The Immune Response of the Mouse to Lymphocytic Choriomeningitis Virus. II. Active Suppression of Cell-mediated Immunity by Infection with High Virus Doses

By FRITZ LEHMANN-GRUBE,* JOSEF CIHAK, MARITA VARHO AND ROLANDO TIJERINA

Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Martinistrasse 52, 2000 Hamburg 20, Federal Republic of Germany

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SUMMARY

Infection of CBA/J mice with low doses of strain WE lymphocytic choriomeningitis (LCM) virus resulted in high cell-mediated and relatively low humoral virus-specific immune phenomena; anamnestic responses were marked. In contrast, infection with high doses of this virus induced no or low degrees of cell-mediated immune phenomena but higher antibody concentrations. Subsequent challenge of these mice did not reveal cell-mediated immunity. However, transfer of spleen cells from mice suppressed as to cell-mediated immunity together with virus into X-irradiated recipients led to marked anamnestic responses. Furthermore, when spleen cells from mice previously infected with low virus doses were injected into mice previously infected with high virus doses, cell-mediated immune phenomena could not be induced by concomitantly inoculated virus, although in controls anamnestic responses were readily evoked. Thus, infection with high doses of LCM virus actively suppressed LCM virus-specific cell-mediated immune responses but led to increased production of antibodies. Suppressor cells could not be demonstrated.

INTRODUCTION

Infection of adult mice with lymphocytic choriomeningitis (LCM) virus either leads to illness often resulting in death or runs a subclinical course. Surviving animals eliminate the virus and are protected against an otherwise lethal challenge inoculation. Murine LCM as well as virus clearance and protective immunity are all T lymphocyte-mediated immune phenomena, and the same is true for delayed-type hypersensitivity (DTH) and cellular cytotoxicity in vitro (for review, see Buchmeier et al., 1980; Lehmann-Grube, 1981).

Intra-uterine transfer of LCM virus from the infected mother to unborn mice or experimental inoculation of the virus to newly born mice is usually well-tolerated. These mice do not clear the virus but remain persistently infected. Also, challenge with LCM virus has no harmful consequences, DTH cannot be elicited, and cytotoxic T lymphocyte (CTL) activity is absent. With respect to the T cell compartment, LCM virus carrier mice fulfill all the criteria of immunological tolerance; elimination of clones is the most likely mechanism, but the details are not known (for review, see Lehmann-Grube et al., 1981 a).

As part of our attempts to elucidate the virus-specific immunological unresponsiveness of carrier mice, we have begun analysing a phenomenon which has been known as 'pre-zone' since 1936 (Bengtson & Woooley, 1936). As a rule, mice die when infected by intracerebral (i.c.) inoculation with LCM virus. One exception is the paradoxical survival after infection with high doses of virus (Bengtson & Woooley, 1936; Hotchin & Benson, 1963;
Lehmann-Grube, 1969; Suzuki & Hotchin, 1971; Lehmann-Grube et al., 1981 b). Because of similarities between adult mice surviving infection with high virus doses and neonatal or congenital carrier mice, it had been assumed that the underlying mechanisms are related (Suzuki & Hotchin, 1971; Dunlop & Blanden, 1977). The findings to be reported show that this is probably not the case. They have, however, revealed a pattern of anti-LCM virus immune responses of the mouse whose further analysis promises to lead to an understanding of how antiviral immunity is regulated.

METHODS

Mice. CBA/J female mice were purchased from The Jackson Laboratory, Bar Harbor, Me., U.S.A., and were used when 8 to 14 weeks old. For the purpose of titrating virus, 4- to 6-week-old NMRI mice were employed.

Cell cultures. L cells were propagated with Eagle’s minimum essential medium plus non-essential amino acids and 5% heat-inactivated calf serum. After infection, the serum concentration was increased to 10%. Mouse fibroblastic cells were obtained by trypsinization of near-term CBA/J foetuses and were cultivated and infected essentially as L cells. In these experiments cells passaged once in vitro were used.

Virus. A clear plaque type variant of the WE strain LCM virus (Rivers & Scott, 1936) was used which had been obtained by picking three times in succession clear plaques of intermediate size (Popescu & Lehmann-Grube, 1976). For the preparation of stock virus, L cells were infected with infectious multiplicities of 0.01. After incubation for 40 h, cell-associated virus was released by ultrasonic treatment and disrupted cells plus medium were centrifuged at about 2000 g. The supernatant fluid was dispensed into ampoules to be snap-frozen and stored at −70 °C. Infectious virus was usually quantified by determining numbers of plaque-forming units (p.f.u.) on L cell Petri cultures (Lehmann-Grube & Ambrassat, 1977), but when the virus concentration was low, infectivity was titrated as 50% infectious dose (ID50) by i.c. inoculation of mice followed by i.c. challenge of the survivors (Lehmann-Grube, 1964). In a series of parallel titrations, 10 ID50 had been found to be equivalent to 1 p.f.u. (F. Lehmann-Grube, unpublished results). Using these figures, p.f.u. were always converted to ID50 values, and in the following all results from titrations are expressed as infectious units (IU).

Mode of infection of mice and determination of virus multiplication in their organs. Mice were infected by intravenous (i.v.) inoculation of 0.3 ml vol. containing the desired quantities of virus. Organs were homogenized 5% (w/v) with Hanks’ balanced salt solution (HBSS) supplemented with 1% heated calf serum. The homogenates were centrifuged for 10 min at about 2000 g and the supernatant fluids were snap-frozen at −70 °C to be titrated in cell cultures or mice. Our method of determining infectious spleen cells (IC) has recently been described by Tijerina et al. (1980).

Mice infected with strain WE LCM virus produce neutralizing antibody (see Results), and it was feared that the true concentrations of infectious virus in the organs, especially late after infection, might be falsified by neutralization. This possibility was tested by the following experiment. Mice were infected by intraperitoneal inoculation and 6 days later their spleen cells were dispersed with a small amount of HBSS. Aliquots of this cell suspension were mixed with equal volumes of three freshly prepared sera from CBA/J mice, 40 days after they had been infected with 105 or 107 IU, and from normal CBA/J mice, respectively. As a further control, heat-inactivated calf serum was also employed. These four serum–cell mixtures were incubated for 2 h at 37 °C with frequent shaking. They were cooled to 0 °C, treated with ultrasound, diluted 1:10, and centrifuged. The supernatant fluids were snap-frozen at −70 °C and p.f.u. were determined a few days later. As compared with the control sera (normal mouse and calf), sera from mice infected 40 days previously with low or
high virus doses reduced the titres of infectious virus in spleen cell homogenates less than twofold and less than fourfold respectively. In a repeat experiment, sera were employed from mice 25 days after their infection with $10^2$ and $10^7$ IU, and these did not at all affect the virus titres of infected and disrupted spleen cells. We conclude that loss of infectivity from organs of LCM virus-infected mice due to the presence of neutralizing antibody need not greatly concern us.

**Adoptive transfer of spleen cells and irradiation of recipient mice.** Spleen cells were separated with a pair of forceps into HBSS supplemented with 1% calf serum. When desired, B lymphocytes were removed by use of nylon wool columns (Julius et al., 1973). Nucleated 'viable' cells were counted with a haemocytometer, viability being based on exclusion of trypan blue. Mice were whole-body irradiated with 250 rad or 850 rad of 200 kVp X-rays (0.5 mm copper filter) at a dose rate of 400 rad/min.

**Methods for measuring immune responses in mice.** In the $^{51}$Cr release assay for determining CTL, target cells were L cells 48 h after infection with LCM virus, effector to target cell ratio was 32:1, and incubation was for 14 h at 37 °C. Maximal and spontaneous releases were determined, respectively, after freezing and thawing three times the contents of four cultures and after incubation of four cultures containing target cells only, and the percentage specific release was calculated with the formula

$$\% \text{ }^{51}\text{Cr release} = \frac{\text{Release in the presence of spleen cells} - \text{spontaneous release}}{\text{Maximal release} - \text{spontaneous release}} \times 100$$

The footpad reaction (FPR) (Hotchin, 1962) was quantified according to Lehmann-Grube & Löhler (1981). The degree of swelling is expressed as the ratio obtained by dividing the mean of thicknesses of inoculated right feet by the mean of thicknesses of left (control) feet. In non-immune mice, thickness does not increase before the fifth day, and in such animals the left foot remained without inoculum. When secondary responses were determined, the left foot received 0.03 ml of diluent.

Neutralizing antibody was measured by a plaque-reduction test (Kimmig & Lehmann-Grube, 1979), and the titre is defined as the reciprocal of that serum dilution which, as calculated by linear interpolation, reduced plaque numbers by 50% in comparison with controls. In our test for measurement of complement-fixing (CF) antibody (Gschwender et al., 1976), the titre is defined as the reciprocal of that serum dilution which, with antigen in excess, binds enough complement to inhibit the lysis of a given quantity of sensitized sheep red blood cells (SRBC) by about 50%.

**RESULTS**

**High virus dose survival of CBA/J mice**

CBA/J mice were infected by i.e. inoculation with either $10^2$ or $10^7$ IU of strain WE LCM virus and deaths were recorded (Table 1). Most of the animals which had received the smaller dose died. When the inoculum contained $10^5$ times more virus, the death rate was significantly lower. Thus, as other mouse strains, CBA/J mice exhibit high virus dose survival.

High virus dose survival is observed with mice infected by i.e. inoculation. Hence, this route should have been chosen to study the role immune responses play in this phenomenon. However, after i.e. infection with LCM virus, many mice were dead by day seven, and in order to allow experimentation during longer time intervals, mice were infected i.v. *A priori*, this change of route of inoculation seemed permissible on the ground that in the case of i.e. administration, also, the greater part of the inoculum immediately enters the circulation (Cairns, 1950), and it is undoubtedly this part which initiates the immune response. If this
Fig. 1. Footpad reactions of CBA/J mice which had received $10^2$ (△) or $10^7$ (●) IU of virus intravenously and immediately thereafter $10^4$ IU into one hind foot. A third group of mice received only the latter treatment (■). Data points are means of measurements from 15 mice.

Table 1. Lethality of CBA/J mice infected by intracerebral inoculation with either high or low doses of LCM virus, strain WE

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Virus dose (IU)</th>
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<tbody>
<tr>
<td></td>
<td>$10^7$</td>
<td>$10^2$</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>11/63*</td>
<td>58/59</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5/51</td>
<td>51/51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16/114 (14%)</td>
<td>109/110 (99%)</td>
<td></td>
</tr>
</tbody>
</table>

* Number of mice dead between 5th and 20th day after infection/number infected.

assumption is correct, mice will survive if inoculated i.c. with a potentially lethal dose and, in parallel, i.v. with a large quantity of virus. The experimental confirmation was obtained in an experiment in which 100 mice were inoculated i.c. with $10^2$ IU. Immediately thereafter half of the animals were given $10^2$ IU i.v. and the other half $10^7$ IU. All 50 mice which had received $10^2$ IU via both routes were dead by day 11, whereas of the 50 mice which had been inoculated with $10^7$ IU i.v. only four had died between the fifth and the 20th day.

Cytotoxic T cell response in mice infected with high or low doses of virus

Already published experiments (Cihak & Lehmann-Grube, 1978; Lehmann-Grube et al., 1981b) have shown that in mice infected with high doses of strain WE virus, the CTL response was comparatively low. In the meantime, this observation was confirmed in numerous similar experiments in which the cytotoxic activity of spleen cells of CBA/J mice against LCM virus-infected syngeneic target cells was measured after their i.v. infection with $10^2$ or $10^7$ IU. Consistently, the lower dose led to strong and characteristic CTL activity, whereas after inoculation of $10^5$ times more virus the response was abortive; sometimes mice infected with $10^7$ IU did not respond at all.

In order to obtain information as to the dose required to reduce CTL activity, CBA/J mice were infected i.v. with different quantities of virus. Activities were high in mice infected with $10^3$ IU, slightly lower in mice infected with $10^5$ IU and virtually absent in mice infected with $10^7$ IU (data not shown). Apparently, at least $10^5$ IU are needed for suppression.
Immune response of mice to LCM virus

Table 2. Development of neutralizing (N) and complement-fixing (CF) antibodies in CBA/J mice infected by intravenous inoculation with low or high doses of LCM virus, strain WE

<table>
<thead>
<tr>
<th>Time after infection (weeks)</th>
<th>N</th>
<th>CF</th>
<th>N</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^2</td>
<td>10^7</td>
<td>10^2</td>
<td>10^7</td>
</tr>
<tr>
<td>1</td>
<td>&lt;1.3</td>
<td>&lt;1.3</td>
<td>&lt;0.7</td>
<td>&lt;0.7</td>
</tr>
<tr>
<td>2</td>
<td>&lt;1.3</td>
<td>&lt;1.3</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
<td>2.7</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.2</td>
<td>2.1</td>
<td>2.8</td>
<td></td>
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<tr>
<td>5</td>
<td>2.4</td>
<td>1.8</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.4</td>
<td>1.9</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>&lt;1.3</td>
<td>2.7</td>
<td>1.7</td>
<td>2.7</td>
</tr>
<tr>
<td>14</td>
<td>~1.3</td>
<td>2.7</td>
<td>1.8</td>
<td>2.6</td>
</tr>
<tr>
<td>Challenge†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2.5</td>
<td>2.7</td>
<td>3.4</td>
<td>2.0</td>
</tr>
<tr>
<td>25</td>
<td>2.3</td>
<td>2.6</td>
<td>2.8</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* log_{10} Titre.
† Challenge by intravenous inoculation of 10^7 IU of virus 14 weeks after primary infection.

Footpad reaction in mice infected with high or low doses of virus

Groups of mice were infected i.v. with 10^2 or 10^7 IU. Immediately afterwards, these, together with mice of a third group which had not received virus i.v., were given 10^4 IU into the right hind foot and at intervals the thicknesses of both feet were measured. The parallel i.v. inoculation of 10^7 IU all but abolished the footpad swelling caused by 10^4 IU (Fig. 1).

Humoral responses in mice infected with high or low doses of virus

LCM virus-specific cell-mediated immunity (CMI) as expressed by lethality, CTL response, and FPR is all but abolished in mice infected with high doses of WE strain virus (always in comparison with the effects of low doses). This contrasts markedly with antibodies which, as has been shown previously, are produced in reverse (Kimmig & Lehmann-Grube, 1979). The humoral response of CBA/J mice against LCM virus was determined again. The results (Table 2) fully confirm our previous observations. Both neutralizing and CF antibodies were much higher in mice infected with high doses than in mice infected with low doses.

Multiplication of LCM virus in spleen after infection with high or low doses of virus

Mice were infected i.v. with 10^2 or 10^7 IU of virus and, at intervals, IU in spleens were determined. The results from three experiments are presented in Fig. 2. With the large dose, highest concentrations were found as early as 24 h after infection and these remained essentially unchanged until the sixth day. When 10^2 IU had been inoculated, maxima were attained later, but on the sixth day they were just as high as after infection with 10^5 times this quantity. On the eighth day, elimination was well under way, infectivity disappearing slightly slower from the mice which had received 10^7 IU.

Search for infected lymphocytes in spleens of mice infected with high or low doses of virus

Inspection of Fig. 2 reveals that 6 to 9 days after infection concentrations of infectious virus in spleens were similar, whether mice had been infected with high or low doses, and we may conclude that the high virus concentration per se does not explain the suppression of CMI. It still appeared possible that the high initial virus concentrations resulted in infection of
increased numbers of T lymphocytes leading to their destruction and/or functional inactivation. An experiment was performed in which mice were infected i.v. with $10^2$ or $10^7$ IU, and 2, 4, and 6 days later their spleens were dispersed, phagocytic elements were removed, and cells were assayed for IC. More were found when the inoculum had contained the greater quantity of virus, but in both groups of mice the numbers of IC were not affected by prior treatment with anti-Thy 1.2 antibody and complement (data not shown), indicating that T lymphocytes had not participated in the infectious process even when the infectious dose had been as high as $10^7$ IU.

We have previously reported that T lymphocytes in acutely infected adult mice are resistant to LCM virus and that the few IC found in the spleens of such animals probably represent contaminating macrophages and related elements and perhaps also fibroblasts and endothelial cells. Their disruption led to release of infectious virus, meaning that they are productively infected (Tijerina et al., 1980). In contrast, the infectious lymphocytes found in LCM virus carrier mice lose their potential to score as IC if treated with ultrasound prior to plating (Doyle & Oldstone, 1978; Popescu et al., 1979). On this basis we performed the following experiment. Mice were infected i.v. with $10^2$ and $10^7$ IU. At intervals, their spleen cells were assayed intact for IC and sonicated for IU. Portions of each spleen were homogenized and titrated for total infectious virus. The results of one such experiment – fully confirmed when repeated – are depicted in Fig. 3. Numbers of IC closely followed total virus concentration. Initially, there were more IC after infection with $10^7$ IU, but on the fifth day their numbers were closely similar to the numbers appearing after infection with $10^2$ IU. Whether the inocula had contained high or low virus doses, always fewer than 0.1% of the spleen cells scored as infectious. The finding that the sonic disruption of cells increased rather than decreased the titres indicated that the infected cells were not T lymphocytes.
**Immune response of mice to LCM virus**

Fig. 3. Numbers of infectious centres in spleens of mice infected with either $10^2$ or $10^7$ IU of LCM virus. Cell-associated infectivity was determined after sonication of dispersed cells and total quantities (upper part of figure) after homogenization of portions of the organs. For mice infected with $10^7$ IU: $\Delta$, homogenate; $\bullet$, intact cells (IC); $\bigcirc$, disrupted cells (IU). For mice infected with $10^2$ IU: $\bigstar$, homogenate; $\blacksquare$, intact cells (IC); $\square$, disrupted cells (IU).

**T cell responses to additional virus inoculations in mice previously infected with high or low doses**

The cytotoxic T cell activities of spleen cells of mice having undergone infections with high or low doses of WE strain virus were tested after challenge. Attempts to elicit responses with $10^2$ IU failed consistently in both groups. When the dose was increased to $10^7$ IU, several experiments differing only with respect to the time intervals between first and second virus inoculations led to closely similar results. There was always a characteristic anamnestic response in mice which had received $10^2$ IU 1 to 4 months (time intervals tested) prior to challenge. In contrast, cytotoxic activity remained at low levels in mice which had been inoculated on either occasion with $10^7$ IU, although a slight increase was sometimes observed (data not shown). The same was found with respect to the FPR. In four out of four experiments accelerated swellings were evoked in mice which had received $10^2$ IU of virus by i.v. inoculation 60 to 70 days (time intervals tested) previously. In contrast, when the primary infection had been established with $10^7$ IU, the subsequent inoculation of $10^7$ IU led to a transient swelling at 6 h, but no enlargement became demonstrable at 24 to 72 h (Fig. 4). Also, no CTL activity was elicited when challenge was attempted by intraperitoneal inoculation (i.v. inoculation killed the recipients) of $2 \times 10^7$ virus-infected syngeneic primary fibroblasts containing (as revealed by parallel titration after sonication of the cells) more than $10^{8.5}$ IU (Fig. 5) or by i.v. inoculation of $10^8$ spleen cells from CBA/J mice which had been infected 3 days previously containing more than $10^7$ IU (data not shown). Nor did the
Fig. 4. Anamnestic footpad reactions to 10^7 IU of CBA/J mice which had been infected 60 days previously by intravenous inoculation of 10^2 (▲) or 10^7 (●) IU of virus or had been left uninfected (■). Data points are means of measurements from 15 mice.

repeated inoculation of 10^7 IU at monthly intervals (up to five times) induce cytotoxic activity (data not shown).

**Detection of memory T cells in mice after their infection with high doses of LCM virus**

Failure to induce secondary responses after primary infection with high virus doses might have one of two possible reasons: (i) LCM virus-specific T lymphocytes are eliminated or (ii) they are present but suppressed. Mice were infected with 10^2 or 10^7 IU and 57, 58, . . . 61 days later their spleen cells were enriched for T cells and inoculated i.v. together with 10^7 IU of virus into X-irradiated (850 rad) recipients whose cytotoxic spleen cell activities were determined in one large test. The results (Fig. 6) were clear-cut: whereas direct challenge of mice previously infected with 10^7 IU failed to activate CTL, transfer of their spleen cells revealed that T cell memory had nonetheless been present and was as high as in mice previously infected with 10^2 IU. This experiment was repeated several times with modifications consisting of use of spleen cells not enriched for T lymphocytes and prolongation of the interval between infection of donor mice and transfer. Spleen cells from irradiated recipients of suppressed spleen cells (donors previously infected with 10^7 IU) consistently expressed cytotoxic activity, although sometimes lower than spleen cells from recipients of responding spleen cells (donors previously infected with 10^2 IU).

These results led to the question as to how soon after infection with high virus doses activation of CTL was demonstrable. Mice were infected with high and low virus doses, and 9, 15, 29, 43 and 57 days later 10^8 of their spleen cells were transferred i.v., together with 10^7 IU of virus, to irradiated (850 rad) recipients whose CTL activities were then determined on the third, fourth and fifth day. In every case, marked responses were observed with cells from recipients irrespective of the interval between infection of donor mice and transfer of their cells and irrespective of the dose of virus with which the donor mice had been infected. A
Immune response of mice to LCM virus

Fig. 5. Anamnestic cytotoxic T lymphocyte responses to the intraperitoneal inoculation of $2 \times 10^7$ virus-infected syngeneic fibroblastic culture cells (containing more than $10^{9.5}$ IU) of CBA/J mice which had been infected (a) 40 or (b) 80 days previously with $10^2$ (■) or $10^7$ (●) IU of virus. Data points are means of two values obtained from individual mice.

Fig. 6. Demonstration of cytotoxic T lymphocyte memory in CBA/J mice infected 60 days previously with $10^2$ (■) or $10^7$ (●) IU of virus by transfer of $5 \times 10^7$ T cell-enriched spleen cells together with $10^7$ IU into lethally irradiated recipients. Note that cytotoxicity did not appear by direct challenge of mice which had been infected with $10^7$ IU (○). Data points are means of two values obtained from individual mice.

Further experiment of the same kind, in which the interval was 10 days, led to essentially the same results (data not shown).

Active suppression of cytotoxic T cell activity in mice infected with high virus doses

The findings described in the previous section were interpreted to mean that in mice infected with high virus doses LCM virus-specific memory CTL are present but suppressed. This conclusion was examined more directly as follows: CBA/J mice were immunized by infection with $10^2$ IU of virus and 30 days later their spleen cells were transferred together with virus either to CBA/J mice infected with $10^7$ IU 80 days previously (suppressed mice) or to normal mice, each animal receiving $10^8$ cells and $10^7$ IU of virus i.v. When the CTL activities were measured 5 and 7 days later (Fig. 7), they were low when suppressed mice had received cells from immune mice but high when normal mice had received such cells. This experiment was repeated twice with minor modifications leading to closely similar results; obviously, infection with high quantities of LCM virus results in active interference with CTL and probably CMI in general.
Fig. 7. Failure to activate memory in cells from CBA/J mice infected 30 days previously with 10^2 IU of virus by transfer, together with 10^7 IU, of 10^8 spleen cells into recipients whose cell-mediated immunity had been suppressed by inoculation 80 days previously with 10^7 IU. Note that memory is readily activated in control mice which had not received virus prior to transfer. Data points are means of two values obtained from individual mice. •, 10^7 IU recipients; □, control recipients.

Fig. 8. Failure to demonstrate suppressor cells for cytotoxic T lymphocytes in lymphoid organs of CBA/J mice whose cell-mediated immunity was suppressed by intravenous infection with 10^7 IU. Donor mice were infected with 10^7 (■, ●) or 10^2 (□, ○) IU and 35 days later 5 x 10^7 spleen cells or 10^7 lymph node cells (■, □) or 5 x 10^7 thymus cells (●, ○) were inoculated intravenously, together with 10^5 IU of virus, into otherwise untreated recipients. ▲, Virus only (10^5 IU). Data points are means of two values obtained from individual mice. (The results with transferred spleen cells were essentially identical but were omitted for the sake of clarity.)

Search for suppressor cells in mice whose cell-mediated immunity was suppressed by infection with high virus doses

One explanation for the block barring the differentiation of LCM virus-specific memory cells into effector CTL was preferential activation of suppressor cells. This possibility was examined by i.v. transfer of lymphoid cells from mice previously infected with 10^7 or 10^2 IU together with virus to recipients whose CTL activity was then tested. Several experiments were performed which differed in time interval between infection of donor mice and their use (37 to 70 days), number (10^7 to 10^9) and source (spleen, lymph nodes, thymus) of cells, pretreatment of recipient mice (X-irradiated with 250 rad or no treatment), and quantity of challenge virus (10^4 or 10^5 IU). In not one case was there even a hint that suppression of LCM virus-specific CMI could be transferred. In other words, whether lymphoid cells came from suppressed mice (previously infected with 10^7 IU) or from responding mice (previously infected with 10^2 IU), the CTL responses of recipients to challenge virus were closely similar. In Fig. 8 one experiment of this kind is depicted for illustration.
DISCUSSION

In comparison with mice infected with low or intermediate doses of strain WE LCM virus, high doses greatly depressed lethality, DTH (footpad reaction to LCM virus), and LCM virus cell-mediated cytotoxicity. Since all these phenomena are T cell-mediated (see Introduction), it is likely that high virus dose survival is but one aspect of general LCM virus-specific T cell suppression.

High virus dose survival has been known for over 40 years (Bengtson & Wooley, 1936) and an inverse relationship between virus dose and cytotoxic T cell response had been noted by Doherty et al. (1974) and Cihak & Lehmann-Grube (1978) and studied by Dunlop & Blanden (1977). A satisfactory explanation, however, has not been given. Our observations show that suppression is long lasting. Since memory was found to be present, we conclude that suppression of CMI in mice infected with high doses of virus does not result from loss of relevant cells, and since activation of memory was impeded when cells from immune mice (previously infected with $10^2$ IU) were transferred to suppressed mice, although it was effectively stimulated when transfer was to normal mice, we conclude that suppression is active.

Presence of (blocked) LCM virus-specific memory cells and ability to eliminate the virus clearly differentiate adult mice surviving acute infection with large quantities of virus from LCM virus carrier mice. Hence, the underlying mechanisms are probably not related. Our observations also effectively invalidate explanations given in the past for high virus dose survival and related phenomena such as mutual destruction of CTL which are both infected and cytolytic for CTL (Dunlop & Blanden, 1977) and dilution of CTL due to extensive replication of the agent throughout the body resulting in their dissipation in various tissues (Zinkernagel & Doherty, 1979). More probably, the suppression in mice of LCM virus-specific CMI by high virus doses is an expression of immune regulation. As to the mechanism, with present knowledge we can only speculate, but since all attempts at demonstrating suppressor cells in these animals have failed and since an inverse relationship exists with respect to cell-mediated and humoral immune responses in mice infected with high and low doses of virus, probably some antibody plays an important part.

Similar patterns of immune responses are seen with other immunogens, for instance SRBC: low quantities induce in mice DTH but little antibody, whereas large quantities have the opposite effects (Mackaness et al., 1974). There is, however, a fundamental difference between both these systems, which makes us reluctant to directly apply knowledge obtained for the much better studied interaction between foreign red blood cells and mouse to the LCM virus-infected mouse. The number of SRBC inoculated is probably a direct measure of the quantity of immunogen which exerts its effects in vivo. In contrast, the LCM virus replicates in the recipient host and the dose which eventually accumulates is different from the one which had been administered, a statement well illustrated by the data in Fig. 2. Assuming that the number of IU correlates with the amount of effective immunogen, essentially identical concentrations were attained irrespective of the initial inoculum, and it is only the time periods needed for reaching maxima which varied. Thus, the difference of the quantity of infectious virus during the first few days after infection and not the total amount attained determines how the mice respond.

This leads to the question whether the marked immunological dissimilarities in mice infected with high or low doses of LCM virus are merely quantitative or whether they point to fundamentally divergent mechanisms; after all, a mouse is probably not equipped by nature to cope with an infective dose of $10^7$ IU of LCM virus. Cytolytic T cell activity is not entirely absent in mice infected with high virus doses; rather, a low degree was often seen between days 5 and 7 (but never on day 9). Natural killer cells could not be detected in these animals (F. Lehmann-Grube et al., unpublished results), and it is likely that CTL precursors
are stimulated and function for a brief period of time. In fact, our experiments indicate that they can be stimulated almost as effectively as in mice infected with 10^2 IU because transfer experiments 9 and 10 days after infection did not reveal marked differences between both groups of recipients.

Nor is the putative suppressor mechanism necessarily unique for mice infected with high virus doses. Already 8 to 9 days after infection with 10^2 IU, lytic activity begins declining, although the concentration of infectious virus (and presumably of effective immunogen) is still very high. This early loss of CTL activity, also, is best explained by active regulation. Also, long-lasting immunological hyporesponsiveness to challenge is found, albeit to a much lesser degree, in mice infected previously with low virus doses. In these animals CTL activities elicited by second inocula appear faster but remain lower than after primary infection (Zinkernagel & Doherty, 1979; F. Lehmann-Grube et al., unpublished observations). Thus, all our findings are compatible with the assumption that the differences of immune responses of mice infected with high and low doses of virus are quantitative, being the consequence of differences of time of appearance and magnitude of some regulatory mechanism.

The interplay of T cells and the products of B cells in LCM virus-infected mice will be discussed on the basis of further data in a forthcoming publication. Here we wish to present a case against the possibility that challenge virus is blocked by neutralizing antibody which, as will be recalled, attains higher concentrations in mice infected with 10^7 IU than in mice infected with 10^2 IU. Findings presented in this communication as well as results from numerous experiments especially performed to clarify this point (F. Lehmann-Grube et al., unpublished results) have all led to the conclusion that suppression of CMI in mice infected with high virus doses is not caused by lack of exposure of the immune system to virus immunogen due to neutralization of challenge virus. The details need not be presented because one observation alone effectively militates against such a mechanism. In mice which had been infected with high doses of strain E-350 ('Armstrong') virus anamnestic cellular immune responses are greatly depressed (Lehmann-Grube et al., 1981b) despite the fact that in these animals neutralizing antibody is hardly detectable (Kimmig & Lehmann-Grube, 1979). We hasten to add that inability to demonstrate this type of immunoglobulin is not interpreted to mean that it is absent. It does mean, though, that its concentration is lower than the one following infection with 10^2 IU of WE strain virus which allows anamnestic responses to proceed.

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