LYMPHOCYTIC CHORIOMENINGITIS VIRUS KILLER T CELLS ARE LETHAL ONLY IN WEAKLY DISSEMINATED MURINE INFECTIONS*

By CHARLES J. PFAU, JEANINE K. VALENTI, DANIEL C. PEVEAR, AND KATHRINE D. HUNT

From the Department of Biology, Rensselaer Polytechnic Institute, Troy, New York 12181

The symptoms and histopathology of many virus diseases depend not so much on damage to cells by virus but on the immunobiological reaction to the infection. Nowhere is this more clearly illustrated than with murine lymphocytic choriomeningitis (LCM) virus (1). The basic features of the system were established by Traub in the 1930's (2): mice that received virus transplacentally or shortly after birth developed a life-long persistent infection, whereas the outcome in mice infected as adults was quite different. In the latter case peripheral injection usually produced an abortive immunizing infection, whereas intracerebral (i.c.) injection resulted in choriomeningitis and death within 7–9 d.

The immunological basis for this choriomeningitis has been investigated intensely during the last 10 yr. The early observations of Rowe (3) showing lack of correlation between serum antibody level and immunity, followed by the finding that thymectomized mice could not be killed by i.c. infection (4), were the first indications of the central role of thymus-derived lymphocytes in fatal lymphocytic choriomeningitis. Following the pioneering work of Volkert (5), Gilden et al. (6) established a model system for adoptive transfer of lymphocytes into adult i.c.-infected mice. In these elegant studies, Cole and co-workers (7) demonstrated, by selective reconstitution of the immune system of cyclophosphamide (CY)-spared mice, that only immune lymphocytes bearing thymus-derived antigens caused rapid convulsive death. Furthermore, Johnson and colleagues (8) conclusively demonstrated that B cells and their secreted products played no role in pathogenesis by showing that mice markedly depleted of immunoglobulin (Ig)-bearing lymphocytes were just as susceptible as normal mice to LCM-induced central nervous system (CNS) disease.

For several years we have focused our attention on the mechanisms of LCM-induced acute CNS disease by studying LCM strains lacking the ability to kill adult i.c.-infected mice in the classic convulsive manner (9, 10). One question asked was: do these so-called “docile” virus strains induce virus-specific T cell responses, and if so, are these responses qualitatively or quantitatively different from those induced by the well-studied so-called “aggressive” strains? It was first necessary to assess T cell

* Supported in part by grant PCM-7917935 from the National Science Foundation, and grant AI-16562 from the National Institutes of Health.

1 Abbreviations used in this paper: CNS, central nervous system; CTL, cytotoxic T lymphocytes; CY, cyclophosphamide; i.e., intracerebral; i.v., intravenous; LCM, lymphocytic choriomeningitis; PFU, plaque-forming units.
function in vitro since an in vivo response, if made, was obviously masked. Using $^{51}$Cr-labeled LCM-infected L cells, the cytotoxic T lymphocyte (CTL) activity was found to be about the same in mice infected with either type of virus (11). CTL are widely considered to be the equivalent of the T cell population responsible for murine LCM pathogenesis (12–14). In view of our results one could conceivably question the equivalence of CTL with killer T cells. It seemed more reasonable, however, to search for a reason why the CTL induced by infection with docile virus were not lethal. This paper shows that the CTL response induced by docile virus can be lethal, but only in mice in which the virus infection has not spread extensively beyond the site of entry.

Materials and Methods

**Viruses and Titrations.** Aggressive and docile viruses were cloned from the blood of an adult ICR mouse infected at birth with tissue-culture-passed LCM virus strain UBC (9). Viruses were quantitated by plaque assay using the previously described (15) Madin Darby canine kidney (MDCK) monolayer technique. Virus content of organs was determined as follows. Mice were killed by cervical dislocation. The brain, liver, kidneys, lungs, spleen, and heart were removed immediately under aseptic conditions. Organs were washed, weighed, ground with sterile sand with a mortar and pestle, and resuspended in tissue culture medium. After the samples were centrifuged at low speed, the supernatants were stored at $-70^\circ$C until titrated by plaque assay.

**Reagents.** CY monohydrate was purchased from Sigma Chemical Company (St. Louis, MO) and diluted in phosphate-buffered saline immediately before use. Antimouse T cell serum (rabbit antimouse brain-associated Thy-1 antiserum) was a product of Cedarlane Laboratories, Hornby, Ontario, Canada.

**Recipients for Adoptive Immunization.** 3-wk-old female C3HeB/FeJ(H-2k) or 4-wk-old female BALB/cByJ(H-2d) mice, from The Jackson Laboratory, Bar Harbor, ME, were infected i.c. usually with 300 (unless stated otherwise) plaque-forming units (PFU) of virus in a volume of 0.03 ml. Either 2 or 3 d later mice infected with the aggressive LCM substrain were given CY intraperitoneally at a dose of 150 mg/kg. In some experiments, mice infected with docile virus were also given CY.

**Donor Mice and Cell Preparation for Adoptive Transfer.** The C3H or BALB/c mice were injected i.c. with 300 PFU of either virus substrain. On the 7th d after infection spleens were removed, and lymphocytes were prepared by standard procedures (11) involving separation on a cushion of sodium ditrizoate-Ficoll. For adoptive transfer cells were resuspended in Eagle's minimal essential medium and 10% heat-inactivated fetal calf serum. Each recipient mouse was given, via the retrobulbar venous plexus, $3-7 \times 10^7$ cells ($\approx 1.5$ mouse equivalents) in a volume of 0.2-0.4 ml. In some adoptive transfers lymphocyte populations were depleted of T cells by incubation with Thy-1 antiserum and complement (11).

**Results**

**Discordance of Plaque-forming Titer with Murine Lethality as a Function of Virus Strain.** Table I shows the mortality pattern in mice injected i.c. with increasing concentrations of virus. The aggressive strain exhibited a phenomenon frequently observed (16–19) but until now not satisfactorily explained: whereas low doses (30–1,800 PFU) invariably killed the mice, high doses ($10^5–10^6$) spared them. On the other hand, a large proportion of mice infected with docile virus were spared when injected with a dose (300–3,000 PFU) that would have invariably been lethal had it been aggressive virus. Virtually all mice surviving injection with docile virus were persistently infected at the end of 28-d observation periods. An i.c. dose of 300 PFU of either substrain was chosen for use in most of our studies.

**Effect of CY on Disease Patterns in LCM-infected Mice.** CY has been used reliably for
TABLE I

Relationship between Virus Dose and Mortality Pattern in Mice Infected with Different Substrains of LCM Virus

| Virus strain | Dose  | Number of mice | Percent mortality | Mean day of death (range)* |
|--------------|-------|----------------|-------------------|---------------------------|
|              | PFU/mouse |                |                   |                           |
| Aggressive   | 3     | 6              | 16                | 12                        |
|              | 30    | 6              | 100               | 10.0 (9-11)               |
|              | 300   | 52             | 100               | 9.7 (8-12)                |
|              | 1,800 | 14             | 100               | 8.9 (8-9)                 |
|              | $10^3-10^6$ | 16          | 0                 | --                       |
| Docile       | 0.01  | 6              | 0                 | --                       |
|              | 0.3   | 8              | 75                | 14.2 (9-23)               |
|              | 3     | 19             | 42                | 12.6 (9-16)               |
|              | 30    | 15             | 53                | 17.0 (11-23)              |
|              | 300   | 79             | 14                | 19.6 (10-17)              |
|              | 3,000 | 20             | 25                | 18.6 (16-23)              |

Data above were compiled based on injection of groups of no less than six C3H mice with a specific virus solution. Mice were counted daily and spun by the tail beginning on day 5 to detect a convulsive behavior as early as possible.

* The experiments were terminated 28 d after infection.

TABLE II

Disease Patterns in CY-treated Mice Injected with Aggressive and Docile Substrains of LCM Virus

| Virus strain | Mouse strain | Day of CY treatment | Percent mortality* | Mean day of death (range) | LCM-like convulsive death |
|--------------|--------------|---------------------|--------------------|---------------------------|--------------------------|
|              | C3H          | 0                   | 0 (14)             | --                        | --                       |
| Aggressive   | C3H          | --                  | 100 (5)            | 9.4 (9-10)                | +                        |
|              | C3H          | 2                   | 100 (7)            | 12.0 (11-14)              | --                       |
|              | C3H          | 3                   | 84 (44)            | 18.4 (11-23)              | --                       |
|              | BALB/c       | --                  | 100 (12)           | 8.7 (8-9)                 | +                        |
|              | BALB/c       | 3                   | 55 (11)            | 13.0 (11-14)              | --                       |
| Docile       | C3H          | --                  | 0 (5)              | --                        | --                       |
|              | C3H          | 2                   | 100 (7)            | 14.4 (14-16)              | --                       |
|              | C3H          | 3                   | 48 (25)            | 17.3 (9-27)               | --                       |
|              | BALB/c       | --                  | 83 (12)            | 13.8 (13-18)              | --                       |
|              | BALB/c       | 3                   | 80 (10)            | 12.6 (10-18)              | --                       |

C3H and BALB/c mice were injected i.c. with 300 PFU of either aggressive or docile virus. The experiment was terminated 28 d after infection. Data were compiled based on injection of no less than five recipient mice per group.

* Parenthesis indicate number of mice killed.

over a decade to prevent lethal choriomeningitis in adult i.c.-infected mice (6). CY was given on the 2nd or 3rd day after infection (for reasons given below), and in all cases such treatment not only delayed death but also prevented neurologic disease (Table II). Unexpectedly, a low survival rate was found among these mice. This has been encountered previously (14, 20), and because, until now, aggressive viruses were used, it had been considered that CY might only partially suppress the immune
response against the infection. However, this explanation seems unlikely because toxicity was apparent in CY-treated C3H mice infected with docile virus, whereas docile virus infection alone was usually not lethal (Table II). Lowering the standard dose of CY (150 mg/kg) in three 5-mg/kg decrements failed to either lower toxicity or prevent CNS disease (data not presented). Docile virus was found to be more pathogenic in BALB/c than C3H mice (Table II). Differences in susceptibility to LCM viruses among mouse strains have been repeatedly observed (17, 21).

Adoptive Transfer of CNS Disease and Identification of the Effector Cell Population. In preparation for four-way adoptive transfer experiments, the basic system was established using the traditional donor-recipient mouse combination in which both are infected with aggressive virus. The data in the upper half of Table III show that adoptive transfer of syngeneic but not allogeneic splenocytes into CY-treated recipients resulted in convulsive death within 6 d. A 5–6-d interval after cell transfer for development of lethal CNS disease has been reported previously (6, 20). After treatment of the donor cells with antibody specific for the Thy-1 antigen, their ability to transfer CNS disease was lost. These mice died in a nonconvulsive manner 14 d after CY treatment (Table III). This was quite close to the mean day of death (15.4, see Table II) caused by the toxicity of CY in control mice infected with aggressive virus. The allogeneic BALB/c recipients also died in a nonconvulsive manner at about the same time as the C3H recipients adoptively transferred with T cell-depleted splenocytes. Again, this was most likely due to CY toxicity.

Disease Outcome and the Timing of Adoptive Transfer in CY-treated Recipients. Because of

| Virus strain* injected into | Day of CY treatment/ day of adoptive transfer | Percent mortality‡ | Days to death in recipient mouse | LCM-like convulsive death |
|---------------------------|---------------------------------------------|-------------------|-------------------------------|--------------------------|
| Donor mice                | Recipient mice                              |                   |                               |                          |
| A                         | A                                           | 3/7               | 89 (61)                       | 5.8 +                    |
| A                         | A                                           | 2/4               | 100 (6)                       | 5.8 +                    |
| A§                        | A                                           | 3/7               | 100 (10)                      | 10.2 –                   |
| A                         | A‖                                          | 3/7               | 50 (7)                        | 11.0 –                   |
| D                         | A                                           | 3/7               | 45 (11)§                      | 9.8 –                    |
| D                         | A                                           | 2/4               | 100 (8)                       | 6.2 +                    |
| A                         | D                                           | 3/7               | 65 (26)                       | 9.4 –                    |
| A                         | D                                           | 2/4               | 100 (6)                       | 5.3 +                    |

Donor and recipient mice were injected i.c. with 300 PFU of either aggressive or docile virus. On the 7th d post-infection, splenocytes were purified from C3H mice and injected into syngeneic, and in one experiment allogeneic, CY-treated recipients, which had been infected 4 or 7 d earlier. As in the previous tables, the mean day of death was used to determine the number of days to death after adoptive transfer.

Data were compiled based on the injection of no less than five recipient mice.

* A, aggressive; D, docile.
‡ Parenthesis indicate number of mice.
§ Donor splenocytes treated with Thy-1 antiserum and complement before use.
‖ BALB/c mice used instead of C3H mice.
¶ The outcome of two experiments. In the third experiment, all 10 mice died in a convulsive manner 4.8 d (mean) after adoptive transfer.
the toxicity of CY we encountered, it was decided to shorten the interval between CY treatment and adoptive transfer. In the first series of experiments recipient mice were infected, treated with CY on day 3, and then injected with donor lymphocytes on day 7. In the second series, the infected donor mice were given CY on day 2 and the adoptive transfer on the 4th d. The protocols produced strikingly different results (lower half of Table III). When adoptive transfers took place in recipients infected 7 d previously, the only adoptive transfer combination that caused CNS disease in the majority of recipient mice was the classic one, i.e., when both donors and recipients were infected with aggressive virus. On the other hand, in each case when adoptive transfers were carried out with recipient mice infected 4 d previously, CNS disease developed ~6 d later. The latter results strengthened our assumption that the docile virus-induced CTL represented a population of lymphocytes with in vivo activity (11). Because each adoptive transfer of some 5 × 10^7 lymphocytes contained ~10^5 PFU of virus, it became important to determine what effect intravenous (i.v.) injection of docile or aggressive virus alone would have on the mice. Over 20 mice were infected i.v. with 10^6 PFU of either aggressive or docile virus, and all survived a 28-d observation period. In fact, this result was obtained when a group of mice injected with aggressive virus was also injected simultaneously with a normally lethal dose (300 PFU) of the same virus by the i.c. route. Furthermore, lymphocyte populations treated with anti-Thy-1 serum retained their initial virus infectivity, while losing their ability to transfer CNS disease (Table III).

**CNS Disease in Mice Infected with Docile Virus as a Function of the Timing of Adoptive Transfer of Donor Lymphocytes Induced by Homologous Virus.** The data presented in Table III clearly show that splenocytes from mice infected with either aggressive or docile virus caused convulsive death in CY-treated recipients infected with heterotypic virus no more than 4 d before adoptive transfer. Because these experiments also showed that mice infected with docile virus could be suitable donors as well as recipients, it seemed logical to assume that mice infected with docile virus (in the absence of CY) might be killed by their own lymphocyte response to the infection if the timing of the interaction between virus and the immune system was appropriate. Table IV shows that this is indeed the case. Virtually all mice infected i.c. with docile virus for no more than 3 d developed CNS disease after receiving Thy-1-bearing lymphocyte populations taken from identical mice infected for 7 d. Although adoptive transfers in recipient mice infected for 4 d resulted in three- to fourfold more deaths than expected from the i.c. infection alone, no convulsive death pattern was observed. Adoptive transfer on the 7th d after infection of the recipients clearly had no effect.

**Multiplication and Distribution of Viruses.** Because we had previously established (10, 11) that docile virus replication commenced more quickly and reached higher titers in the brain than aggressive virus, the patterns of replication were established in other organs. At 24-h intervals for 7 d after i.c. infection with 300 PFU of either aggressive or docile substrains, virus contents in the brain, heart, kidneys, liver, lungs, and spleen were determined by plaque titration. As shown in Fig. 1, the patterns of replication established in the brain were generally observed in other organs. The responses could be roughly categorized into three groups. In the first group (lung and liver), docile virus was easily detected by the 2nd d after infection, whereas aggressive virus was not seen until the 4th to 6th d. At all times aggressive virus titers lagged behind docile virus 1-4 log units. In the second group (spleen and brain), both virus substrains
Table IV
Correlation between Day of Adoptive Transfer and Ability to Kill Mice Infected with Docile Virus

| Days recipients infected before adoptive transfer | Number of mice | Percent mortality | Days to death in recipient mice (range) | LCM-like convulsive death |
|--------------------------------------------------|----------------|------------------|----------------------------------------|--------------------------|
| 0                                                | 8              | 100              | 7.0 (6-9)                              | +                        |
| 1                                                | 15             | 100              | 7.2 (6-9)                              | +                        |
| 2                                                | 16             | 88               | 7.3 (6-9)                              | +                        |
| 3                                                | 28             | 93               | 7.5 (5-13)                             | +                        |
| 3*                                               | 8              | 0                | -                                      | -                        |
| 4                                                | 19             | 58               | 12.4 (6-22)                            | -                        |
| 7                                                | 30             | 23               | 11.9 (4-17)                            | -                        |

The protocol was exactly as given in Table III except for the exclusive use of C3H mice as both donors and recipients. All splenocytes used for the adoptive transfer were taken from mice on the 7th d after infection. Data were compiled based on injection of no less than five recipient mice per group.

* Lymphocytes treated with Thy-1 antibody and complement.

Fig. 1. C3H mice were injected i.c. with 300 PFU of either aggressive or docile LCM substrain. At 24-h intervals for 7 d, two mice (occasionally one) in each group were killed. Each of the organs was separately weighed and titrated by plaque assay. These data were combined with the results of four other experiments in which specific organs were titrated on various days after infection. Thus, the average titers were usually based on three to four individual titrations and sometimes as many as six. When no range is given, there was only one titration.
could be detected at the same time, either on the 1st or 2nd day after infection. Again, aggressive virus titers usually lagged behind docile virus by 1–4 log units of infectivity. The highest titers were generally encountered in the spleens of mice infected with docile virus (in one case reaching $2 \times 10^{10}$ PFU/g on the 4th day after infection). In the third group (kidney and heart), little, if any, virus of either type could be detected until the 4th or 5th day after infection. Because this time interval coincided with the detection of virus in the blood, these organs may not have been truly infected. It should be pointed out that differences in the content of aggressive and docile viruses in the visceral organs would have been larger by ~100-fold if titrations were carried out using LD$_{50}$ assays (1 LD$_{50} = \sim 10$ PFU of aggressive strain but only 0.1 PFU of docile strain, as indicated in Table I).

**Aggressive Virus Replication, Distribution, and Pathogenicity in Mice as a Function of Inoculum Size.** The data in Fig. 1 and Table I, taken together, indicated an inverse correlation between spread of the virus infection and lethality. With identical 300-PFU inoculum doses the rapidly spreading docile virus was not lethal, whereas the slowly disseminated aggressive virus killed. Because very high inocula of aggressive virus, unlike low doses, did not kill (Table I), we determined whether this same inverse correlation would hold in this case as well. The data in Table V clearly indicated that on the 3rd and 4th day after infection, the sparing dose of aggressive virus resulted in very high titers of virus in all organs examined; higher than those caused by docile virus (Fig. 1).

**The Time of Adoptive Transfers that Induce CNS Disease Varies with the Concentration of Docile Virus Used to Infect Recipient Mice.** The previous results (Table V and Fig. 1) showed a correlation between rapid virus spread (whether docile or aggressive substrains) and relatively benign infection. Suggestive of this correlation was the fact that increasing death rates were observed in mice infected with decreasing amounts of docile virus (Table I). Thus, mice were infected with either 30 or 300 PFU of docile virus (the former concentration causing four times the death rate as the latter). On

### Table V

| Organ     | 3 d post-infection (inoculum [PFU/mouse]) | 4 d post-infection (inoculum [PFU/mouse]) |
|-----------|------------------------------------------|------------------------------------------|
|           | 300                                      | 1.5 $\times$ 10$^6$                      | 300                                      | 1.5 $\times$ 10$^6$                      |
| Liver     | $<10^3$                                  | $6.9 \times 10^3$                        | $<10^3$                                  | $2.9 \times 10^7$                       |
| Lung      | $1.7 \times 10^2$                        | $7.5 \times 10^6$                        | $9.0 \times 10^9$                        | $1.5 \times 10^8$                       |
| Spleen    | $2.2 \times 10^5$                        | $>4.8 \times 10^8$                       | $3.9 \times 10^9$                        | $>3.5 \times 10^8$                      |
| Brain     | $5.8 \times 10^3$                        | $3.2 \times 10^9$                        | $3.8 \times 10^4$                        | $1.8 \times 10^5$                       |
| Kidney    | $<10^3$                                  | $9.4 \times 10^2$                        | $<10^3$                                  | $3.7 \times 10^6$                       |
| Heart     | $<10^3$                                  | $10^3$                                   | $<10^3$                                  | $1.5 \times 10^5$                       |
| Serum     | $<10^3$                                  | $2.0 \times 10^3$                        | $<10^3$                                  | $1.8 \times 10^5$                       |

10 C3H mice were divided equally into two groups; one injected with 300 PFU/mouse, and the other with 1.5 $\times$ 10$^6$ PFU/mouse. On the 3rd and 4th d after infection, one mouse from each group was bled and killed. The three remaining mice in each group were observed daily. All mice receiving the low dose of virus died on day 9, whereas all mice receiving the high dose were healthy on the 28th d after infection, when the experiment was terminated.
LYMPHOCYTIC CHORIOMENINGITIS VIRUS KILLER T CELLS

The Interrelationship between Virus Inoculum Size, Spread of the Infection, and Loss of Susceptibility to Being Killed by Adoptive Transfer

| Days recipients infected before adoptive transfer | Infection dose (PFU/recipient) | Number of mice | Percent mortality | Days to death in recipient mice (range) |
|--------------------------------------------------|-------------------------------|----------------|------------------|----------------------------------------|
| 3                                               | 300                           | 5              | 100              | 7.4 (6-10)                             |
| 4                                               | 300                           | 5              | 40               | 12.5 (7-18)                            |
| 4                                               | 30                            | 5              | 100              | 7.2 (5-10)                             |
| 5                                               | 30                            | 5              | 100              | 7.6 (4-10)                             |

Protocol was exactly as described in Table IV.

The adoptive transfer experiments presented here clearly indicate that an insufficient T cell response is unlikely to be the reason why adult mice live after i.c. infection with a given strain of LCM virus. Before these studies the possibility existed that the strong CTL response observed in our docile virus-infected mice (11) did not necessarily represent a population of effector cells capable of causing CNS disease. On the other hand, although the CTL responses induced by docile or aggressive viruses were comparable against those induced by target L cells infected with homologous or heterotypic virus, we proposed that their inability to kill in vivo could be due to “target defects” in mice infected with docile virus (11). The target organ has been assumed to be the brain because of the infiltration of lymphocytes and macrophages in the virus-infected choroid plexus, ependyma, and meninges. Although there is no rigorous proof for this, meningeal inflammatory exudate may be isolated from the cisterna magna and constitutes a potent source of LCM-specific cytotoxic T cells (22). The data presented in this paper raise the possibility that these defects may be in the abundance rather than in the lack of target cells and organs for virus-specific T cells. What we can say definitely is that a strong inverse correlation exists between lethality and spread of the infection. This was shown in a number of ways: (a) Only when docile virus dissemination and replication was at an early stage in recipient mice was it possible to induce CNS disease by adoptive transfer (Tables III, IV, and VI). (b) Only very high i.c. doses of aggressive virus, which mimicked the normally rapid spread of docile virus (Fig. 1 and Table V), were not lethal. (c) Only very low doses of docile virus, which mimicked the normally slow spread of aggressive virus (Table I), were highly lethal.

The hypothesis that disease outcome in mice is determined by the number of target
organs "seen" by the T cell response against LCM infection, has received increasing attention over the last 10 yr. It was first advanced by Gilden and colleagues (6) in their classic studies using adoptive transfers to show the central role of T cells in inducing CNS disease in mice. They noted that such passive immunization in neonatally infected adult carrier mice, while suppressing viremia and inducing high levels of CF antibodies (23), failed to cause CNS disease. They suggested that because neonate carrier mice had more widespread tissue infection with higher levels of viremia than CY carriers, immune lymphoid cells would be diverted so that fewer reached the choroid plexus. Consistent with this view was their finding of the importance of the timing of adoptive transfer in CY-spared mice (6). Whereas 100% mortality was obtained when adoptive transfer was carried out 5–9 d after infection, the severity of choroiditis was much lower, survival time longer, and mortality lower when drug-induced carriers were given cells a month or more after infection when viremia was always present. Thomsen and colleagues (20) also noted the loss of the ability of adoptive transfers to kill when delayed for too long. They suggested that immunosuppression allowed the infection to spread to many organs so that the brain was no longer the main target for the immune attack. These views were also expressed by Zinkernagel and Doherty (24), who further stated that focusing the immune attack on the brain could also be the explanation why i.c. infection with low doses of viscerotropic virus (defined as a strain that grew widely in the body) were fatal. They pointed out that this postulate was based on the assumption that a maximal cytotoxic T cell response is made regardless of the strain of virus used.

Our data showing that rapid spread of an LCM infection assures survival is consistent with, and lends further support to the hypothesis that multiple target organs divert the focused assault of virus-specific T cells on the brain. The next step in testing this hypothesis is to find where CTL are at various stages of lethal and nonlethal LCM infections. Preliminary results in our laboratory already show that CTL activity in the lungs of mice infected with docile virus is much higher than when aggressive virus is used. Supportive evidence also comes from recent data, published by Lehmann-Grube and colleagues (25), using a strain of LCM virus (WE) showing i.e. high virus dose survival in CBA/J mice. They found that CTL recoverable from spleens were markedly fewer when mice were injected (i.v.) with $10^7$, rather than $10^2$, infectious U of virus. Although the conclusion was made that the high virus dose suppressed cell-mediated immunity, an alternate explanation is that the high virus dose established multiple target organs very quickly with a concomitant recruitment of virus-specific lymphocytes from the spleen.

Because the greater part of an intracerebral inoculum spills over immediately into the blood stream (26), and because both strains of LCM show no great difference in growth rate in tissue culture (27), it is not clear why aggressive virus lags so dramatically behind docile virus in its appearance and replication in the visceral organs. We have considered the role of interferon here. Its central, but as yet undefined, importance in the adult LCM infection can be shown by comparing the docile and aggressive virus infections. On the one hand, mice infected with docile virus will develop CNS disease with appropriately timed injections of interferon inducers (10). On the other hand, adult mice infected with aggressive virus strains will be spared by treatment with anti-interferon serum (28; C. J. Pfau and K. D. Hunt, unpublished observations). This sparing is accompanied by a 100- to 1000-fold
rise in viremia (28; Pfau and Hunt, see above), and we are currently determining the kinetics of spread of the aggressive virus under these conditions. Continued investigations of the interplay between interferon, virus spread, and the immune response may be important in understanding how human disorders could be caused by virus tropisms and immunological recognition of infected targets.

Summary

Two types of lymphocytic choriomeningitis (LCM) viruses were studied which, upon intracerebral injection into adult C3H mice, provoked either (a) acute fatal central nervous system (CNS) disease or (b) life-long persistent infection. Both virus types, (a) aggressive and (b) docile, had been found to induce LCM-specific lymphocytes with comparable in vitro lytic activity (11). Because the requirement for T cells in the development of adult LCM disease has been extensively documented, we sought other reasons for the lack of acute disease in mice infected with docile virus. A striking correlation was found between the outcome of the infection and spread of virus to visceral organs. Adoptive transfer experiments showed that a 300-plaque-forming unit inoculum of docile virus induced a population of T cells in donor mice fully capable of causing CNS disease in identically infected recipients. This disease-causing ability was lost if the interaction was delayed beyond 3 d after infection of the recipients, but could be preserved by lowering the size of the viral inoculum in the recipients. Furthermore, without adoptive transfer, very low intracerebral doses of docile virus (which mimicked the normally slow spread of aggressive virus) were lethal. On the other hand, very high doses of aggressive virus, which mimicked the normally rapid spread of docile virus, did not induce fatal CNS disease. The results suggest that rapid dissemination of the LCM infection creates multiple target organs which divert the focused lethal T cell attack on the brain.

Received for publication 8 February 1982 and in revised form 1 April 1982.

References

1. Buchmeier, M. J., R. M. Welsh, F. J. Dutko, and M. B. A. Oldstone. 1980. The virology and immunobiology of lymphocytic choriomeningitis virus infection. Adv. Immunol. 30:275.
2. Traub, E. 1973. LCM virus research, retrospect and prospect. In Lymphocytic Choriomeningitis Virus and Other Arenaviruses. F. Lehmann-Grube, editor. Springer-Verlag, Heidelberg. 3-10.
3. Rowe, W. P. 1954. Studies on pathogenesis and immunity in lymphocytic choriomeningitis infection of the mouse. Res. Rep. Nav. Med. Res. Inst. 12:167.
4. Rowe, W. P., P. H. Black, and R. H. Levey. 1963. Protective effect of neonatal thymectomy on mouse LCM infection. Proc. Soc. Exp. Biol. Med. 114:248.
5. Volkert, M. 1962. Studies on immunological tolerance to LCM virus. A preliminary report on adoptive immunization of virus carrier mice. Acta Pathol. Microbiol. Scand. 65:305.
6. Gilden, D. H., G. A. Cole, and N. Nathanson. 1972. Immunopathogenesis of acute central nervous system disease produced by lymphocytic choriomeningitis virus. II. Adoptive immunization of virus carriers. J. Exp. Med. 135:674.
7. Cole, G. A., N. Nathanson, and R. A. Prendergast. 1972. Requirement for θ-bearing cells in lymphocytic choriomeningitis virus-induced central nervous system disease. Nature (Lond.). 235:333.
8. Johnson, E. D., A. A. Monjan, and H. C. Morse. 1978. Lack of B-cell participation in acute lymphocytic choriomeningitis disease of the central nervous system. Cell. Immunol. 36:143.
9. Jacobson, S., and C. J. Pfau. 1980. Viral pathogenesis and resistance to defective interfering particles. *Nature (Lond.)* 283:311.

10. Jacobson, S., R. M. Friedman, and C. J. Pfau. 1981. Interferon induction by lymphocytic choriomeningitis viruses correlates with maximum virulence. *J. Gen. Virol.* 57:275.

11. Pfau, C. J., J. K. Valenti, and S. Jacobson. 1982. Cytotoxic T cells are induced in mice infected with lymphocytic choriomeningitis virus strains of markedly different pathogenicities. *Infect. Immun.* 36:598.

12. Cole, G. A., and E. D. Johnson. 1975. Immune responses to LCM virus infection in vivo and in vitro. *Bull. W. H. O.* 52:465.

13. Doherty, P. C., and R. M. Zinkernagel. 1975. H-2 compatibility is required for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. *J. Exp. Med.* 141:502.

14. Doherty, P. C., and R. M. Zinkernagel. 1975. Capacity of sensitized thymus-derived lymphocytes to induce fatal lymphocytic choriomeningitis is restricted by the H-2 gene complex. *J. Immunol.* 114:30.

15. Jacobson, S., F. J. Dutko, and C. J. Pfau. 1979. Determinants of spontaneous recovery and persistence in MDCK cells infected with lymphocytic choriomeningitis virus. *J. Gen. Virol.* 44:113.

16. Bengston, I. A., and J. G. Wolley. 1936. Cultivation of the virus of lymphocytic choriomeningitis in the developing chick embryo. *Public Health Rep.* 51:29.

17. Hotchin, J., and L. Benson. 1963. The pathogenesis of lymphocytic choriomeningitis in mice: the effects of different inoculation routes and the footpad response. *J. Immunol.* 91:460.

18. Lehmann-Grube, F. 1969. Dose-response relationships of lymphocytic choriomeningitis viruses in mice and L cell tube cultures. *J. Hyg.* 67:269.

19. Suzuki, S., and J. Hotchin. 1971. Initiation of persistent lymphocytic choriomeningitis infection in adult mice. *J. Infect. Dis.* 123:603.

20. Thomsen, A. R., M. Volkert, and O. Marker. 1979. The timing of the immune response in relation to virus growth determines the outcome of the LCM infection. *Acta Pathol. Microbiol. Scand. Sec. C.* 87:47.

21. Riviere, Y., I. Gresser, J.-C. Guillon, M.-T. Bandu, P. Ronco, L. Morel-Maroger, and P. Verroust. 1980. Severity of lymphocytic choriomeningitis virus disease in different strains of suckling mice correlates with increasing amounts of endogenous interferon. *J. Exp. Med.* 152:633.

22. Zinkernagel, R. M., and P. C. Doherty. 1973. Cytotoxic thymus-derived lymphocytes in cerebrospinal fluid of mice with lymphocytic choriomeningitis. *J. Exp. Med.* 138:1266.

23. Volkert, M., J. Hannover Larsen, and C. J. Pfau. 1964. Studies on immunological tolerance to LCM virus. 4. The question of immunity in adoptively immunized virus carriers. *Acta Pathol. Microbiol. Scand.* 61:258.

24. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. *Adv. Immunol.* 27:51.

25. Lehmann-Grube, F., M. Varho, and J. Cihak. 1981. Reciprocal patterns of humoral and cell-mediated anti-viral immune responses of mice infected with high and low doses of lymphocytic choriomeningitis virus (LCM). In The Replication of Negative Strand Viruses. D. H. L. Bishop and R. W. Compans, editors. Elsevier/North-Holland, New York. 85.

26. Mims, C. A. 1960. Intracerebral infections and the growth of viruses in the mouse brain. *Brit. J. Exp. Med.* 41:52.

27. Jacobson, S. 1980. Properties of in vitro and in vivo long term persistent infections with lymphocytic choriomeningitis virus. Ph.D. Dissertation, Rensselaer Polytechnic Institute, Troy, New York.

28. Saron, M.-F., Y. Riviere, I. Gresser, and J.-C. Guillon. 1981. Prevention of death in mice fatally infected with LCM virus by anti-interferon globulins. In Abstracts, Fifth International Congress of Virology. 99.