly recurred. When leukemic animals were given two injections of T-cells cultured against normal syngeneic fibroblast cell lines, the leukemia in some of the animals regressed but quickly recurred, as observed with animals given normal spleen cells.

The reactive T-cells in the experiments described above were cultured for 14 days in the presence of stimulator cells and interleukin 2. It has been shown by Gillis and Watson (27) that T-cell populations can change functionally with time in culture. We thus examined the therapeutic effectiveness of CTL/RFB that had been carried in culture for 60 days. As shown in Table 3, the 60-day CTL/RFB remained significantly cytotoxic for the NIH/RFB cell line. Despite their in vitro activity, the T-cells from 60-day cultures were incapable of causing regression of FV leukemia (data not shown).

Characterization of T-Cell-induced Regressed Mice. In RFV-regressed mice, the spleen and liver return to normal size and architecture (20) and infectious virus disappears from blood and tissue. Fully disease-regressed mice are histopathologically indistinguishable from normal animals. Productive virus infection of macrophage progenitors in the bone marrow has also been shown to correlate with leukemic status: regressed and regressor animals (leukemic animals that will experience regression) have uninfected bone marrow CFU-C, whereas progressor mice (leukemic animals that will not experience regression) have infected CFU-C (14).

To characterize the regressions induced in leukemic mice by CTL/RFB, their histopathological, hematological, and virological features were compared with leukemic spontaneously (RFV)-regressed animals, and normal controls. Spleen size and appearance, virus titers, and other manifestations of the disease returned to normal in the CTL-regressed animals. Productive virus infection of CFU-C was determined by depletion using monospecific antibody directed against gp70 (14), the major viral envelope protein expressed on infected cells. As shown in Table 4, CFU-Cs in FV-leukemic animals induced to regress permanently with CTL/RFB were not productively infected with virus. The CTL-regressed mice were thus similar to animals in which the disease spontaneously and completely regressed when RFV was used as the inducing virus. When temporary regressions were induced with two injections of normal spleen cells, bone marrow CFU-C remained productively infected with virus, in concert with the observation that the disease would rapidly recur in most of these animals.

As described above, CTL/RFB themselves did not demonstrate a significant blastogenic response to virus antigens. Rosenberg and Rosenberg (28), using the Friend virus FBL-3 lymphoma system, found that T-cell cultures rarely exhibit a proliferative response to syngeneic tumor but that T-cells from treated animals were proliferative in response to antigen. We determined therefore whether spleen cells directly isolated from mice in which the disease was induced to regress with CTL/RFB exhibited a proliferative response to virus-infected cells. As shown in Table 5, spleen cells from CTL-induced disease-regressed animals demonstrated a significant proliferative response specific to the original stimulator cell used in immunization in vivo and in vitro culture. This activity was consistently observed in all CTL-induced disease-regressed animals. Spleen

![Graph](image-url)

**DAYS POST VIRUS**

Chart 3. Treatment of FV-leukemic mice with two injections of \(5 \times 10^6\) cultured T-cells each at 14 and 21 days post-virus inoculation. Leukemic animals (15-20/group) received CTL/RFB (C), normal spleen cells (A), or no treatment (O). The incidence of regression observed in the CTL/RFB group was significantly different from the no treatment group, at \(P < 0.01\) by Kruskal and Wallis' test. No significant difference was observed between the CTL/RFB and normal spleen cell groups.

### Table 3

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>Target cells</th>
<th>% chromium release</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL/RFB</td>
<td>NIH/RFB23</td>
<td>22 ± 6^c</td>
</tr>
<tr>
<td></td>
<td>NIH/3T3</td>
<td>12 ± 2</td>
</tr>
<tr>
<td></td>
<td>BALB/c/MuLV</td>
<td>5 ± 2</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Normal spleen cells</td>
<td>NIH/RFB23</td>
<td>4 ± 3</td>
</tr>
<tr>
<td></td>
<td>NIH/3T3</td>
<td>5 ± 4</td>
</tr>
<tr>
<td></td>
<td>BALB/c/MuLV</td>
<td>2 ± 1</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>2 ± 1</td>
</tr>
</tbody>
</table>

^a Plated at 100:1, effector:target cell ratio.  
^b Mean ± SD of 4 replicate samples calculated as

\[
\text{Experimental cpm} - \text{spontaneous cpm} \times 100
\]

\[
\text{Maximum cpm} - \text{spontaneous cpm}
\]

^c Significantly different from the same target cell plus normal spleen cells (raw cpm data), at \(P < 0.001\) (Student's t test).

### Table 4

<table>
<thead>
<tr>
<th>Animals</th>
<th>Treatment</th>
<th>No. of CFU-C/10^5 bone marrow cells</th>
<th>% colony reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT/LRFB regressed</td>
<td>Anti-gp70 + C'</td>
<td>98 ± 6°</td>
<td>9</td>
</tr>
<tr>
<td>Normal spleen cell regressed</td>
<td>Anti-gp70 + C'</td>
<td>96 ± 11</td>
<td>0</td>
</tr>
<tr>
<td>RFV regressed</td>
<td>Anti-gp70 + C'</td>
<td>82 ± 8</td>
<td>40</td>
</tr>
<tr>
<td>FV leukaemic</td>
<td>Anti-gp70 + C'</td>
<td>74 ± 3</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>Anti-gp70 + C'</td>
<td>78 ± 7</td>
<td>59</td>
</tr>
</tbody>
</table>

^c', complement.  
^d Mean ± SD.  
^e Significantly different from control, \(P > 0.001\) (Student's t test).
T-cell subsets and regression. To identify the Lyt subpopulation(s) responsible for regression in FV-leukemic mice, we tested in vivo activity of depleted populations of the cultured T-cells, CTL/RFB. As shown in Chart 4, transfer of CTL/RFB treated with complement and either monoclonal anti-Lyt-1.2 or anti-Lyt-2.2 resulted in regression; however, in all of the disease-regressed animals given cells treated with anti-Lyt-1.2 the disease recurred. In animals given CTL/RFB treated with complement only or with anti-Lyt-2.2 and complement, the disease regressed and did not recur. Antiserum treatment resulted in cytotoxicity of 20, 50, and 51% in complement-only, anti-Lyt-1.2-treated, and anti-Lyt-2.2-treated samples, respectively. The samples were adjusted so that equal numbers of live cells were inoculated in each group of leukemic mice.

The specific cytotoxicity against NIH/RFB observed with CTL/RFB was abrogated in cell populations treated with anti-Lyt-2.2, whereas treatment with anti-Lyt-1.2 had no effect. No significant blastogenic activity was obtained from either untreated CTL/RFB or antibody-treated populations of T-cells (data not shown).

Production of interferon by T-cell cultures. Murine γ-interferon is produced by T-cells and has been shown to augment many T-cell and other immunological functions (29, 30). Forni and Giovarelli (31) have implicated helper cells and γ-interferon in causing in vivo regression of methylcholanthrene-induced sarcomas. To determine whether in our system interferon is produced by the reactive T-cell cultures, media from the cultures were assayed for interferon at day 14 when the cells were used for treatment of leukemic mice. Media from six independently derived CTL/RFB cultures contained interferon, from 10 to a maximum of 433 units/ml, whereas media from cultured normal T-cells contained <10 units/ml. (Titers are expressed as the reciprocal of the supernatant dilution causing a 50% reduction in the number of plaques compared with the controls and are given per ml of supernatant from cultures containing 5 × 10⁶ cells/ml, the dose used for in vivo immunotherapy.) After treatment at pH 2, the interferon activity in the supernatants was unchanged, suggesting that the detectable interferon was not γ-interferon (Table 6).

### DISCUSSION

Several studies have shown that T-cells specifically activated in vitro against tumor cell antigens possess antitumor effects in vivo (5–7). These effects, however, are often short-lived and usually require pretreatment of recipients with cytotoxic drugs or irradiation. We report here permanent regression of disease in fully leukemic mice following a single transfer of T-cells specifically sensitized to antigens on virus-infected cells and expanded and stimulated in vitro.

Mice inoculated with 100 spleen focus-forming units of RFV uniformly develop extensive leukemia within 7–14 days. The disease is characterized by massive splenomegaly, hepatomegaly, viremia, and the presence of immature erythroid precursors in the peripheral blood (8) and is thus initially indistinguishable from the progressive lethal disease induced by the conventional strain of virus (32, 33). Between 4 and 8 wk after inoculation with RFV, the disease spontaneously regresses in 50% of the
leukemic mice. In disease-regressed mice, the spleen and liver return to normal size and architecture (20) and infectious virus disappears from the blood and tissues (8).

Intact T-cell and macrophage function are required for leukemia regression (11–14). Based on these results, we attempted to induce leukemia regression in conventional FV-infected animals with an acute disease, using direct passive transfer of T-cells from disease-regressed and immune mice. We found that specifically reactive cells were effective in therapy but that in the majority of disease-regressed animals, the disease eventually recurred.

To expand the cell populations responsible for induction of regression, to characterize them, and to improve their therapeutic efficacy, we utilized established techniques for in vitro culture of T-cells. Cells that were cytotoxic to targets productively infected with virus but not significantly proliferative in response to them were grown in these conditions. These cells when inoculated into fully leukemic mice caused permanent regression of the disease. No adjunctive treatment was required to achieve the response.

Animals in which the leukemia had been successfully treated with cultured T-cells expressed no histopathological remnant of the disease; spleen size and architecture returned to normal, virus was not detectable in plasma or tissue extracts, and hematopoietic progenitors infected with virus in the course of the disease were normal.

The identity of the antigens recognized in the immune response generated to ecotropic C-type viruses has been the subject of many studies. It has been shown by Plata et al. (34) that CTL sensitized against FV antigens recognize the major viral envelope protein gp70 and to a lesser extent the gag internal virion proteins p30 and p12. In the Moloney leukemia virus system, CTL recognize gp70 (21), while the T-cell proliferative response recognizes both gp70 and p12 in animals undergoing tumor regression (35). Also associated with tumor regression is a consistent humoral response against the virion envelope proteins, gp70 and p15(E). Lee and Ihle (36) have shown that in the virome highly leukemic strains of AKR mice, gp70-specific blastogenesis in vitro begins in the preleukemia stage, at about 5 mo of age. We believe that the specificity of the effector CTL/RFB in our system is directed against gp70, as this is the primary target of the humoral and cellular immune responses detected in spontaneous disease-regressed mice (10, 12, 14), but further studies will be required to establish this.

Identification and characterization of the lymphocyte subpopulations that mediate tumor regression are necessary for further development and understanding of successful immunotherapeutic methods. To date, there exists a diversity of results in this area. The Lyt-2* cytotoxic cell has been implicated in mediating the regression of allogeneic tumors (37) as well as skin allografts (38). The helper Lyt-1* cell has also been shown to have activity in both tumor and skin allogeneic systems (2). Others (1, 28) have implicated both Lyt-1* and Lyt-2* cell populations. In the FBL-3 tumor system (28), mice are cured only by combination of Lyt-1* and Lyt-2* cells. In the treatment of the Meth A sarcoma (2), the mixture of Lyt-1* and Lyt-2*-depleted cells was not capable of curing mice, but a cell of the Lyt-1*2* type was effective.

Using our model system, populations of cultured T-cells were depleted of specific Lyt subpopulations with specific monoclonal antisera. By this technique, Lyt-1* cells were found to cause permanent regressions, whereas Lyt-2* cells caused temporary remissions of the disease. A plausible explanation is that the Lyt-2* cytotoxic cells can kill off large numbers of leukemic cells leading to an initial remission but cannot sustain the effect or recruit host cells to the activity. Lyt-1* cells, possibly helper cells, could by recruiting host cells mount a more effective, more long-lasting response leading to cure. Other possibilities include interactions with suppressor functions, help in generating a humoral immune response, activation of macrophages, or production of factors directly affecting the leukemia.

Another mechanism by which passively transferred sensitized T-cells may cause leukemia regression is through the production of lymphokines (soluble mediators) that either directly or indirectly affect the disease (39). Among the lymphokines of interest in this regard are y-interferon, IL-2, interleukin 3, and granulocyte-macrophage colony-stimulating factor. Our results suggest that the effector T-cells do not make large quantities of y-interferon in vitro (or interleukin 3 or granulocyte-macrophage colony-stimulating factor, data not shown). However, whether the transferred cells stimulate recipient cells in vivo to produce y-interferon or other lymphokines remains to be determined.

That it is the Lyt-1* helper population that contains the primary effector cells in this system is also in accord with the finding that T-cells in culture for 60 days were incapable of causing regression in vivo in spite of the fact that they retained significant cytotoxic activity in vitro. Additionally spleen cells of CTL/RFB-induced disease-regressed mice were significantly proliferative in response to viral antigens as were cells from spontaneously disease-regressed RFV mice but not cells from untreated leukemic animals. The lack of blastogenic activity in the cultured effector cells prior to inoculation could be due to suppression and has been observed in other systems (28).

Transient regressions were induced by large doses of normal T-cells or T-cells sensitized with normal fibroblasts. This could be due to a nonspecific or even nonimmunological effect. We have shown previously (13) that temporary regression of leukemia can be induced by the transfer of macrophages which involves their hematopoietic regulatory and not immunological functions.

Inherent in these studies are questions concerning why, in our system, we can elicit complete disease remissions and permanent cures in fully leukemic animals without pretreatment of the recipients or donors as required in other systems. The explanation often provided for the need for irradiation or chemotherapeutic treatment is a requirement to eliminate suppressor cells in the host that would abrogate the effects (7). FV-infected animals are severely immunosuppressed, with impaired general T- and B-cell and macrophage function (13, 14, 40). It is possible that the suppression in FV leukemic mice is not effective against the transferred T-cells or is abrogated by them.

Another factor which may be relevant is our use of a mixed system for therapy; the T-cells are cultured in vitro with virus-infected fibroblast cell lines as antigen and then are used to treat an autologous leukemia in vivo primarily involving infected hematopoietic cells. The T-cells are initially obtained from RFV-infected and -regressed or RFV-immunized animals, expanded in culture with RFV antigens, and used to treat antigenically very closely related but distinguishable CFV or FV-A leukemias (12, 16). Small differences in immunogenicity, antigenic specificity, or
antigen presentation as possibly manifest in the immunologically mediated spontaneous regression of RFV leukemia may contribute to the therapeutic efficacy. The use in our system of an autoclonal leukemia versus a transplanted tumor that may have undergone selection and progression with passage could also influence the results.

In summary, using the FV-induced erythroleukemia model system, we developed an effective immunotherapeutic protocol for the treatment of Friend virus leukemia. The cultured T-cells are cytotoxic but not proliferative in vitro to virus-specific antigens. The in vivo reactive T-cells that induce permanent regressions appear to be Lyt-1\(^{+}\), possibly helper cells. The Lyt-2\(^{-}\) subset including cytotoxic cells induces temporary regressions of leukemia. The immunotherapy is effective in acutely leukemic mice and requires no pretreatment of recipients. This system thus provides an opportunity to elucidate the mechanisms involved in highly effective immunotherapy of cancer.

ACKNOWLEDGMENTS

We thank Cathrine Allen for preparation of the manuscript.

REFERENCES


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