Infection of Murine T Lymphocytes with Lymphocytic Choriomeningitis Virus: Effect of Age of Mice on Susceptibility

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Infectious T lymphocytes were determined in thymuses and spleens of mice acutely infected with LCM virus. Such cells appeared in newborn animals, but their numbers declined when the age at infection increased and none were found in adult mice. Either susceptibility to LCM virus is associated with some degree of immaturity or the probability of murine T lymphocytes to encounter the virus as an infectious entity rather than an immunogen is higher in very young than in immunologically mature mice.

Introduction

In adult mice the lymphocytic choriomeningitis (LCM) virus induces specific immune phenomena which can be revealed both *in vivo* and *in vitro*. In the T cell compartment these are virus elimination, protective immunity, footpad response, and acute illness as well as destruction of cultivated virus-infected target cells [1–6]. Antibodies also appear [7, 8], but their biological relevance is uncertain [9]. In contrast, infection of unborn or newly born mice with LCM virus does not result in acute illness and the virus is not eliminated but persists lifelong [10]. Nor can other LCM virus-specific T cell activities be demonstrated by available methods [11, 12, and F. Lehmann-Grube, unpublished].

LCM virus carrier mice respond normally to most other antigens [9, 13]; hence, every explanation for absence of LCM-viral T cells must account for specificity. It has been assumed that elimination of LCM virus-specific T cell clones from congenitally or neonatally infected mice is comparable with elimination of clones directed at "self" or other tolerogens [14–16, 9]. The finding that a proportion of lymphocytes in carrier mice are infected [17–19] suggested an alternative explanation, namely direct viral action. The natural and experimentally easiest way to induce persistent LCM virus infection is to introduce the virus *in utero* or shortly after birth [10, 20]. We therefore have begun to investigate the effect of age of the mouse on the susceptibility of its white blood cells to LCM virus and have concentrated on T cells.

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Materials and Methods

Colony bred NMRI mice were purchased from Zentralinstitut für Versuchstiere, Hannover, and CBA/J mice from Dr. Ivanovas, Versuchstierzuchten, Kisslegg im Allgäu, Federal Republic of Germany. The WE strain of LCM virus [21] had been triple plaque purified. For use in these experiments it was propagated and its quantity determined as plaque-forming units (PFU) in L cells. Anti-LCM-viral antiserum, directed against all immunogenic components of the agent, was obtained from rabbits. Its neutralizing titer, determined in a plaque reduction assay, was 5000. Monoclonal antibody to theta antigen (anti-Thy 1.2 antibody) was purchased from New England Nuclear, Dreieich, Federal Republic of Germany. Cell-associated viral infectivity was determined by an infectious center (IC) assay [19]. Details which appear important in this context were as follows. Cells were dispersed mechanically and, in the case of the spleen, phagocytic elements were removed with a magnet after they had taken up carbonyl iron. The concentration was then adjusted to 4×10^6 "viable" cells per ml, "viability" being based on exclusion of trypan blue. If T lymphocytes were to be removed, the cells were held for 30 min at 37 °C with suitably diluted anti-Thy antibody plus

guinea-pig complement, which had previously been absorbed with agarose and mouse spleen cells. They were washed and – whether treated with anti-Thy antibody or not – were incubated for 1 h at 37 °C with anti-LCM virus antiserum to neutralize free virus. They were washed again twice and were resuspended in medium at a concentration of 2×10^6 cells per ml. Cells were diluted and transferred in 0.5 ml volumes onto L cell monolayers in plastic dishes and plaques were counted after incubation for 5 days.

Results

Newborn NMRI mice were infected and at intervals total infectious virus and infectivity associated with all cells as well as infectivity remaining after treatment of cells with anti-Thy antibody were determined in thymus and spleen. Fig. 1 depicts the results obtained with thymuses. The number of IC increased parallel with PFU and reached highest values between days 5 and 7 after infection. Throughout, the numbers of IC which were resistant to treatment with anti-theta antibody were much lower. Based on the mean of the differences on days 5, 6, 7, 10, and 14, approximately 98% of the IC from the thymus of neonatally LCM virus-infected NMRI mice were positive for theta antigen. Results obtained with the spleens were similar except that both the plateau of IC formation and the maximum of total infectivity were reached earlier, namely on days 3 and 4, respectively (data not shown). Furthermore, the proportion of theta-positive IC in the spleen was lower than in the thymus; based on the mean of the differences on days 5, 6, 7, 10, and 14, 72% of all IC were T lymphocytes. In both thymus and spleen the numbers of infectious T cells attained plateaus around the 5th day after infection, and these did not change much when the mice grew older (data not shown).

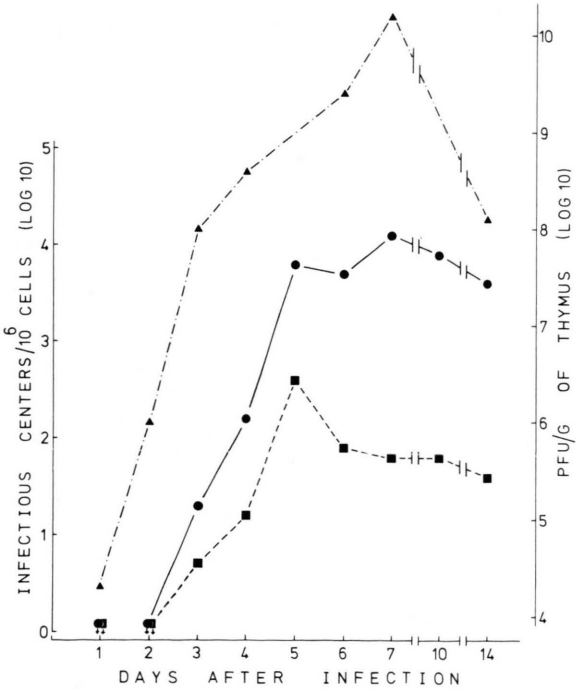

Fig. 1. Appearance of infectious cells (IC) in the thymuses of mice infected neonatally with LCM virus and the effect on their numbers of selective removal of T cells. NMRI mice, less than 24 h old, were infected by intraperitoneal inoculation of 10^3 PFU. At intervals of multiples of 24 h the numbers of IC prior to (●——●) and after (■– – –■) treatment of the cells with anti-Thy antibody plus complement were determined as described in the text. To measure the total amount of infectious virus contained in the tissue, pieces of the thymuses were weighed and homogenized with a known volume of diluent. Infectious virus was then determined as PFU on L cell monolayer cultures (▲–·–▲).

The next experiment in this series was similar, except that virus was inoculated at intervals after birth of the mice and cell-associated infectivities were determined 5 days after infection. The data presented in Fig. 2 show a striking dependency of susceptibility on age: the older the mouse, the lower the number of infectious T cells. This was marked in the case of the thymus but not less obvious for the spleen. When the virus was inoculated on the 8th day after birth, in both organs the numbers of infectious T cells were as low as approximately 30 per 10^6 and none at all were found when the mice were infected at age 23 days. Further attempts with NMRI and CBA/J mice infected at this age or when older have consistently given the same negative results.

Results of previous experiments [19 and F. Lehmann-Grube, unpublished] as well as the present observations indicate that T cells in adult NMRI and CBA/J mice cannot be infected in the sense that they score as infectious centers. Doyle and Oldstone [18] reported to have succeeded in infecting macrophages, B cells, and T cells of adult BALB/WEHI and SWR/J mice inoculated by the intraperitoneal route with strain E-350 ("Armstrong") of LCM virus. It has repeatedly been shown that cells of the mononuclear phagocytic system can be infected with

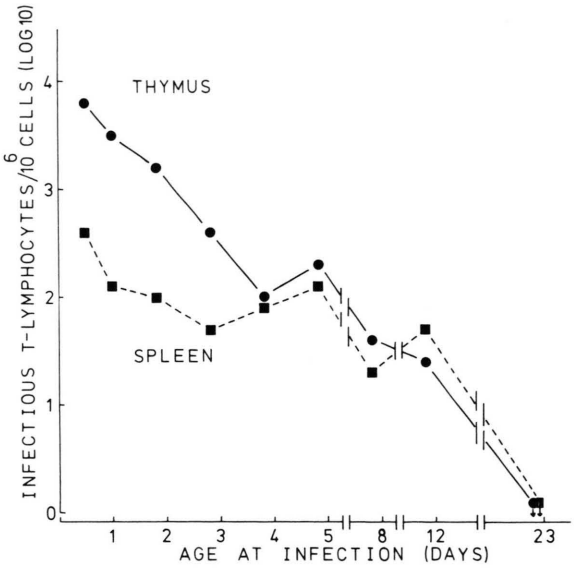

Fig. 2. Effect of age of mice at infection with LCM virus on numbers of infectious T lymphocytes in thymus and spleen. The values were calculated from data obtained with an experiment which was essentially performed as has been described in the legend of Fig. 1 except that virus was inoculated at intervals after birth and IC were always determined 5 days later. ●——●, IC in thymus; ■---■, IC in spleen.

LCM virus both *in vitro* and *in vivo* [22–25], and our data (presented here and unpublished) are inconclusive as to B cells. But there is an obvious discrepancy between the present results and the ones of Doyle and Oldstone with respect to T cells. We have therefore considered a property of infected lymphocytes on which (if they come from congenitally or neonatally infected mice) both groups have made similar observations. This is loss of the capacity to score as IC if disrupted, *e. g.* by sonication. The results of a number of similar experiments employing NMRI and CBA/J mice infected intravenously or intraperitoneally with strains WE or E-350 of LCM virus are exemplified in Fig. 3 and can be summarized as follows: (1) Appearance and disappearance of IC closely followed rise and fall of concentration of total infectious virus in the organ; (2) the numbers of IC remained very low and never exceeded 0.01%; (3) sonication did not reduce infectivity, even increased it somewhat, a pattern reminiscent of the one seen with cultivated peritoneal exudate cells. We conclude that the IC found in spleens of acutely infected adult mice are not lymphocytes. Probably they are elements of the mononuclear phagocyte system which had escaped iron treatment and perhaps also contaminating endothelial cells and fibroblasts.

The data presented in this communication show that murine T lymphocytes can be infected by LCM virus but only in newborn or very young mice. Perhaps these animals' immunological immaturity increases the probability for a T lymphocyte to make contact with the LCM virus as an infectious entity rather than an immunogen. Alternatively, the T cells themselves are immature and thereby susceptible. Nor do we know whether immunologic stimulation is a necessary precondition and whether the infected T lymphocytes in LCM virus carrier mice are indeed the ones which are (potentially) immunologically specific for LCM virus, as has previously been hypothesized [19]. Hopefully, the answers will come from work now under way in this laboratory.

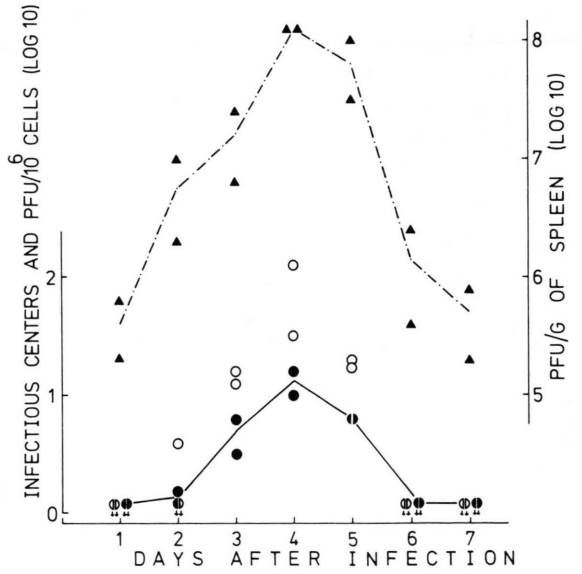

Fig. 3. Appearance of IC in the spleens of adult mice acutely infected with LCM virus and the effect of their disruption on infectivity. Six-week-old NMRI mice were infected by intravenous inoculation of 10^3 PFU. At intervals of multiples of 24 h cells of the spleens were dispersed, the phagocytic elements were removed, and the infectivity of the remaining cells was determined before (●——●) and after (○——○) sonication, which was applied under conditions known to disintegrate the cells but not to affect viral infectivity. Total amounts of infectious virus (▲–·–▲) were measured as has been described in the legend of Fig. 1.

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