3'-Azido-3'-Deoxythymidine Prevents Induction of Murine Acquired Immunodeficiency Syndrome in C57Bl/10 Mice Infected with LP-BM5 Murine Leukemia Viruses, a Possible Animal Model for Antiretroviral Drug Screening

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Creative Products Research Laboratories, Kissei Pharmaceutical Co., Ltd., Matsumoto-city,1 and Department of Enteroviruses2 and Department of Blood Products,3 AIDS Research Center, National Institute of Health, 2-10-35, Kamiosaki, Shinagawa-ku, Tokyo, 141, Japan

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Adult C57Bl/10 mice (H-2b Fv-1a) inoculated with LP-BM5 murine leukemia virus develop a disease which has many features in common with human acquired immunodeficiency syndrome (AIDS), in particular abnormal lymphoproliferation and severe immunodeficiency. In the present study, we examined the possibility that this murine AIDS (MAIDS) model would be useful for evaluating antiretrovirus drugs in vivo through the use of a well-defined antiretrovirus drug, the reverse transcriptase (RT) inhibitor (H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. St. Claire, S. Nusinov-Lehman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder, Proc. Natl. Acad. Sci. USA 82:7096-7100, 1985) 3'-azido-3'-deoxythymidine (AZT). We evaluated the effect of AZT treatment on de novo virus infection as well as on the induction of immunodeficiency by various parameters, including RT activity in serum, splenomegaly, proliferative responses against alloantigens and mitogens, soluble-antigen-presenting cell activity, and immunoglobulin G levels in serum. Our results demonstrated that AZT treatment of C57Bl/10 mice infected with LP-BM5 murine leukemia virus efficiently prevented the induction of immunodeficiency if started at the time of virus inoculation. Starting AZT treatment 1 week later provided only a partial protective effect. Starting AZT treatment 2 weeks later was associated with suppression of RT activity in serum but no prevention of immunosuppression. This MAIDS model may allow rapid and cost-effective screening for antiretrovirus drugs targeted against retroviral functions shared between human AIDS and MAIDS, such as those encoded by gag, pol, or env.

The retrovirus-induced human acquired immunodeficiency syndrome (AIDS) has been regarded as an incurable disease so far. Although a number of efforts in the identification, isolation, and characterization of human immunodeficiency virus (HIV) have been successfully carried out, relatively little is known about the pathogenesis of AIDS. Since nonhuman primates infected with HIV do not develop the immunodeficiency syndrome seen in humans, there have been many difficulties in investigating the pathogenesis of AIDS as well as in evaluating anti-HIV drugs by using animal models.

The LP-BM5 murine leukemia virus (MuLV) originally isolated by Laterjet and Duplan (8) causes a syndrome, termed murine AIDS (MAIDS), characterized by hypergammaglobulinemia, lymphadenopathy, severe immunodeficiency, enhanced susceptibility to infection, and the development of B-cell lymphomas in adult mice of susceptible strains (2, 3, 5, 6, 11, 12, 15, 24). One of the unique features of LP-BM5 MuLV is that it induces a persistent immunosuppression not only in newborn mice but also in adult mice. Since many features of this syndrome are common to those defined in human AIDS, as described above, MAIDS may serve as a useful experimental model for understanding the pathogenesis of AIDS as well as for searching for anti-HIV drugs. In another study with the MAIDS system, we demonstrated that antigen-presenting cells (APCs) from LP-BM5 MuLV-infected mice failed to stimulate the proliferation of antigen-specific helper T-cell clones (10a). This result may explain why functional abnormalities of CD4+ helper T cells are observed before the numbers of these cells decline (1, 7) and supports the notion that APCs are one of the initial targets for HIV infections in humans. The present study was undertaken to establish an experimental system for screening antiretrovirus drugs in vivo with MAIDS.

MATERIALS AND METHODS

Mice. Male C57Bl/10 mice (B10 mice) were used at 4 weeks of age. Mice were purchased from Japan SLC Inc., Shizuoka, Japan. The body weight of each mouse was checked on the day of LP-BM5 MuLV inoculation and on the day of sacrifice. Both B6.C-H-2b1 and B6.C-H-2b12 mice were originally purchased from Jackson Laboratory, Bar Harbor, Maine, and then bred in our own animal colony. All the mice were specific pathogen free and were housed in an air-conditioned room. The room temperature was kept at about 22°C, and each cage of experimental groups of mice was supplied with a separate air flow and isolated from the others by use of a mouse Polysulfone cage system. Mice were given pelleted feed sterilized by gamma irradiation.

LP-BM5 MuLV. An SC-1 clone chronically infected with LP-BM5 MuLV, termed the G6 cell line, was kindly supplied by H. C. Morse III, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Virus was prepared from the supernatant of this G6 clone. A 24-h culture supernatant of G6 clone cells contained approximately 5 × 106 PFU of ectropic virus per ml, as determined by the XC
plaque assay described by Rowe et al. (16). The virus preparation was stored at −70°C until use.

**Virus inoculation.** B10 mice were inoculated by the intraperitoneal route with 0.5 ml of the LP-BM5 MuLV preparation. Mice were sacrificed at 3 or 5 weeks after virus inoculation, according to experimental schedules. Sera and spleen cells from each experimental mouse were subjected to immunological examinations.

**Allogeneic mixed-lymphocyte responses.** For determination of allogeneic mixed-lymphocyte responses, responder spleen cells (4 × 10^6) and irradiated (3,000 rads) stimulator spleen cells (4 × 10^6) were cultured together in 0.2 ml of culture medium in 96-well round-bottomed microculture plates at 37°C in 7.5% CO₂. The culture medium consisted of RPMI 1640 supplemented with 10% fetal calf serum, penicillin (5,000 IU/100 ml), streptomycin (5,000 μg/100 ml), nonessential amino acids, sodium pyruvate (11.0 mg/100 ml), 2-mercaptoethanol (5 × 10⁻³ M), and 1-glutamine (29.2 mg/100 ml). On day 4, cultures were pulsed with 1 μCi of [³H]thymidine, incubated for an additional 12 to 18 h, and harvested. Data are expressed as the arithmetic mean counts per minute of triplicate cultures. Standard errors are indicated in parentheses.

**Mitogenic responses.** Phytohemagglutinin (PHA) and lipopolysaccharide (LPS) were purchased from Difco Laboratories, Detroit, Mich. Concanavalin A (ConA) was obtained from Pharmacia, Uppsala, Sweden. Responder spleen cells (10⁵) were cultured with PHA (10 μg/ml), LPS (50 μg/ml), or ConA (5 μg/ml) in 0.2 ml of culture medium in 96-well flat-bottomed microculture plates at 37°C in 7.5% CO₂. Proliferation was determined on day 3 by the same method as described above.

**RT assay.** Portions (0.1 ml) of serum samples were centrifuged at 16,000 × g for 90 min in tubes from Beckman Instruments, Inc., Fullerton, Calif. The pellets were suspended in 15 μl of a detergent solution containing 0.6% Triton X-100, 50 mM Tris hydrochloride, 100 mM NaCl, and 1 mM EDTA. Portions (10 μl) of this suspension were mixed with reverse transcriptase (RT) buffer (40 μl) containing 50 mM Tris hydrochloride (50 mM KCl, 10 mM MgCl₂ (0.25 mM MnCl₂), 5 mM dithiothreitol, 10 μM (703 GBq/mmol) [³H]dTTP (ICN Pharmaceuticals Inc., Irvine, Calif.), and 25 μg of poly(rA)·dT (Pharmacia) per ml. The reaction mixtures were incubated at 37°C for 1 h in Beckman tubes and spotted on DE81 filters (Whatman, Inc., Clifton, N.J.). The filters were washed five times with 0.5 M Na₂HPO₄, five times with double-distilled water, and twice with alcohol. The dried filters were placed in vials with 10 ml of aqueous scintillation fluid, and radioactivities were counted with a Tri-Carb counter (Packard Instrument, Co., Inc., Rockville, Md.).

**Determination of the IgG level in serum.** Immunoglobulin G (IgG) levels in serum for each experimental mouse were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was carried out with the PhastSystem (Pharmacia) and 8 to 25% PhastGel (Pharmacia) under nonreducing conditions. Purified human IgG and albumin fractions were used to identify the IgG band. Relative concentrations of IgG in serum samples (expressed as a percentage of total serum protein) were determined with an UltraScan XL densitometer (LKB Pharmacia).

**AZT treatment.** 3'-Azido-3'-deoxythymidine (AZT) (10) was purchased from Sigma Chemical Co., St. Louis, Mo. Mice were treated with AZT according to the schedule indicated in Results. AZT (1 mg/ml) in drinking water was given to LP-BM5 MuLV-inoculated mice. By monitoring water consumption, we estimated that this concentration provided a dose of approximately 250 mg/kg per day.

**RESULTS**

**Immunodeficiency induced in B10 mice by LP-BM5 MuLV inoculation.** For the rapid evaluation of antiviral effects of test drugs, it would be advantageous to use viruses that are able to induce a rapid and severe immunodeficiency in host experimental mice. In the first experiment, we examined the effect of LP-BM5 MuLV inoculation on the proliferative responses of spleen cells from B10 mice against alloantigens or mitogens. Spleen cells from untreated B10 mice or B10 mice intraperitoneally inoculated with LP-BM5 MuLV were stimulated in vitro with class I alloantigens (B6.C-H⁻²⁺⁻¹⁻), class II alloantigens (B6.C-H⁻²⁻²⁻), ConA (5 μg/ml), or LPS (50 μg/ml). The cultures were assayed for their proliferative responses by measurement of [³H]thymidine incorporation on day 3 (mitogenic responses) or day 4 (allogeneic responses). The stimulation index was calculated by the following formula: stimulation index = experimental counts per minute (with alloantigen or mitogen)/control counts per minute (without alloantigen or mitogen).

<table>
<thead>
<tr>
<th>Stimulator</th>
<th>Control</th>
<th>3 wk</th>
<th>5 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6.C-H⁻²⁻¹⁻ (class I)</td>
<td>6.4 (0.01)</td>
<td>1.4 (0.1)</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td>B6.C-H⁻²⁻²⁻ (class II)</td>
<td>3.6 (0.6)</td>
<td>1.0 (0.1)</td>
<td>0.6 (0.01)</td>
</tr>
<tr>
<td>ConA</td>
<td>7.7 (0.4)</td>
<td>2.8 (0.3)</td>
<td>1.4 (0.04)</td>
</tr>
<tr>
<td>LPS</td>
<td>8.1 (0.2)</td>
<td>2.5 (0.2)</td>
<td>1.6 (0.08)</td>
</tr>
</tbody>
</table>

* Spleen cells from untreated control or LP-BM5 MuLV-inoculated (intraperitoneally) B10 mice were cultured with class I alloantigens (B6.C-H⁻²⁻¹⁻), class II alloantigens (B6.C-H⁻²⁻²⁻), ConA (5 μg/ml), or LPS (50 μg/ml). The cultures were assayed for their proliferative responses by measurement of [³H]thymidine incorporation on day 3 (mitogenic responses) or day 4 (allogeneic responses). The stimulation index was calculated by the following formula: stimulation index = experimental counts per minute (with alloantigen or mitogen)/control counts per minute (without alloantigen or mitogen).

**TABLE 2. APC activity of spleen cells from B10 mice inoculated with LP-BM5 MuLV**

<table>
<thead>
<tr>
<th>KLH (10 μg/ml)</th>
<th>[³H]Thymidine incorporation by 8-5 clone cells (SE) at the indicated time after LP-BM5 MuLV inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3 wk</td>
</tr>
<tr>
<td>Absent</td>
<td>0.7 (0.1)</td>
</tr>
<tr>
<td>Present</td>
<td>42.5 (3.0)</td>
</tr>
</tbody>
</table>

* KLH-specific, I-A⁻K⁻-restricted cloned helper T cells (8-5 clone cells) were cultured with mitomycin C-treated spleen cells from untreated or LP-BM5 MuLV-inoculated (intraperitoneally) B10 mice in the absence or presence of antigen (KLH). The proliferation of cloned helper T cells was assayed on day 3. [³H]thymidine incorporation is reported in counts per minute (thousands).
as the APC source for testing the proliferative responses of keyhole limpet hemocyanin (KLH)-specific, I-A<sup>e</sup>-restricted helper T-cell clones (8-5 clone cells) (Table 2). In the presence of APCs from untreated control B10 mice, 8-5 clone cells proliferated very well in an antigen-specific manner. In the presence of APCs from LP-BM5 MuLV-infected mice, however, the antigen-specific proliferative response of 8-5 clone cells decreased progressively during the course of virus infection. The results indicated that LP-BM5 MuLV is able to induce a rapid and severe immunodeficiency in terms of both T-cell proliferative responses and APC activity in B10 mice.

**Table 3.** Effect of AZT administration on LP-BM5 MuLV-induced immunodeficiency in B10 mice<sup>a</sup>

<table>
<thead>
<tr>
<th>Group</th>
<th>LP-BM5 MuLV inoculation</th>
<th>AZT administration</th>
<th>Body wt (g)</th>
<th>Spleen wt (mg)</th>
<th>No. of spleen cells (10&lt;sup&gt;6&lt;/sup&gt;)</th>
<th>PHA response (change in cpm [10&lt;sup&gt;5&lt;/sup&gt;])</th>
<th>APC activity (change in cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>No</td>
<td>27.5 (0.5)</td>
<td>70.7 (1.4)</td>
<td>105 (8)</td>
<td>61.6 (13.9)</td>
<td>4,964 (1,993)</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>No</td>
<td>26.3 (0.3)</td>
<td>310.2 (14.2)</td>
<td>266 (10)</td>
<td>-0.3 (0.2)</td>
<td>186 (104)</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>0.3 mg/ml</td>
<td>26.5 (0.3)</td>
<td>123.8 (17.6)</td>
<td>143 (26)</td>
<td>90.2 (30.6)</td>
<td>3,906 (1,430)</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>1.0 mg/ml</td>
<td>27.2 (0.4)</td>
<td>107.8 (10.1)</td>
<td>140 (6)</td>
<td>48.6 (22.2)</td>
<td>3,567 (1,081)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Untreated control B10 mice (group 1), LP-BM5 MuLV-inoculated B10 mice (group 2), or B10 mice treated with AZT beginning 3 days before LP-BM5 MuLV inoculation (groups 3 and 4) were sacrificed 5 weeks after viral inoculation. Body weight, spleen weight, and spleen cell number were determined for each experimental mouse (four mice per group). Spleen cells from each experimental mouse were prepared for the mitogen response determination as well as for the APC assay. The PHA response was assayed on day 3 of the culture, while APC activity was assessed by the proliferative responses of cloned helper T cells (8-5 clone cells) on day 3 as described in Materials and Methods. Data for the PHA response and APC activity are expressed as the arithmetic mean change in the counts per minute of triplicate cultures (counts per minute of mitogen- or antigen-stimulated culture minus counts per minute of culture without mitogen or antigen, respectively). Standard errors are shown in parentheses.

**Table 4.** Statistical analyses of the data reported in Table 3<sup>a</sup>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P value for group:</th>
<th>1 vs 2</th>
<th>1 vs 3</th>
<th>1 vs 4</th>
<th>2 vs 3</th>
<th>2 vs 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Spleen wt</td>
<td>&lt;0.001</td>
<td>0.05 &lt; P &lt; 0.1</td>
<td>0.02 &lt; P &lt; 0.05</td>
<td>0.001 &lt; P &lt; 0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of spleen cells</td>
<td>&lt;0.001</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>PHA response</td>
<td>&lt;0.001</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>APC activity</td>
<td>0.1 &lt; P &lt; 0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
<td>&gt;0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Statistical analyses were carried out with the Student t test.
TABLE 5. Effect of duration of AZT administration on LP-BM5 MuLV-induced immunodeficiency in B10 mice

<table>
<thead>
<tr>
<th>Group</th>
<th>CPB inoculation</th>
<th>AZT administration</th>
<th>PHA response (change in cpm)</th>
<th>APC activity (change in cpm)</th>
<th>IgG concn (% of total serum protein)</th>
<th>RT activity (cpm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>No</td>
<td>27,669 (7,870)</td>
<td>19,036 (1,740)</td>
<td>7.1</td>
<td>4,170</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>No</td>
<td>813 (163)</td>
<td>4,505 (694)</td>
<td>27.6</td>
<td>33,190</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Days 0 to 15</td>
<td>25,738 (4,613)</td>
<td>28,558 (2,321)</td>
<td>9.5</td>
<td>370</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Days 8 to 15</td>
<td>3,823 (2,009)</td>
<td>18,004 (4,235)</td>
<td>14.1</td>
<td>1,270</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>Days 15 to 35</td>
<td>1,500 (181)</td>
<td>4,862 (593)</td>
<td>18.1</td>
<td>4,040</td>
</tr>
</tbody>
</table>

* AZT treatment was started on the day of LP-BM5 MuLV inoculation (group 3), 1 week later (group 4), or 2 weeks later (group 5). See Table 3, footnote a, for details on the PHA response and APC activity. Each group contained six mice, and data are expressed as the arithmetic mean change in the counts per minute of triplicate cultures. Standard errors are shown in parentheses. Determination of the IgG level and RT activity in serum is described in Materials and Methods. The data presented were determined with pooled serum samples from mice in each group.

group 1 versus group 5). Although the level of RT activity was as low as that in untreated control mice, however, immunological activities were not recovered in such mice (see above). The findings suggest that the IgG level in serum but not the RT activity in serum correctly reflected the immunological status of experimental mice.

DISCUSSION

The use of murine models for screening anti-HIV drugs is restricted to antiviral agents designed to inhibit the functions of gene products which are commonly shared between MuLV and HIV, such as gag, pol, or env. Since no murine lentiviruses have been identified so far, it has been impossible to evaluate anti-HIV drugs targeted against specialized HIV genes, such as tat, vif, nef, and rev, which are not present in MuLV. In the present study, one of the most powerful RT inhibitors, AZT, was chosen as a positive control test drug. Our results demonstrated that B10 mice infected with LP-BM5 MuLV responded to AZT treatment and were protected from severe immunodeficiency when the treatment was initiated at the time of virus inoculation (Tables 3 to 6).

We propose this MAIDS system as a possible screening protocol for antiretrovirus drugs in vivo for the following reasons. First, and most importantly, it is not necessary to use expensive, valuable primates. Recent studies confirmed the existence of an immunodeficiency virus in cats, termed feline immunodeficiency virus (14, 23), which is closely related to HIV. Other recent studies demonstrated that mice with severe combined immunodeficiency and reconstituted with human hematopoietic stem cells (SCID-hu mice) were successfully infected with HIV (9, 13). Although cats or SCID-hu mice might be excellent experimental animals for AIDS studies, we believe that laboratory mice are the best animals for drug screening because of their genetic homogeneity and their availability. Second, this MAIDS system takes a relatively short time (less than 6 weeks) to reach an evaluation of a test drug. This short test time would aid rapid drug screening. Third, even without sacrificing experimental mice, we can estimate whether a drug treatment is effective by analyzing IgG levels in serum (Tables 5 and 6).

LP-BM5 MuLV is a somewhat unique C-type retrovirus that induces a long-lasting immunodeficiency status rather than progressive tumors in adult mice of susceptible strains (3). With such a virus it is thus possible to evaluate antiviral drugs by examining the time course of changes in the biological and immunological parameters rather than mortality. For example, as already mentioned above, IgG levels in serum showed a good correlation with the immunodeficiency status of LP-BM5 MuLV-infected B10 mice. We also found that the serum-soluble interleukin-2 receptor levels were elevated during the course of virus infection (unpublished observation). This phenomenon was recently reported by Honda et al. (4), who analyzed serum-soluble interleukin-2 receptor levels in the serum of AIDS patients, and might be another good indicator of the immunodeficiency status of MAIDS mice. In contrast, RT activity in serum was found to be an inappropriate parameter for the evaluation of antiretrovirus drugs, because even when RT activity in serum was depressed by AZT, host immune responses were not restored by the treatment (Tables 5 and 6). We assume that once the immunodeficiency was triggered by the virus infection, it persisted regardless of whether viruses continuously existed.

A number of studies have used murine models for antiretroviral therapy as well as prophylaxis (17–20). Most of them, however, used MuLVs which had very short latent periods and which therefore rapidly induced leukemia rather than immunodeficiency. The LP-BM5 MuLV used in the present study was able to induce an immunodeficiency which might not have been caused by tumor development or by an immunosuppressive component derived from retroviral envelope protein p15E (22). Although little is known about the induction mechanisms for LP-BM5 MuLV-induced immunodeficiency, this MAIDS system may allow rapid and inexpensive screening for antiretrovirus agents that would be also effective for human AIDS.

ACKNOWLEDGMENT

We thank Herbert C. Morse III for providing the LP-BM5 MuLV-infected stroma cell line G6 and for helpful suggestions and kind encouragement during the course of this study.

TABLE 6. Statistical analyses of the data reported for the PHA response and APC activity in serum in Table 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 vs 2</th>
<th>1 vs 3</th>
<th>1 vs 4</th>
<th>1 vs 5</th>
<th>2 vs 3</th>
<th>2 vs 4</th>
<th>2 vs 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA response</td>
<td>0.05 &lt; P &lt; 0.1</td>
<td>&gt;0.2</td>
<td>0.1 &lt; P &lt; 0.2</td>
<td>0.05 &lt; P &lt; 0.1</td>
<td>0.01 &lt; P &lt; 0.02</td>
<td>&gt;0.2</td>
<td>0.5 &lt; P &lt; 0.1</td>
</tr>
<tr>
<td>APC activity</td>
<td>0.001 &lt; P &lt; 0.01</td>
<td>0.05 &lt; P &lt; 0.1</td>
<td>&gt;0.2</td>
<td>0.001 &lt; P &lt; 0.01</td>
<td>&lt;0.001</td>
<td>0.05 &lt; P &lt; 0.1</td>
<td>&gt;0.2</td>
</tr>
</tbody>
</table>

* See Table 4, footnote a.
LITERATURE CITED


