T Lymphocyte Function as the Principal Target of Lymphocytic Choriomeningitis Virus-Induced Immunosuppression

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Plaque-forming cell responses against sheep erythrocytes, Escherichia coli lipopolysaccharide, pneumococcal polysaccharide, and polyvinylpyrrolidone were examined in mice infected with lymphocytic choriomeningitis virus. A 92 to 96% reduction of the thymus-dependent anti-sheep erythrocyte responses was observed 2 to 4 weeks after infection. However, the thymus-independent responses against the three other antigens were close to normal at all stages of the infection. Studies on allograft immunity of infected C57Bl/6 mice against DBA/2 mastocytoma cells revealed a severe suppression of the T cell-mediated cytotoxic response which was temporally related to the impaired humoral responsiveness against sheep erythrocytes. The capacity of spleen cells from infected mice to restore immune responsiveness of lethally irradiated recipients against sheep erythrocytes was significantly reduced. The adoptive responses, however, were clearly improved when normal thymus cells were added to the inferior spleen cells. Moreover, it appeared that the spleen cells from immunosuppressed donor mice could not confer suppression to normal lymphoid cells. The presented findings are consistent with the assumption that a numeric deficiency of T cells, or cells belonging to some T cell subpopulation, is the primary cause of lymphocytic choriomeningitis virus-induced immunosuppression.

The mechanisms by which viruses may depress the function of the immune apparatus have remained a controversial subject (10, 32, 36, 44). In a recent paper (5) on the immunosuppressive effect of the lymphocytic choriomeningitis (LCM) virus it was reported that apparently this virus acted by causing a numerical deficiency of cells belonging to the population of radiosensitive immunocompetent cells. Moreover, it was suggested that this cellular deficiency was probably a consequence of an LCM virus-induced suppression of the lymphoid precursor cells (4, 5). These investigations, however, were not designed to ascertain whether the LCM virus infection affected preferentially bone marrow-derived (B) or thymus-processed (T) lymphocytes, or whether perhaps both these cell populations were influenced.

From an earlier study on the LCM virus (27), it appears that the virus interferes with humoral immune responses against thymus-dependent antigens such as sheep erythrocytes (SRBC) and human serum albumin. Moreover, some observations have been reported which might suggest a concomitant suppression of cell-mediated immunity. Thus, the footpad responses of LCM virus-infected mice against unrelated viruses were reduced (27), and data on skin allograft survival indicated a significant, although very moderate, enhancing effect of the virus (24). In the present work, B and T cell functions in LCM virus-infected animals were investigated by employing a number of thymus-independent antigens and by studying the allograft responses in infected mice by means of the in vitro measurements of cytotoxic cells. The observations indicated a significant suppression of T cell-dependent immune functions exclusively. The findings were supported by data which were obtained in cell transfer experiments. These data were consistent with the assumption that a numerical deficiency of T cells was the primary cause of the LCM virus-induced immunosuppression and, in addition, they argued against mechanisms involving suppressor cells or suppressive humoral factors which have been reported to play a role in antigenic competition (12, 40, 41) and in immunosuppression with other viruses (14, 22).

MATERIALS AND METHODS

**Virus.** The LCM virus strain employed was the Traub strain. This strain was obtained from E. Traub (Tübingen, Germany) in 1960. In this laboratory the
Virus was kept at -70° C as 10% stock suspensions of spleens from intraperitoneally (i.p.) infected C57/BL6 mice. A total of 20 mouse passages were carried out. The virus preparations used in this study were tissue culture fluid obtained after three or four additional passages of the virus in monolayers of L cells grown in Eagle minimum essential medium. The preparations were kept at -70° C. Dilutions of the virus were prepared in phosphate-buffered saline (PBS), and virus titrations were carried out as previously described (5) by intracranial inoculation into white Swiss mice.

Mice. Except for those used for titration purposes, all animals employed were strictly inbred C57/BL6 mice (originally obtained from Statens Seruminstitut, Denmark). The mice were 2 to 3 months old, and in transplantation experiments cell donors and recipients were of the same sex. Infections were produced by i.p. inoculation of 10^6 mean lethal doses (LD_{50}) of LCM virus. This injection causes a symptomless and transient infection with maximum viremia on day 6 to 8 (5).

Antigens and immunizations. SRBC stored in Alsever solution were washed three times in PBS. The suspension was adjusted to contain about 2 x 10^8 SRBC per 0.5 ml, which was injected i.p. or intravenously (i.v.) as described. Lipopolysaccharide (LPS) of Escherichia coli O55:B5 was obtained from Difco Laboratories, Detroit, and was extracted by the method of Westphal et al. (43). A solution containing 1 mg of LPS per ml of PBS was boiled for 1 h immediately before use. After centrifugation the solution was adjusted to contain 50 µg of LPS per 0.5 ml, which was injected i.v. Type III pneumococcal polysaccharide (S III), made as described by Katz and Pappenheimer (20), was obtained from Wellcome, England, and 0.5 µg of S III in 0.5 ml of PBS was used for i.v. immunization. Polyvinylpyrrolidone (PVP) K 90, from Fluka AG, Switzerland, was dissolved in PBS, and amounts of 0.5 µg in 0.5 ml were given i.v. The immunization doses employed were all selected so as to give rise to near-optimal immune responses against the respective antigens.

P-815-X2 mastocytoma cells of DBA/2 origin were kindly donated by K. T. Brunner and J.-C. Cerottini, Lausanne, Switzerland. The ascites tumor was maintained by serial transplantations into the strain of origin. For immunization, 5 x 10^4 cells in 0.5 ml of PBS were injected i.p.

Assays for PFC. Individual lymphoid cell suspensions were prepared by passing the spleens through stainless-steel meshes. After a wash in Eagle minimum essential medium with Hanks salts, appropriate dilutions of the spleen cells were assayed for plaque-forming cells (PFC), using SRBC or antigen-coated SRBC (see below). Agarose A 37 (L'Industrie Biologique Francaise S.A.), 0.5% in Eagle minimum essential medium (0.4 ml), 10% SRBC (0.05 ml), and the spleen cell suspensions (0.1 ml) were mixed and poured onto microscope slides precoated with 0.1% agarose in water. The slides were incubated for 1 h at 37° C in a humid atmosphere containing 5% CO_2. Guinea pig serum diluted 1:10 in Eagle medium was added, and the incubation was continued for 2 h. The plaques were counted by indirect illumination with a magnifying glass.

LPS-coated SRBC were prepared as described by Möller and Michael (30). Packed and washed SRBC (0.1 ml/ml) were added to a boiled solution containing 1 mg of LPS per ml of PBS. After incubation at 37° C for 30 min, the SRBC were washed three times before use in the PFC assay. S III-coated SRBC were made as described by Baker et al. (2). Amounts (0.5 ml) of packed SRBC were suspended in 1.0 ml of saline containing 1 mg of S III. Then 1.0 ml of 0.1% chromium chloride (CrCl_2·6H_2O) in saline was added under mixing. Incubation was carried out at room temperature for 5 min and followed by four washes with saline. The coating with PVP was performed essentially as described by Anderson and Blomgren (1). SRBC pretreated with 1:20,000 tannic acid at 37° C for 15 min were washed twice in PBS. To a 4% suspension of the tanned cells was added an equal amount of PBS containing 800 µg of PVP K 15 (Fluka AG, Switzerland) per ml. After incubation of the mixture at room temperature for 15 min, the labeled cells were washed three times with PBS.

Splenic PFC were always calculated from the counts of duplicate assays of individual spleen cell suspensions. As to the PFC responses against LPS, S III, and PVP, the numbers of PFC were corrected for background PFC, which were determined by testing the spleen cells against normal or tannic acid-treated SRBC. PFC counts of normal nonsensitized mice did not exceed 200 PFC per spleen in tests carried out against any of the four antigens under study.

In vitro measurement of cytotoxic cells. Spleen cells suspensions were prepared by homogenizing pooled spleens by one stroke by hand in a Ten-Broek grinder. After sedimentation for 45 min at 4°C, the cells of the supernatant fluid were washed four times in Parker medium without serum added by centrifugation at 400 x g for 10 min, and finally the lymphoid cells were resuspended in Parker medium with 10% inactivated fetal calf serum so as to contain 10^6 cells per ml. DBA/2 mastocytoma target cells were grown in suspension culture in Eagle minium essential medium with 10% inactivated fetal calf serum. For labeling, the technique described by Cerottini and Brunner (6) was followed by incubating 5 x 10^4 cells at 37° C for 30 min in 1 ml of medium containing 50 µCi of ^51Cr as Na_2CrO_4 (Amersham, England, specific activity > 200 mCi/mg). After five washes the cells were resuspended in medium at a concentration of 10^4 cells per ml.

The quantitation of the ^51Cr release from target cells was performed as previously described (19). Briefly, volumes of 0.6-ml reaction mixtures containing 3 x 10^4 labeled target cells and 50 or 100 times 3 x 10^4 spleen cells were incubated in test tubes set up in roller drums (20 rotations/h). After incubation for 18 h at 37° C, the tubes were centrifuged for 10 min at 1,000 x g, and 0.4 ml of the supernatant was added to the scintillation fluid containing the nonionic detergent BBS-3 (Beckman). Radioactivity was measured in a Beckman LS-233 liquid scintillation counter. The total and the spontaneous ^51Cr release were determined for each preparation of target cells employed.
Total release was determined by adding $2 \times 10^4$ target cells to the scintillation fluid, and ranged between 19,000 and 21,400 counts/min. Spontaneous release was measured in target cell cultures incubated as described but without spleen cells added, and ranged between 5,900 and 7,900 counts/min. The cytotoxicity of a given spleen cell suspension was expressed as the mean counts per minute obtained from five parallel cultures minus the mean counts per minute spontaneously released. The variation which was recognized between parallel cultures was always minimal and has therefore been omitted from the tables.

**Lymphoid cell transfer experiments.** Cell suspensions were prepared by passing pooled donor spleens or thymuses through stainless-steel meshes. After two washes with Hanks salt solution, cell counting, adjusting, and mixing were performed so that the desired cell transplantation doses were contained in volumes of 0.5 ml. Recipient mice were treated with 800 R of X rays. The irradiation was administered by a Siemens Stabilinpan therapy machine operated with the following factors: 200 kV, 15 mA, 1.0-mm copper filtration. The dose rate was 47 R/min, and half-value layer was 1.5-mm copper. Within 3 h after irradiation the recipients were injected i.v. with the donor cells, and immediately afterwards they received $2 \times 10^4$ SRBC i.p.

**Complement fixation test.** Complement-fixing antibodies against LCM virus antigen were measured by a technique previously described (3).

**RESULTS**

**PFC responses of LCM virus-infected mice against thymus-dependent and thymus-independent antigens.** In the first series of experiments, the thymus-dependent immune responses to SRBC and the thymus-independent immune responses to LPS (1, 30), S III (17), and PVP (1) were examined at various times postinfection with LCM virus. In all experiments the same time schedule was employed. On days 24, 14, and 4 before immunization, groups of five mice were inoculated i.p. with $10^3$ LD$_{50}$ of the virus. On day 0 the antigen was injected i.v. into uninfected controls and into each of the infected groups. Four days later, i.e., days 28, 18, and 8 postinfection, all the mice were killed, and their spleens were assayed for PFC employing SRBC or SRBC coated with the respective antigen as described above. The experimental data are recorded in Table 1. In accordance with previous findings (5, 27), the PFC responses against SRBC were unaffected during the first week of infection but were reduced by 92 to 96% in animals examined 18 and 28 days after virus infection. With regard to the thymus-independent antigens, experiments were performed in duplicates. It may be noted that relatively few PFC appeared in all mice sensitized with S III, or PVP as compared with those that could be achieved with SRBC or LPS. To evaluate the data, the Wilcoxon rank test was used to test the PFC counts of infected groups versus the corresponding control groups. The 0.05 significance level was reached in the case of two groups sensitized with thymus-independent antigens: one group showed a threefold increase and the other showed a twofold reduction as compared with the control groups (Table 1). For the evaluation of the results, however, the relatively large number of groups (24) should

<table>
<thead>
<tr>
<th>Table 1. PFC responses against SRBC, LPS, SIII, and PVP in the course of acute LCM virus infection*</th>
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<tbody>
<tr>
<td>Antigen</td>
</tr>
<tr>
<td>---------</td>
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<tr>
<td></td>
</tr>
<tr>
<td>SRBC</td>
</tr>
<tr>
<td>LPS</td>
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<tr>
<td>LPS</td>
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<tr>
<td>S III</td>
</tr>
<tr>
<td>S III</td>
</tr>
<tr>
<td>PVP</td>
</tr>
<tr>
<td>PVP</td>
</tr>
</tbody>
</table>

*LCM virus ($10^3$ LD$_{50}$) was given i.p.; $2 \times 10^4$ SRBC, 50 μg of LPS, 0.5 μg of S III, or 0.5 μg of PVP was given i.v.
*Means and ranges of groups (each comprising five mice). p.i., Postinfection.
Significance at the 0.01 level relative to uninfected controls.
Significance at the 0.05 level relative to uninfected controls.
also be considered. Taking this into account, it seems justified to conclude that the responsiveness against the thymus-independent antigens was either unaffected by the LCM virus infection or, at most, very slightly influenced as compared with the pronounced suppression of the anti-SRBC response.

To complete the report on the described experiments, it should be added that the development of LCM infection after virus inoculation was checked by demonstrating the appearance of specific complement-fixing antibodies against LCM virus antigen.

Cytotoxic responses of LCM virus-infected mice against tumor allografts. In the following experiments the T cell-dependent cytotoxic response against allogeneic cell transplants was examined in LCM virus-infected mice. Groups of six C₃H mice were inoculated i.p. with 10⁶ LD₅₀ of virus 10 and 0 days before sensitization. On day 0 three uninfected C₃H mice and one-half of the infected mice received 5 x 10⁴ DBA/2 mastocytoma cells by the i.p. route. Ten days later, that is days 20 and 10 postinfection, the mice were killed together with three normal C₃H control mice, and the pooled spleen cell suspensions from the six different groups were tested for in vitro cytotoxicity against DBA/2 target cells as described above. The data from the experiment, performed in duplicate, are recorded in Table 2. It can be seen that the results obtained with nonsensitized mice, whether infected or not, were almost identical. LCM virus which might contaminate the spleen cell suspensions prepared from infected mice seemed, therefore, not to affect significantly the chromium-51 release from the target cells. It is apparent from the table that the cytotoxic responses achieved in allografted infected mice were close to normal when tested on day 10 postinfection but were severely suppressed when tested on day 20 postinfection.

It has been shown previously (5, 27) that the LCM virus-induced suppression of the anti-SRBC response is transient and that gradual restoration takes place 1 to 2 months after infection. To further investigate the time course of suppression of the cytotoxic responsiveness, groups of three C₃H mice were infected with LCM virus at various intervals (Table 3). All the animals were injected i.p. with 5 x 10⁶ DBA/2 mastocytoma cells on the same day, and 10 days after this allografting the mice were killed, and their spleen cells were assayed for cytotoxicity as mentioned above. From Table 3 it is evident that a decreased response could be observed as early as day 15 postinfection, and that gradual restoration occurred about 1 month after infection.

### Table 2. Cytotoxicity of spleen cells from LCM virus-infected C₃H mice against DBA/2 mastocytoma cells

<table>
<thead>
<tr>
<th>Expt</th>
<th>Allografting</th>
<th>No virus</th>
<th>Day 10 p.i.</th>
<th>Day 20 p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>0</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>5 x 10⁴ DBA/2 cells i.p.</td>
<td>107</td>
<td>94</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>5 x 10⁴ DBA/2 cells i.p.</td>
<td>75</td>
<td>79</td>
<td>11</td>
</tr>
</tbody>
</table>

* Allografting of C₃H mice was performed 10 days before measurement of cytotoxic cells.

Expressed as (mean counts per minute of five parallel cultures minus mean counts per minutes released spontaneously) x 10⁻². Spleen cell pools were obtained from groups of three mice. p.i., Postinfection.

### Table 3. Cytotoxic responses of LCM virus-infected mice at various intervals after virus inoculation

<table>
<thead>
<tr>
<th>No virus</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 20</th>
<th>Day 24</th>
<th>Day 30</th>
<th>Day 38</th>
<th>Day 49</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>67</td>
<td>0</td>
<td>5</td>
<td>33</td>
<td>33</td>
<td>39</td>
<td>67</td>
</tr>
</tbody>
</table>

* Expressed as (mean counts per minute of five parallel cultures minus mean counts per minute released spontaneously) x 10⁻². Spleen cell pools were obtained from groups of three mice. DBA/2 mastocytoma cells (5 x 10⁴) were given i.p. (DBA/2 mastocytoma cells were also used as target cells).

Lymphoid cell transfer experiments. In a previous report (5) it was suggested that the LCM virus-induced immunosuppression might be accounted for by a simple numeric deficiency of immunocompetent cells. However, in the light of recent reports on Moloney virus-infected mice (14, 22), it seems to be urgent also to consider suppressor cell activity as a possible explanation of the immune defects associated with LCM virus infection. In an attempt to demonstrate suppressor cells in infected immunosuppressed mice, the spleen cells from such mice were examined in the following transplantation assay. Pooled spleen cell suspensions were prepared from 5 to 6 normal mice, and from similar numbers of mice inoculated with 10⁴ LD₅₀ of virus 14 days previously. Then cell preparations were made which contained the following numbers of cells per 0.5 ml: (i) 50 x 10⁴ normal cells, (ii) 50 x 10⁴ cells from infected mice, and (iii) a mixture containing 50 x 10⁴ of each of these cells. These cell doses were injected i.v. into groups of 5 to 6
recipient mice which had previously been treated with 800 R of X rays. After transplantation the recipients received $2 \times 10^8$ SRBC i.p., and 6 days later their spleens were assayed for PFC. From the data recorded in Table 4 it appears that the donor cells from the infected mice gave rise to significantly decreased responses and moreover that the PFV numbers achieved with mixtures of infected and normal cells were close to the sum of PFC produced by the separate cell fractions. The results, therefore, made it unlikely that suppressor cells could play any significant role for the suppressed adoptive responses seen in recipients of cells from infected donors. The findings seemed to exclude the possibility that virus contamination of the transferred cell suspension might have interfered with the results obtained. Blood virus titrations performed just before sacrifice showed that the recipients of cells from infected donors might carry small amounts of virus, ranging from no trace to $10^2$ LD$_{50}$ per 0.03 ml, whether they received such cells alone or in mixtures with normal cells.

The findings presented in this paper have strongly suggested the T lymphocyte population to be the primary target of the LCM virus-induced immunosuppression. This assumption was further tested in the final experiments. From donor groups comprised of eight to nine normal mice and five mice injected with virus 14 days previously, cell preparations were produced which contained the following numbers of cells per inoculation dose: (i) $50 \times 10^6$ normal thymocytes, (ii) $50 \times 10^6$ spleen cells from infected donors, (iii) a mixture of $50 \times 10^6$ of each of these cells, and (iv) $50 \times 10^6$ normal spleen cells. The cell preparations were injected into groups of five lethally irradiated mice, which were then sensitized with SRBC and tested 6 days later for PFC as described above (Table 5). The numbers of PFC found in the recipients of thymus cells were minimal and within the background level. However, when thymus cells were added to the inferior spleen cell populations prepared from infected mice, they caused a significant restoration of the responsiveness against SRBC. The PFC numbers obtained with such mixtures were close to those produced by the normal spleen cells. It is apparent that these results are consistent with the assumption that the restriction of the immune capacity is caused by a T cell deficiency, whereas they are inconsistent with the possibility that the B cells should be the limiting component.

### DISCUSSION

From previous papers (5, 27) on the LCM virus it is apparent that this virus can cause pronounced suppression of the thymus-dependent humoral immune response against SRBC and human serum albumin. The inhibition is expressed during the second week of the acute infection and persists for about 2 months. The LPS, S III, and PVP, which were employed in

<table>
<thead>
<tr>
<th>Cell transplants</th>
<th>PFC per recipient spleen</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(× 10$^{-4}$)</td>
<td></td>
</tr>
<tr>
<td>Normal spleen cells (50 × 10$^6$)</td>
<td>289 (148-444)</td>
<td>0.05</td>
</tr>
<tr>
<td>Suppressed spleen cells (50 × 10$^6$)</td>
<td>62 (33-103)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Normal spleen cells (50 × 10$^6$) + suppressed spleen cells (50 × 10$^6$)</td>
<td>337 (184-516)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Spleen cell pools were obtained from groups of five to six normal mice and five to six mice inoculated i.p. with $10^3$ LD$_{50}$ of virus 14 days previously.

* After irradiation and i.v. transplantation the recipients received $2 \times 10^8$ SRBC i.p.; PFC were scored 6 days later and are given as means and ranges of groups of five to six recipients.

* Wilcoxon rank test versus recipients of $50 \times 10^6$ normal spleen cells (each experiment tested separately).

### TABLE 4. Interaction between LCM virus-suppressed spleen cells and normal spleen cells in the anti-SRBC response after transfer to 800 R-irradiated recipients

<table>
<thead>
<tr>
<th>Cell transplants</th>
<th>PFC per recipient spleen</th>
<th>P*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(× 10$^{-4}$)</td>
<td></td>
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<tr>
<td>Normal thymocytes (50 × 10$^6$)</td>
<td>0.31 (0.05-0.65)</td>
<td>0.05</td>
</tr>
<tr>
<td>Suppressed spleen cells (50 × 10$^6$)</td>
<td>54 (42-68)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Normal thymocytes (50 × 10$^6$) + suppressed spleen cells (50 × 10$^6$)</td>
<td>128 (68-220)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Normal spleen cells (50 × 10$^6$)</td>
<td>178 (72-304)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Thymus and spleen cell pools were obtained from groups of eight to nine normal mice and five mice inoculated i.p. with $10^3$ LD$_{50}$ of virus 14 days previously.

* After irradiation and i.v. transplantation the recipients received $2 \times 10^8$ SRBC i.p.; PFC were scored 6 days later and are given as means and ranges of groups of five recipients.

* Wilcoxon rank test versus recipients of $50 \times 10^6$ normal thymocytes plus $50 \times 10^6$ suppressed spleen cells (each experiment tested separately).
the present work, have all been demonstrated to be strictly thymus-independent antigens (1, 17, 30). Surprisingly, it was found that when these antigens were administered to mice at various stages of the LCM virus infection they gave rise to humoral immune responses which were similar to those found in normal mice. On the other hand, the allograft responses of the infected mice revealed a significant impairment which was temporally correlated to the hyporesponsiveness against the thymus-dependent antigens. The allograft responses, as tested by the in vitro cytotoxicity technique employed, has been clearly defined as a function mediated by T cells without requirement for B cells or macrophages (7, 13). The present findings, therefore, make it justified to assume that the central event in the LCM virus-induced immunosuppression is a disorder affecting the T lymphocytes. The significant restoration in the cell transfer experiments of the immunological capacity of spleen cells from infected animals by the addition of normal thymus cells is consistent with this assumption.

A number of other viruses are also known to depress thymus-dependent immune functions in animals (10, 32; for further references see below) and in humans (21, 23, 32). In most cases, however, the available information is incomplete, and evaluations and generalizations as regards the immunodepressive mechanisms are, therefore, difficult to carry out.

The immunosuppression induced by murine oncornavirus infections has been studied most extensively. The humoral immune responsiveness of radiation leukemia virus-infected mice was recently investigated by Peled and Haran-Ghera (35). Their findings show convincing similarity to the present observations with the LCM virus with regard to clearcut restriction of the viral immunosuppression to thymus-dependent immune functions. Also, Friend leukemia and the Moloney sarcoma viruses are known to cause distinct depression of a variety of in vitro correlates of T cell immunity (14, 22, 29) but affect only to a relatively limited degree responses against bacterial somatic antigens (9, 16). In the papers by Gorczynski (14) and Kirchner et al. (22) on the Moloney virus it was strongly suggested that the immunosuppressive activity of this virus was mediated by a population of suppressor cells, probably belonging to the monocyte-macrophage series. The concept of suppressor cells (12, 41), however, seems not to afford any ready explanation of the present findings with the LCM virus. The cell transfer data recorded in Tables 4 and 5 clearly demonstrate that the immunosuppressed spleen cell population from infected mice would not confer suppression to normal spleen or thymus cells. These experiments also argue against mechanisms involving the elaboration of humoral immunosuppressive factors, which have been suggested to play a role in the antigenic competition phenomenon (40).

Very recently it has been claimed that T cells may carry receptors for certain viruses, e.g., measles, influenza, parainfluenza, and cytomegalovirus (45; A. M. Denman, personal communication; H. F. McFarland, manuscript in preparation). In relation to the present observations, it may seem attractive to propose such T cell receptors for the LCM virus as affording an explanation of a selective destruction of the T lymphocytes. However, the small frequency of infected lymphocytes as estimated in immunofluorescent studies (less than 5% [25]), the noncytopathic nature of the LCM virus, and the demonstration of almost intact immune functions in the heavily infected persistent LCM virus carriers (33) make it rather unlikely that the LCM virus should act by any direct extensive damage to the T lymphocytes.

Certain parallel observations make it interesting to compare the LCM virus with the lactic dehydrogenase (LDH) virus, which is known to depress cell-mediated immunity (18). While both these viruses may cause persistent infections in mice, it is notable that their immunosuppressive effects are largely confined to the acute stage of infection. Shortly after virus inoculation they cause a dramatic decrease in thymocytes (5, 15, 37) and also reduce the number of lymphocytes in the peripheral thymus-dependent regions (15, 37). The studies on the LDH virus indicate that this cellular depression is mediated by the early production of some intermediate soluble factor affecting the T cell population, and that this factor is not adrenocortical steroid (37). In the LCM virus infection there is also some evidence for the existence of an early intermediate depressive factor. This evidence has come from the study of hemopoietic precursor cells, which are severely suppressed in the early stage of the LCM virus infection (4, 5). It was suggested that the factor might have an analogous effect on lymphoid precursor cells, causing inhibition of their proliferation and differentiation and leading to a numeric deficiency of mature immunocompetent cells. In the light of the present findings, this hypothesis might be extended by postulating a selective interference with the T lymphocyte population. Such selectivity might be explained by a preferential effect of the inhibiting factor on T cell precursors, or perhaps more
likely, by assuming a higher cellular turnover rate within the T cell population than within the B cells. The turnover rate of T and B cells is as yet not very well defined. Attempts to estimate the life-span of unprimed B cells have given conflicting results (39), and information about the rate of conversion of stem cells to B cells is poor (31). As to the lymphoid cells within the thymus, their rapid turnover rate is well known. With regard to the peripheral T cells, it has become clear that different subpopulations exist, and that cooperative interaction between these is involved in immune reactions (11, 38). The recent convincing demonstration of peripheral T cell subsets endowed with a short life-span (28, 34) may, therefore, afford support to the foregoing discussed hypothesis.

In connection with the present demonstration of the T lymphocytes as the target of the LCM virus-induced immunosuppression, it should be emphasized that there is strong evidence that T cells play the central role in the recovery from LCM virus infections, and also that they are indispensable in the production of antibodies against the virus (8, 26, 42; M. Volkert, K. Bro-Jørgensen, O. Marker, B. Rubin, and L. Trier, manuscript in preparation). It may, therefore, be suggested that the suppressive effect described could be a significant event in the induction of tolerant and persistent infections with this virus. Moreover, we have recently obtained strong evidence that the effect may also account for a distinct transient impairment of the tumor resistance in acutely LCM virus-infected mice (F. Gütter, K. Bro-Jørgensen, and P. N. Jørgensen, manuscript in preparation).

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LITERATURE CITED


