Adoptive Transfer of Antiviral Resistance by Lymphoid Cells from Mice Protected against Friend Leukemia Virus-induced Disease by Passive Serum Therapy

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ABSTRACT

Spleen and bone marrow cells from DBA/2 mice protected against infection with a leukemogenic dose of Friend leukemia virus (FLV) by passive therapy with chimpanzee anti-FLV serum were shown to be able to adoptively transfer antiviral resistance to unimmunized irradiated syngeneic recipients. Similar to the results obtained with donor mice immunized against a syngeneic FLV erythroleukemia cell line, spleen cells from serum-protected donor mice were more effective in transferring antiviral resistance than were the corresponding bone marrow cells. In contrast to the previously demonstrated close correlation between passive serum therapy protection and the development of a host antiviral humoral response, adoptive cell transfer-mediated resistance to FLV challenge was not accompanied by the presence of detectable antiviral antibodies. While the cell type(s) responsible for transfer of antiviral resistance have not been conclusively identified, studies presented in this report indicate that elimination of T-cells by treatment with anti-Thy 1.2 antibodies plus complement does not affect the ability of spleen cells from serum-protected mice to adoptively transfer antiviral resistance. Furthermore, the idea that mature T-cells do not play a critical role in the FLV serum protection mechanism is further supported by the fact that the serum therapy protocol operates effectively in nude athymic mice. In the course of the adoptive transfer studies, it was observed that sublethal irradiation of DBA/2 mice prevented the development of FLV-induced splenomegaly (which reflects the activity of the defective spleen focus-forming virus component) without affecting the replication of the helper nondefective lymphatic leukemia virus. This irradiation effect could be reversed by reconstituting the mice with spleen or bone marrow cells from normal or FLV-infected syngeneic mice, suggesting that the irradiation effect is due to the inactivation of a spleen focus-forming virus target cell, presumably of an erythroid stem cell nature, which can be replaced by normal or leukemic spleen or bone marrow cells.

INTRODUCTION

We and others have previously reported that mice challenged with a leukemogenic dose of FLV4 can be protected against the development of disease by subsequent passive therapy with xenogeneic antisera raised against disrupted virus or the purified major viral envelope glycoprotein, gp71 (9, 38, 40). A close correlation has been shown to exist between protection by xenogeneic immune serum and the development of host (mouse) antiviral humoral immunity, with both of these parameters being inversely related to serum and spleen infectious virus titers (9).

Our current efforts are aimed at determining the precise mechanism by which passively administered xenogeneic serum leads to resistance against virus-induced disease, particularly the identification of those components of the host's immune system which are involved in protection. Recent experiments using agents which abrogate macrophage functions suggest that these cells do not play a central role in the passive serum therapy protective mechanism (10), although their minor involvement has not been ruled out. The present paper reports a series of studies aimed at determining whether virus-specific resistance mechanisms are generated in serum-protected FLV-infected mice. The results indicate that spleen cells from mice protected against FLV infection by passive serum therapy or from animals immunized against syngeneic FLV erythroleukemia cells can transfer antiviral resistance to unimmunized irradiated recipients, although a host antiviral humoral response did not appear to accompany adoptive transfer protection, in contrast to passive serum therapy (9). This report also describes preliminary experiments utilizing T-cell depletion techniques and nude athymic mice aimed at determining which components of the host immune system are required for successful serum therapy (and thus which cell populations may be involved in the adoptive transfer of antiviral resistance). These studies demonstrated that T-cell-depleted spleen cells from serum-protected mice efficiently transfer antiviral resistance to syngeneic recipients and that T-cell-deficient nude mice are as amenable as are normal animals to serum therapy protection, again accompanied by the induction of host antiviral humoral immunity, thereby arguing against the necessity for intact T-cell functions in this system. Finally, in the course of the adoptive transfer studies, an interesting divergent effect of sublethal irradiation on the susceptibility of DBA/2 mice to the 2 components of the FLV complex [the defective SFFV and the nondefective helper LLV (12, 37)] was observed, and this is also described.

4 The abbreviations used are: FLV, Friend leukemia virus; gp71, glycoprotein with a molecular weight of 71,000; SFFV, spleen focus-forming virus; LLV, lymphatic leukemia virus; CpaFLV, chimpanzee anti-disrupted Friend leukemia virus; Gagp71, goat anti-Friend leukemia virus m.w. 71,000 glycoprotein; RIA, radioimmunoassay.
MATERIALS AND METHODS

Virus

Preparation of stock FLV was as described (9). Briefly, the spleens of FLV-infected DBA/2 mice (Microbiological Associates, Inc., Bethesda, Md., or The Jackson Laboratory, Bar Harbor, Maine) were collected when they became grossly palpable (usually 2 to 3 weeks after infection, at which time they weighed >900 mg each) and used to prepare a 20% (w/v) preparation in Roswell Park Memorial Institute Medium 1640 by homogenization for 30 sec and clarification by centrifugation at 2000 × g for 15 min. The supernatant was removed and filtered successively through 1.2-μm- and 0.8-μm-pore size Millipore filters, and the cell-free filtrate was stored in small aliquots at −70°C.

Cells

The monolayer derivative of the FLV-infected Eveline STU mouse embryo cell line (denoted FEM) producing very large amounts of virus, which was used as the target for the antibody-mediated complement-dependent cytoxicity assay (see below), has been described in detail elsewhere (8, 41). The suspension-grown DBA/2 FLV erythroleukemia FLC-745 cell line, obtained from the Genetic Mutant Cell Repository, Institute for Medical Research, Camden, N. J., has also been described previously (20, 22).

Antisera

CpaFLV antiserum was prepared as described previously (9). Gaαp71 antiserum, described in detail elsewhere (40), was kindly supplied by Professor Werner Schäfer, Max-Planck-Institut für Virusforschung, Tübingen, Germany.

Tumor Cell Immunization

DBA/2 mice were immunized with the syngeneic FLV erythroleukemia FLC-745 cells in order to serve as donors of tumor-immune lymphoid cells in adoptive transfer experiments. Immunization was carried out by 6 weekly i.p. inoculations of 5 × 10⁶ lethally irradiated (6000 R) FLC-745 cells, a protocol which has already been shown to induce antiviral immunity (21, 22).

Passive Serum Therapy

The basic serum therapy protocol has been described in detail elsewhere (9). Briefly, 7- to 8-week-old DBA/2 mice were inoculated i.p. with ~2 × 10³ plaque-forming units of FLV [as measured by the FG-10 sarcoma-positive, leukemia-negative cell plaque assay (2, 9)] in 0.2 ml phosphate-buffered saline (0.15 μM NaCl 0.1 μM phosphate, pH 7.2), on Day 0. Mice were then given 0.2 ml of the appropriate serum i.p. on Days 3, 6, 9, and 12 after virus inoculation. Animals were sacrificed at indicated times, blood was collected, and spleens were removed aseptically for processing as described below and by Collins et al. (9). All blood samples were allowed to clot for 30 min at room temperature and then overnight at 4°C; sera were collected and stored at −70°C.

Adoptive Transfer of Antiviral Resistance

Spleens were removed aseptically from appropriate donor mice (e.g., tumor-immune, serum-protected, and various control groups) at the time of sacrifice, a portion was saved for analysis of infectious virus content by the FG-10 assay, and spleen cells collected in Roswell Park Memorial Institute Medium 1640 by forcing spleen fragments through a sterile steel mesh screen. Only spleens of the appropriate size (i.e., >250 mg for nonprotected FLV-infected mice and <250 mg for normal and serum-protected mice) were used to supply cells for adoptive transfer experiments. The splenocytes were washed once in medium (1200 rpm for 5 min), and the pelleted cells were resuspended in 0.83% NaClCl and incubated for 3 min at 37°C in order to lyse RBC. The resulting cell preparation was then counted and washed twice in medium before inoculation into recipient mice. Bone marrow cells were obtained by washing the femoral bone marrow cavities of mice with medium, a procedure which yielded ~2 × 10⁷ cells/mouse with a viability by trypan blue exclusion of ~80 to 90%. As with the splenocytes, the bone marrow cells were washed twice in medium before inoculation.

Recipient DBA/2 mice received 550 R whole-body irradiation using a ¹³⁷Cs-source γ-iradiator (Gamma Cell 40; Atomic Energy of Canada, Ltd., Ottawa, Ontario, Canada) just prior to the adoptive transfer of spleen or bone marrow cells and were maintained on antibiotics. Unless otherwise indicated, recipient mice received 5 × 10⁷ spleen or 2 × 10⁶ bone marrow cells i.p. in 0.5 ml medium. In the case of recipient mice also challenged with FLV, the standard virus dose was inoculated i.p. in 0.2 ml medium 2 days after the cell inoculation. Animals were sacrificed at indicated times, with blood collected and spleens processed as in the serum therapy procedure.

In the case of pretreatment of adoptively transferred spleen cells with normal or immune (Gaαp71) goat serum plus complement, 4 × 10⁷ spleen cells were incubated for 30 min at 4°C with a 1:10 dilution of the appropriate serum in a total volume of 0.6 ml. A 0.4-ml volume of rabbit complement (Pel Freeze, Rogers, Ark.) diluted 1:2 was then added, and the cells were incubated at 37°C for a further 45 min. After this incubation period, the cells were washed in medium, resuspended to the desired concentration, and adoptively transferred as previously described. An identical procedure was used for the depletion of T-cells before adoptive transfer of splenocytes except that the first treatment utilized a 1:100 dilution of mouse anti-Thy 1.2 monoclonal antibody (Catalog No. NEI-001A; New England Nuclear, Boston, Mass.). The efficacy of this procedure in eliminating T-cells was monitored by determining the blastogenic response of the treated cells to the T-cell mitogen concanavalin A and the B-cell mitogen lipopolysaccharide using an [³²P]thymidine incorporation assay described in detail elsewhere. 5

Serological Assays

RIA. The double-antibody RIA procedure described by Strand and August (43) utilized purified FLV gp71 labeled with ¹²⁵I according to the method of Greenwood et al. (24). The specific activity of the labeled antigen ranged from 5 × 10³ to 2 × 10⁵ cpm/ng protein. Between 1 and 5 ng of ¹²⁵I-FLV gp71 in a 10-μl volume were used in the RIA. All dilutions were made

in RIA buffer [20 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 0.01 mM EDTA containing bovine serum albumin, 2 mg/ml]. Normal mouse serum (30 μl) and test serum (10 μl) were added to the labeled antigen and incubated at 37° for 2 hr. Rabbit anti-mouse immunoglobulin antiserum (30 μl) (Cappel Laboratories, Downingtown, Pa.) was then added and incubated at 4° overnight. Immunoprecipitates were washed once with RIA buffer (without bovine serum albumin), collected by centrifugation at 8000 × g for 2 min, and counted in a Packard Auto-Gamma Counter (Packard Instrument Co., Downers Grove, Ill.).

**Cytotoxicity.** The [14C]nicotinamide release microcytotoxicity assay using the FLV-producing FEM cells as targets has been described in detail (9). Briefly, 2 × 10⁴ FEM cells were seeded in each well of a Falcon No. 3034 microtest plate (Falcon Plastics, Oxnard, Calif.) in 10 μl minimal essential medium supplemented with 10% fetal calf serum and [14C]-nicotinamide (25 μCi/ml; 60 nCi/mmol; Amersham/Searle, Arlington Heights, Ill.). After 24 hr of growth, when the cells had just reached confluence, the plates were washed 3 times with cold minimal essential medium (without serum), wash fluid was aspirated from the plates and wells, and 5 μl of the appropriate test serum dilution were added to triplicate wells. The plates were incubated for 15 min at 37° in a CO₂ incubator, and then 10 μl of rabbit complement (Pel Freeze, Rogers, Ark.) diluted 1:6 were added to appropriate wells. After a further 15 min incubation at 37° in a CO₂ incubator, 5 μl were removed from each well for counting in a Beckman LS-350 β-irradiation counter (Beckman Instruments, Palo Alto, Calif.).

Maximum release of radioactivity was determined by the addition of 0.5% Triton X-100, 15 μl/well, at the start of the first incubation. Results are expressed as percentage of specific release (SR), where:

\[
\% \text{ of SR} = \frac{\text{Test cpm} - \text{background cpm}}{\text{maximum release cpm} - \text{background cpm}}
\]

Background is either complement control or medium control, and specific release of ≥20% reflects significant cell lysis.

**RESULTS**

**Effect of Sublethal Irradiation on FLV-induced Disease.** In the course of carrying out preliminary experiments aimed at establishing the optimal conditions for determining whether antiviral resistance could be transferred by lymphoid cells from serum-protected mice, it was repeatedly observed that sublethally irradiated control mice not reconstituted with any lymphoid cell population failed to develop FLV-induced disease, as monitored by the development of splenomegaly, a point which is illustrated by mice of Groups 5 and 6 in Table 1. These results also demonstrate that inoculation of either 5 × 10⁷ normal spleen cells or 2 × 10⁷ normal bone marrow cells reversed the inhibitory effect of 550 R irradiation on the ability of FLV to induce splenomegaly, regardless of whether virus was inoculated 3 days before or 2 days after donor cell transfer. Analysis of the infectious virus content in spleen cell extracts from the various groups of cell recipient mice using the FG-10 sarcoma-positive, leukemia-negative cell plaque assay (which detects only the nondefective LLV) yielded the unexpected result that even the normal-sized spleens of inoculated nonreconstituted mice of Groups 5 and 6 contained high levels of infectious virus. Thus, despite the inability of the inoculated virus to induce splenomegaly in sublethally irradiated nonreconstituted mice (such splenomegaly induction reflecting the activity of the defective SFFV component of FLV), there is no effect on the replication of the nondefective helper LLV. This suggests that sublethal irradiation inactivates a target cell for the SFFV which is present in, and can be replaced by, normal spleen or bone marrow cells, while such irradiation has no apparent effect on the target cell(s) for LLV replication.

Regardless of the identity of the radiation-sensitive SFFV target cell, since splenomegaly induction is taken as the end point for FLV infection in the serum therapy model (9, 10), the data presented in Table 1 indicate that control mice must receive at least some lymphoid cell population (e.g., spleen or bone marrow cells from normal mice or animals with progressive disease) if they are to develop virus-induced splenomegaly, thereby allowing a comparison between these animals and those receiving comparable cell populations from serum-protected donor mice. It was thus essential for these studies to determine whether hematopoietic cells from FLV-infected mice could mimic normal spleen or bone marrow cells in replacing the radiation-sensitive cell necessary for FLV-induced splenomegaly. The results presented in Table 2 demonstrate that both spleen and bone marrow cells from FLV-infected mice treated with normal chimpanzee serum can support the induction of splenomegaly in the virus-challenged recipients in a fashion comparable to that seen with normal spleen and bone marrow.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cells transferred</th>
<th>FLV on Day</th>
<th>Radiation dose (R)</th>
<th>Av. spleen wt (mg)</th>
<th>Splenomegaly/total</th>
<th>Day of sacrifice</th>
<th>Virus titerb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>550</td>
<td>1799 ± 610</td>
<td>5/5</td>
<td>18</td>
<td>Small</td>
</tr>
<tr>
<td>1</td>
<td>Normal spleen</td>
<td>2</td>
<td>550</td>
<td>668 ± 325</td>
<td>9/11</td>
<td>31</td>
<td>Large</td>
</tr>
<tr>
<td>2</td>
<td>Normal spleen</td>
<td>3</td>
<td>550</td>
<td>749 ± 481</td>
<td>4/5</td>
<td>31</td>
<td>&lt;25</td>
</tr>
<tr>
<td>3</td>
<td>Normal spleen</td>
<td>4</td>
<td>550</td>
<td>1006 ± 419</td>
<td>8/8</td>
<td>31</td>
<td>1.3 ± 10⁷</td>
</tr>
<tr>
<td>5</td>
<td>Normal bone marrow</td>
<td>5</td>
<td>550</td>
<td>184 ± 67</td>
<td>1/12</td>
<td>31</td>
<td>7.1 ± 10⁷</td>
</tr>
<tr>
<td>6</td>
<td>Normal bone marrow</td>
<td>6</td>
<td>550</td>
<td>144 ± 77</td>
<td>1/8</td>
<td>31</td>
<td>2.39 ± 10⁸</td>
</tr>
</tbody>
</table>

b Spleen weight ≥250 mg is considered to represent significant splenomegaly.

FLV titer determined by the FG-10 sarcoma-positive, leukemia-negative cell plaque assay described in "Materials and Methods" and expressed as plaque-forming units/g. Spleen tissues from mice of the same group with either small (<250 mg) or large (>250 mg) spleens were pooled before preparation of the cell extracts for assessment of infectious virus content.

Mean ± S.E.
cells. Furthermore, spleen or bone marrow cells (latter not shown) from virus-infected mice could themselves induce splenomegaly in cell recipient mice not subsequently challenged with FLV (Group 3), not unexpected considering the high levels of infectious virus present in these tissues of the leukemic donor mice. As previously shown, sublethally irradiated nonreconstituted mice subsequently challenged with FLV demonstrated high levels of infectious virus in splenic tissue despite the absence of splenomegaly development (Group 6). Thus, the results presented in Tables 1 and 2 indicate that both normal and FLV-infected splenocytes and bone marrow cells could reverse the SFFV-inhibitory effect of sublethal irradiation, thereby allowing a determination of whether those same cell populations obtained from serum-protected donor mice could transfer antiviral resistance to irradiated recipients.

**Infectious Virus Carryover to Sublethally Irradiated Recipients by Adoptively Transferred Spleen Cells from Serum-protected Mice.** An additional point which required examination before the adoptive transfer experiments could be performed concerned the possible carryover of infectious virus in spleen or bone marrow cells from serum-protected donors. This is especially pertinent since previous results (9, 10) have demonstrated that, while serum-protected mice usually have undetectable levels of infectious FLV in spleen tissue (as determined by the FG-10 plaque assay), in some cases infectious virus was present, although at much lower levels (~10³ to 10⁴ times less) than in nontreated infected control mice. As demonstrated in Table 3, when low levels of infectious virus are present in serum-protected donor spleen cells (e.g., Groups 1 and 3), then a portion of the irradiated mice receiving these cells can develop marginal splenomegaly (Groups 5 and 8). Elimination of virus-producing donor cells by pretreatment with high-titer Gagp71 serum in the presence of rabbit complement was investigated, and the results suggest that this may be useful in reducing, if not completely preventing, the carryover of FLV in those donor spleen cell preparations containing

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**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cells transferred</th>
<th>FLV on Day 2</th>
<th>Day of death or sacrifice</th>
<th>Av. spleen wt (mg)</th>
<th>Splenomegaly/total</th>
<th>Virus titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal spleen</td>
<td>+</td>
<td>30</td>
<td>668 ± 325</td>
<td>9/11</td>
<td>&lt;25</td>
</tr>
<tr>
<td>2</td>
<td>FLV + NCS spleen</td>
<td>+</td>
<td>30</td>
<td>1298 ± 190</td>
<td>6/6</td>
<td>1.29 x 10⁸</td>
</tr>
<tr>
<td>3</td>
<td>FLV + NCS spleen</td>
<td>–</td>
<td>24–30</td>
<td>1568 ± 124</td>
<td>6/6</td>
<td>5.70 x 10⁸</td>
</tr>
<tr>
<td>4</td>
<td>Normal bone marrow</td>
<td>+</td>
<td>30</td>
<td>1007 ± 454</td>
<td>3/3</td>
<td>6.60 x 10⁸</td>
</tr>
<tr>
<td>5</td>
<td>FLV + NCS bone marrow</td>
<td>+</td>
<td>30</td>
<td>1250 ± 431</td>
<td>2/2</td>
<td>2.70 x 10⁸</td>
</tr>
<tr>
<td>6</td>
<td>FLV + NCS bone marrow</td>
<td>+</td>
<td>30</td>
<td>147 ± 23</td>
<td>0/6</td>
<td>6.58 x 10⁶</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>–</td>
<td>30</td>
<td>98 ± 22</td>
<td>0/6</td>
<td>&lt;25</td>
</tr>
</tbody>
</table>

NCS, normal chimpanzee serum.

**Table 3**

<table>
<thead>
<tr>
<th>Donors</th>
<th>Virus titer</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Small</td>
</tr>
<tr>
<td>1</td>
<td>4.68 x 10³</td>
</tr>
<tr>
<td>2</td>
<td>&lt;25</td>
</tr>
<tr>
<td>3</td>
<td>&lt;25</td>
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Adoptive transfer of anti-FLV resistance by spleen and bone marrow cells from serum-protected and tumor-immune DBA/2 mice to syngeneic recipients

Donor DBA/2 mice were normal, leukemic (FLV-infected treated with NCS8), serum-protected (FLV infected, treated with CpaFLV serum), or immunized with the syngeneic FLV erythroleukemia FLC-745 tumor. Only leukemic spleens weighing >250 mg and serum-protected spleens weighing <250 mg were used to furnish cells for adoptive transfer. All recipient mice received 550 R whole-body dose on Day 0 and except for Group 7 were challenged with the standard FLV dose on Day 2. See Table 1, Footnotes a and b and legend for further details.

The combined results of 3 such adoptive transfer experiments control mice receiving serum-protected spleen cells from serum-protected donor DBA/2 mice immunized against the syngeneic FLV erythroleukemia cells (Groups 11 and 12), although again the bone marrow cells from tumor-immune mice did demonstrate partial protection when compared to normal or leukemic cells (see Groups 8 and 9). It should also be noted that the transfer of fewer bone marrow cells as compared to spleen cells (2 x 10^7 versus 5 x 10^7) may have contributed to the less efficient transfer of antiviral resistance seen with the former cells.

While the effect of adoptive transfer of lymphoid cells from serum-protected mice on virus replication was reminiscent of that seen with the passive serum therapy procedure itself (9, 10), namely, the marked elimination of infectious virus in mice failing to develop significant splenomegaly (Table 4), the host antiviral humoral immune response previously shown to accompany serum therapy protection (9, 10) was not evident in the adoptively protected mice (Chart 1). Thus, of the 24 mice receiving either serum-protected or FLC-745 tumor-immune spleen or bone marrow cells which did not develop significant

detectable levels of infectious virus (compare Groups 8 and 9). In any event, the results in Table 3 underscore the necessity of monitoring infectious FLV in all preparations of donor spleen cells, especially those from serum-protected mice, as well as including in all adoptive transfer experiments control mice receiving serum-protected spleen cells without subsequent FLV challenge in order to detect any infectious virus carryover to the cell recipients.

Adoptive Transfer of Anti-FLV Resistance by Lymphoid Cells from Serum-Protected and Tumor-Immune Mice. While it has been shown previously (9, 11, 38) that the passive serum therapy of FLV infection is accompanied by the induction of host antiviral humoral immunity, as measured in RIA versus 125I-FLV gp71 or with a complement-dependent cytotoxicity assay using FLV-infected FEM mouse embryo target cells, little information has been available as to whether an antiviral cellular immune response is also generated. Studies to determine whether spleen and/or bone marrow cells from serum-protected DBA/2 mice can adoptively transfer anti-FLV resistance to sublethally irradiated syngeneic mice were performed using the protocol described above. As a positive control for the adoptive transfer technique, mice receiving spleen or bone marrow cells from DBA/2 donor mice immunized against the syngeneic FLC-745 FLV erythroleukemia cells were included since this immunization has been shown to induce resistance to direct FLV challenge in addition to the expected rejection of the FLC-745 cells themselves.

The combined results of 3 such adoptive transfer experiments are presented in Table 4: they clearly indicate that spleen cells from serum-protected donor DBA/2 mice previously rejecting FLV challenge after treatment with CpaFLV serum could markedly inhibit FLV-induced splenomegaly in irradiated recipient animals inoculated with FLV 2 days after cell transfer (Group 6). While 13 of 22 mice in this group did demonstrate spleens weighing >250 mg at the time of sacrifice, it is obvious from the average spleen weight for the group that these were only marginally splenomegalic and much smaller than those seen in recipient mice receiving normal or leukemic spleen cells [the latter from donor mice treated with normal chimpanzee serum after FLV inoculation (Groups 4 and 5)]. In accord with the absence of detectable infectious FLV in the serum-protected donor spleen cells (Group 3), all mice of recipient control Group 7 not challenged with FLV after receiving these splenocytes demonstrated normal-sized spleens, which were themselves devoid of infectious virus.

The data in Table 4 concerning the adoptive transfer of bone marrow cells suggest that these cells from serum-protected mice were less efficient in their ability to transfer anti-FLV resistance than were the corresponding spleen cells. Nevertheless, recipients of bone marrow cells from serum-protected donor mice (Group 10) had considerably smaller spleens than did similarly challenged mice previously receiving normal or leukemic bone marrow cells (Groups 8 and 9). It is interesting to note that the greater ability of spleen cells to transfer anti-FLV resistance as compared to bone marrow cells was also seen when these cell populations were obtained from donor mice previously immunized with syngeneic FLC-745 FLV erythroleukemia cells (Groups 11 and 12), although again the bone marrow cells from tumor-immune mice did demonstrate partial protection when compared to normal or leukemic cells (see Groups 8 and 9). It should also be noted that the transfer of fewer bone marrow cells as compared to spleen cells (2 x 10^7 versus 5 x 10^7) may have contributed to the less efficient transfer of antiviral resistance seen with the former cells.

Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Protocol #</th>
<th>FLV challenge</th>
<th>Day of sacrifice</th>
<th>Av. spleen wt (mg)</th>
<th>Splenomegaly/total</th>
<th>Virus titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NCS</td>
<td>–</td>
<td>29</td>
<td>107 ± 23</td>
<td>0/41</td>
<td>&lt;25</td>
</tr>
<tr>
<td>2</td>
<td>CpaFLV</td>
<td>+</td>
<td>29</td>
<td>1100 ± 576</td>
<td>21/23</td>
<td>&lt;25</td>
</tr>
<tr>
<td>3</td>
<td>CpaFLV</td>
<td>+</td>
<td>29</td>
<td>157 ± 44</td>
<td>2/47</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Recipients</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>Normal spleen</td>
<td>+</td>
<td>30-40</td>
<td>782 ± 337</td>
<td>20/22</td>
<td>&lt;25</td>
</tr>
<tr>
<td>5</td>
<td>FLV + CpaFLV spleen</td>
<td>+</td>
<td>30-32</td>
<td>1084 ± 745</td>
<td>13/13</td>
<td>&lt;25</td>
</tr>
<tr>
<td>6</td>
<td>FLV + CpaFLV spleen</td>
<td>–</td>
<td>30-32</td>
<td>286 ± 113</td>
<td>13/22</td>
<td>&lt;25</td>
</tr>
<tr>
<td>7</td>
<td>FLV + CpaFLV spleen</td>
<td>–</td>
<td>30-32</td>
<td>125 ± 35</td>
<td>0/15</td>
<td>&lt;25</td>
</tr>
<tr>
<td>8</td>
<td>Normal bone marrow</td>
<td>+</td>
<td>30-40</td>
<td>954 ± 463</td>
<td>6/6</td>
<td>&lt;25</td>
</tr>
<tr>
<td>9</td>
<td>FLV + NCS bone marrow</td>
<td>+</td>
<td>40-53</td>
<td>1250 ± 431</td>
<td>2/2</td>
<td>&lt;25</td>
</tr>
<tr>
<td>10</td>
<td>FLV + CpaFLV bone marrow</td>
<td>+</td>
<td>40-53</td>
<td>434 ± 173</td>
<td>3/4</td>
<td>&lt;25</td>
</tr>
<tr>
<td>11</td>
<td>FLC-745 immune spleen</td>
<td>+</td>
<td>40-53</td>
<td>116 ± 80</td>
<td>1/14</td>
<td>&lt;25</td>
</tr>
<tr>
<td>12</td>
<td>FLC-745 immune bone marrow</td>
<td>+</td>
<td>40-53</td>
<td>304 ± 117</td>
<td>7/9</td>
<td>&lt;25</td>
</tr>
</tbody>
</table>

* NCS, normal chimpanzee serum; NA, not available.
* Donor protocol, serum treatment; recipient protocol, cells transferred.
* Mean ± S.E.

splenomegaly after FLV challenge, only 6 demonstrated a detectable antiviral humoral response measured both in the cytotoxicity test and by RIA with 125I-FLV gp71. Note that all 6 of these serologically reactive sera were obtained from recipients of tumor-immune spleen cells; thus, in no case did a recipient of serum-protected spleen cells rejecting subsequent challenge with FLV demonstrate the presence of detectable levels of antiviral antibody.

In order to determine whether T-cells are required for the adoptive transfer of antiviral resistance by spleen cells from serum-protected donor mice, preliminary depletion experiments have been performed by pretreating the donor splenocytes with anti-Thy 1.2 monoclonal antibodies plus rabbit complement before inoculation into irradiated recipients. Under conditions in which the blastogenic response of the treated cells to the T-cell mitogen, concanavalin A, was reduced >90% (data not shown), with no effect on responsiveness to lipopolysaccharide, a B-cell mitogen, the T-cell-depleted spleen cells from serum-protected donors demonstrated an undiminished ability to protect syngeneic recipients against a subsequent challenge with FLV (Table 5, Groups 5 and 6). Note that the low level of splenomegaly induction in normal spleen cell recipients (Groups 3 and 4) is frequently seen in FLV-challenged irradiated reconstituted mice, which only occasionally manifest spleen enlargement comparable to that seen in control FLV-infected animals. In any event, the results presented in Table 5 strongly suggest that T-cells are not required for the adoptive transfer of antiviral resistance by spleen cells from serum-protected donor mice.

Passive Serum Therapy in Nude Athymic Mice. In parallel with the adoptive transfer experiments using fractionated spleen cell populations from serum-protected donor mice, the possible role of T-cells in the serum therapy system has been further investigated by examining the ability of nude athymic mice to be protected against FLV infection by treatment with xenogeneic anti-FLV or anti-gp71 antisera. The data presented in Table 6 demonstrate that T-cell deficient NIH/Swiss nude mice can be protected against FLV pathogenesis, as measured by both mortality and splenomegaly induction, by treatment with either the CpaFLV or the Gaggp71 antisera. It should be noted that the nude mice are much more susceptible to the lethal effects of FLV than are immunointact animals (e.g., DBA/2 mice), accounting for the significant mortality by Day 30 in these experiments. Thus, 62% of the control nude mice treated with normal chimpanzee or goat serum subsequent to FLV infection were dead by Day 30, compared to only 16% of the
animals treated with immune sera (CpaFLV or Gagp71). While nearly one-half (21 of 46) of the immune serum-treated mice succumbed with an average latent period of 38 days (after FLV inoculation), mice treated with immune sera and the appearance of a mouse antiviral humoral response indistinguishable from that seen with serum-protected DBA/2 mice, measurable with both RIA and the complement-dependent cytotoxicity test (Chart 2), suggesting that intact host T-cell functions are not necessary either for passive serum protection or for the induction of the antiviral humoral response which accompanies it in this system. Preliminary results indicate that the antibody produced in serum-protected nude mice is IgG,7 and studies are currently under way to further characterize the nature of this antibody response in both nude athymic and immunointact mice, especially its possible role in the passive serum therapy mechanism.

DISCUSSION

The present report demonstrates that DBA/2 mice protected against a leukemogenic challenge with FLV by the subsequent passive administration of xenogeneic antiviral antiserum generate immune spleen cells capable of adoptively transferring anti-FLV resistance to sublethally irradiated immununized syngeneic recipients. Parallel experiments indicated that spleen cells from donor DBA/2 mice immunized with the syngeneic FLV erythroleukemia FLC-745 cells were also capable of transferring anti-FLV resistance with apparently somewhat greater efficiency than that of the serum-protected splenocytes (Table 4). With both serum-protected and tumor-immune donors, transfer of bone marrow cells was less effective than were the corresponding spleen cells in inducing resistance to FLV challenge, suggesting the involvement of mature primed immune cells, although some protection was seen as compared to mice receiving bone marrow cells from either normal or leukemic (FLV plus normal chimpanzee serum-treated) donors before FLV inoculation. It should be noted that the transfer of fewer bone marrow cells as compared to spleen cells (2 x 10^7 versus 5 x 10^7) may have contributed to the less efficient transfer of antiviral resistance seen with the former cells, possibly reflecting the presence of fewer immunocompetent cells or, conversely, of more erythroid target cells for FLV infection in the bone marrow cell population used. The necessity of determining the infectious virus content of adoptively transferred spleen cells from serum-protected donor mice, as well as including in all adoptive transfer experiments control recipient mice receiving these spleen cells without subsequent FLV challenge in order to monitor any possible infectious virus "carryover," has also been demonstrated (Table 3). Such transfer of infectious

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7 J. J. Collins and F. Santilippo, unpublished observations.
virus to the cell recipient mice would tend to artificially reduce the efficacy of the adoptive transfer procedure and could falsely indicate the lack of lymphoid cells in serum-protected mice capable of transferring antiviral resistance.

The results presented suggest that serum therapy protection is accompanied by the induction of virus-specific host resistance mechanisms which may be distinct from the previously reported antiviral humoral response (9, 10, 38) and that these mechanisms may play a central role in this serum therapy model. The failure of mice protected against FLV challenge by the adoptive transfer of spleen cells from serum-protected or tumor-immune donors to consistently demonstrate antiviral humoral activity (Chart 1) argues against the notion that this adoptively transferred protection reflects the activity of mature antibody-producing cells or of primed T-helper cells capable of supporting antibody synthesis in the irradiated recipients. However, it is recognized that the inability to detect antibody, even with a sensitive RIA technique, does not conclusively rule out the possibility that antibody and/or antibody-producing cells play a role in this system. Viral antigen may be present in excess in the cell recipient FLV-challenged mice precluding detection of free circulating antibody, or antibody may not have been assayed for at the appropriate times after transfer of lymphoid cells. Studies are in progress utilizing virus-specific antibody-forming cell assays to determine whether synthesis of antiviral antibodies is completely absent in adoptively protected mice.

The adoptive transfer approach described in this report provides an excellent means for identifying those components of the serum-protected host’s immune system which are specifically activated against FLV. Preliminary results with T-cell-depleted spleen cells from serum-protected donors (Table 5) suggest that these cells are not required for the adoptive transfer of antiviral resistance to irradiated recipients, and more extensive fractionation studies using both positive and negative selection techniques are currently under way. In any event, the apparent noninvolvement of T-cells in this serum therapy system, as suggested by the adoptive transfer experiments, is supported by the finding that T-cell-deficient nude athymic mice can be efficiently protected against FLV challenge by the passive administration of CpaFLV or Qaqp71 antiserum (Table 6). Furthermore, the serum-protected nude mice demonstrated a host antiviral humoral (IgG) immune response (Chart 2) essentially indistinguishable from that seen previously in immunointact DBA/2 mice (9, 10), suggesting that this antibody response is not highly T-cell dependent. Good IgG responses by nude athymic mice to other antigens have been reported previously (34). However, given the fact that serum protection of nude mice is associated with the characteristic host antiviral humoral response while adoptive immunity with serum-protected spleen cells is not (Chart 1), it remains possible that different mechanisms are involved in these 2 systems.

Also with respect to the possible involvement of T-cells in the serum therapy mechanism, it should be noted that, although we have previously reported that virus-specific cytotoxic effector cells are generated (subject to secondary stimulation in vitro) in serum-protected DBA/2 mice (11), more recent experiments demonstrate this to be a variable occurrence, in contrast to the consistent antiviral humoral response. In addition, the possible role of macrophages in the serum therapy mechanism is argued against by experiments in which agents inhibiting macrophage functions, including silica and a tumor-produced macrophage chemotaxis inhibitor, failed to interfere with the ability of passively administered CpaFLV serum to protect against FLV infection (10).

It is certainly possible that mechanisms such as antibody-dependent cellular cytotoxicity or natural killing may be involved in this serum therapy model system, in accord with the observation that both of these functions are at normal or even higher levels in nude athymic mice (25, 39). Also consistent with the possible role of antibody-dependent cellular cytotoxicity in the serum protection of FLV-induced disease is the finding that mouse anti-FLV antiserum, which has a much lower titer than does the xenogeneic CpaFLV serum, is more efficient in its passive therapy capability (11), possibly reflecting better recognition of homologous antibodies by and more efficient interaction with host immune cells, e.g., via Fc receptors (27, 33). In this regard, the ability of antibody fragments [F(ab')2 and F(ab')2] to function in this serum therapy system is presently under examination.

A considerable amount of work has been carried out by a number of investigators to establish the mechanisms operative in a closely related immunotherapy model system, namely, that involving the induction of solid tumors by murine sarcoma viruses. While passive serum therapy has been shown to significantly suppress the development of virus-induced sarcomas in immunologically compromised hosts (5, 16, 17, 32), effective long-term protection requires the adoptive transfer of T-cells from murine sarcoma virus-immune mice (6, 7, 17, 18, 23). Other studies have confirmed that T-lymphocytes are of predominant importance in the adoptive transfer of immunity to tumors induced by a variety of oncornaviruses, including FLV (4, 46). Of particular interest is the recent report by Leclerc and Cantor (26) demonstrating that different T-cell subpopulations are responsible for the adoptive transfer of resistance to murine sarcoma virus challenge as opposed to challenge with virus-induced lymphoma cells, the latter of which correlates with cytolytic activity in vitro. This could account for the difference that we have seen in the resistance of serum-protected DBA/2 mice to rechallenge with FLV or with cells of an established FLV erythroleukemia cell line.6 In any event, while the preponderance of available evidence supports a central role for T-cells in the adoptive transfer of resistance to challenge with oncornaviruses or oncornavirus-induced tumors, it remains possible that other immunocompetent cells, acting either alone or in concert with T-cell populations, are responsible for the transfer of anti-FLV resistance with spleen cells from serum-protected mice. The expanded use of fractionated donor cells should allow identification of the active cell population(s).

Although considered unlikely, the present results do not completely rule out the possibility that the ability of spleen cells from serum-protected mice to transfer antiviral resistance to irradiated recipients might reflect nonimmunological effects of the passive serum therapy protocol. Thus, if serum-protected mice are characterized by a diminution in the number of splenic erythroid stem cells serving as FLV targets (19, 36), this could explain the failure of irradiated mice receiving these cells to support the development of FLV-induced splenomegaly. While an analogous inhibitory effect of stem cell deprivation has been described by others (44), it should be noted that we have failed to demonstrate appreciable differences in peripheral blood
DBA/2 mice were compared at times after infection similar to parameters when serum-protected and age-matched normal DBA/2 mice were compared at times after infection similar to those in the present study (38), in conflict with the notion that serum-protected mice develop marked alterations in their hematopoietic environment. Lastly, should the defective SFFV persist in spleen cells of serum-protected mice after the elimination of replicating helper virus, these cells could be resistant to infection with LLV upon transfer to recipient mice, possibly due to the production of defective interfering particles (14), which would tend to reduce the levels of exogenous helper virus and thus inhibit splenomegaly development (13, 15). Studies are in progress to determine whether the SFFV does persist in the spleen cells of serum-protected mice.

In the course of carrying out the reported adoptive transfer studies, an effect of sublethal irradiation on FLV tumorigenesis was observed, namely, the inability of virus to induce splenomegaly in DBA/2 mice receiving 550 R whole-body γ-irradiation unless these mice also received syngeneic spleen or bone marrow cells, whether from normal or from FLV-infected donors (Tables 1 and 2). Of particular interest was the finding that, despite the absence of splenomegaly development in FLV-challenged irradiated nonreconstituted mice, the nondefective LLV component of the FLV complex (37) replicated in spleen tissue to levels comparable to that seen in unirradiated control mice. Thus, the effect of the sublethal irradiation seems to be specifically directed toward the splenomegaly-inducing defective SFFV component of the FLV complex (12), probably reflecting the inactivation of a radiation-sensitive target cell necessary for the development of splenomegaly. While in vivo studies have indicated that the target cell for SFFV-induced transformation is a radiosensitive erythropoietin-responsive erythroid stem cell found in the bone marrow and spleen (1, 19, 31, 37, 44, 45), formal proof that SFFV can transform erythroid target cells in the absence of coinfection with the helper LLV is not yet available. Nevertheless, it is reasonable to assume that it is this SFFV erythroid stem cell target which is being inactivated by sublethal irradiation and replaced by the adoptive transfer of spleen or bone marrow cells in the present studies.

The fact that sublethal irradiation inhibited splenomegaly induction, whether administered before or after FLV challenge (Table 1), suggests that the putative radiosensitive target cell is necessary for virus reinfection and spread prior to the appearance of overt disease. However, at the present time, it is not known whether the SFFV component persists in the tissues of sublethally irradiated mice. Note that SFFV has been shown to replicate and persist in spleen cells of SI/SI′ anemic mice which are genetically resistant to FLV-induced erythroleukemia and splenomegaly development (3, 30). SFFV has also been shown to persist in the hematopoietic tissues of susceptible mice in the absence of LLV (15), with its erythroid cell-transforming ability dependent on actively replicating helper virus (13, 15, 42). This observation tends to argue against SFFV persistence in the sublethally irradiated mice of the present study since LLV replication appears to be unimpaired (Tables 1 and 2). Nevertheless, the reported effect of sublethal irradiation on the splenic microenvironment necessary for the development of FLV-induced erythroleukemia (28, 29, 35) could also prevent the appearance of virus-induced splenomegaly under conditions in which the helper LLV was actively replicating and the SFFV genome was present.

In conclusion, it has been shown that spleen cells from serum-protected DBA/2 mice can adoptively transfer anti-FLV resistance to sublethally irradiated immununized syngeneic recipients, while bone marrow cells are considerably less active. While the identity of the cell(s) active in the transfer of antiviral immunity is not yet known, preliminary fractionation experiments (Table 5) suggest that T-cells are not involved. Furthermore, if the inability to detect antiviral antibody in adoptively protected mice (Chart 1) can be shown to actually reflect the absence of antibody synthesis, then it would appear that the mechanism responsible for adoptive protection may be different from that operating in mice protected against FLV infection by the passive administration of xenogeneic antisera. Studies are in progress to identify the components of the host immune system and the relevant mechanisms responsible for resistance to FLV-induced disease subsequent to both passive serum therapy and adoptive cell transfer protection.

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Adoptive Transfer of Antiviral Resistance by Lymphoid Cells from Mice Protected against Friend Leukemia Virus-induced Disease by Passive Serum Therapy


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