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Effects of Anti-Thymocyte Serum on Lymphocytic Choriomeningitis (LCM) Virus Infection in Mice. (32254)

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Several studies(1,2,3) have suggested that lethality in acute lymphocytic choriomeningitis (LCM) virus infection of mice is a result of host immunologic response, rather than a direct effect of virus multiplication. Mice inoculated neonatally are protected from lethal effects of the virus, while adult morbidity and mortality can be reduced or delayed by various immunosuppressive methods, including X-irradiation, antimetabolite therapy, and neonatal thymectomy(1,2). In the present study, the role of cellular immunity in LCM pathogenesis was further examined, using rabbit anti-mouse thymocyte (RAMT) serum to inhibit host response.

Materials and methods. Three- to four-week-old ICR mice of both sexes were used in all experiments. RAMT serum was prepared as described previously(4), by immunizing rabbits over a several-week period with dispersed suspensions of viable mouse thymus cells. Serum effectiveness was assayed by its ability to (a) diminish peripheral blood lymphocyte counts by 50% within a 4-hour period, and to (b) double the mean survival time of AKR skin grafts on C_3H mice. All RAMT sera were shown to be free of anti-LCM activity as assayed by mouse neu-

TABLE I. Experimental Protocol for Comparison of the Effects of Anti-Thymocyte Serum (RAMT) and Normal Rabbit Serum (NRS) on LCM Infection of Mice.

Group	No. of mice	Treatment
A	12	0.3 ml RAMT serum every 3rd day (A_1, −6 to +6; A_2, −6 to +12; A_3, −6 to +30)
B	18	0.3 ml RAMT serum days 0, +1, +2
C	18	0.3 ml RAMT serum days +3, +4, +5
D	12	0.5 ml RAMT serum days −1, 0, +1
E	7	0.5 ml RAMT serum day −6
Control	74	None, or NRS in schedules comparable to those in experimental groups A-E

Day 0 represents day of intracerebral inoculation of 1000 LD_{50} of LCM virus.

tralization and indirect immunofluorescent techniques. The virus suspension used was the Armstrong E-350 strain obtained from the American Type Culture Collection, and contained 10^6 LD_{50}/.03 ml when titrated by intracerebral inoculation in 3-week-old ICR mice. In all experiments, mice were inoculated intracerebrally with 1000 LD_{50} of virus on day 0. The design of experiments performed is shown in Table I. From day +5 onwards, the animals were observed twice daily for signs of LCM infection (convulsions induced by spinning, ruffled fur, facial edema, hunched posture). Gross and light microscopic examinations were performed on all dying mice, and brains of these animals were titrated for presence of virus. Survivors were tested for anti-LCM antibodies, the presence of virus in blood and brain, and resistance to rechallenge.

Results. The results of the experiments are graphically summarized in Fig. 1 and 2. Normal rabbit serum (NRS) had no effect on the development of LCM infection, which was uniformly fatal in controls between days +5 and +8. Differing RAMT serum treatment schedules, however, markedly altered the course.

When RAMT serum was given at 3-day intervals (Group A), starting on day −6, no clinical signs developed so long as injections were continued. Animals of this group sacrificed during their period of unresponsiveness showed no histologic evidence of infection, despite high brain (10^6-10^7) and blood (10^3-10^5) virus titers. When RAMT serum was discontinued, characteristic clinical and histologic choriomeningitis developed in 7 animals within 1-2 weeks, although 4 of these recovered with active immunity following prolonged infection. This prolonged illness, lasting 1-2 weeks, resembled acute LCM infection in its clinical signs and sometimes even included convulsions. In the remainder of Group A animals, no clinical illness developed when RAMT serum was discontinued, despite histologic evidence of mild meningitis and choroiditis. With the indirect immunofluorescent technique, all surviving animals were found to have anti-LCM antibodies. They also were resistant to intracerebral rechallenge with 1000 LD_{50} of virus, and had gradually diminishing virus titers. By day 40, brain titers had diminished to 10^3 LD_{50}, and by day 76 they were undetectable. Ani-

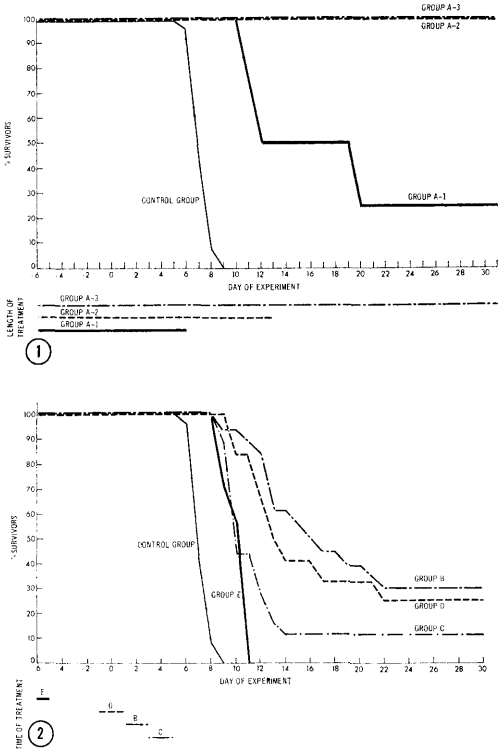

FIG. 1. Effect of prolonged anti-thymocyte (RAMT) serum treatment on survival from LCM infection in mice.

FIG. 2. Effect of varying short treatment schedules of anti-thymocyte (RAMT) serum on survival from LCM infection in mice.

mals of this group treated with RAMT serum for 36 days (A_3) showed evidence of small lymphocyte depletion from thymus dependent areas of spleen and lymph nodes(5,6) at termination of treatment, and replacement of these cells by large mononuclear and plasma cells. There was also a moderate depletion of small thymocytes from the thymic cortex. Nineteen-day treatments (A_2) produced similar but less pronounced changes. By 7-14 days following discontinuation of RAMT serum therapy, repopulation of lymphoid organs by small lymphocytes and restoration of peripheral lymphocyte counts was complete in all Group A animals.

All other RAMT serum treatment schedules employed (Groups B-E) were able to delay the onset of clinical and histologic signs of infection. Even one dose of RAMT serum 6 days prior to virus inoculation was able to prolong life significantly (Group E). There was no significant difference between the two groups intensively treated early in the course of infection (B and D). However, a marked difference ($p<0.01$, Kolmogorov-Smirnov non-parametric test) was found between the effects of RAMT serum therapy early and late in the LCM virus incubation period. Treatment on days 0, +1 and +2 (Group B) resulted in a median prolongation of life of 10 days over that of controls, while treatment on days +3, +4, and +5 (Group C) resulted in a prolongation of less than 4 days.

Discussion. The mechanisms of action of anti-thymocyte and anti-lymphocyte sera are not fully understood. Possible actions include lymphocytolysis, blindfolding or sterile activation of lymphocytes, and interference with a thymic humoral factor(7,8,9). Anti-thymocyte serum is more effective than anti-lymphocyte serum in depressing peripheral lymphocyte counts and delaying homograft rejection(9). This augmented effectiveness, together with an apparent selectivity of anti-thymocyte serum for thymic cells(1), strongly suggests that part of its action is directed against the thymus itself, possibly by suppression of a thymic hormone(9). The promptness of the effect noted, however, indicates that immunologic thymectomy alone cannot explain the action of the antiserum. A more appropriate analogy may be that between the effects of antithymocyte serum and those of thymectomy plus irradiation(9). This would account both for thymic suppression and a more rapid peripheral lymphoid depletion.

The importance of the thymus in LCM pathogenesis has been repeatedly demonstrated. Neonatal thymectomy protects mice of differing strains against later morbidity from LCM infection, despite its inability to prevent virus multiplication, or complement fixing antibody formation(2). Thymectomized animals fail to develop the characteristic mononuclear cell infiltration of the leptomeninges, ependymal lining of the ventricular system, and choroid plexus, and it has been proposed that they cannot produce a "clone" of small lymphocytes to react to new exposure with LCM virus(3). Intraperitoneal cell-tight diffusion chambers containing thymic tissue restore susceptibility of neonatally thymectomized mice to lymphocytic infiltration and lethality following LCM inoculation, indicating that a thymic humoral factor is necessary to allow full responsiveness of competent cells to the virus(3). Suppression of this factor, together with peripheral lymphocyte depletion, could account for the suppressive effects described in this report. Although it was not possible to induce a permanent state of tolerance in adults using RAMT serum, preliminary data from unpublished experiments with newborn mice suggest early and prolonged treatment can permit development of neonatal-type tolerance to LCM at a later age than ordinarily possible. This suggests an anti-thymocyte serum induced maturation arrest of the lymphoid system.

It has been proposed that anti-thymocyte serum also acts on the afferent limb of the immune pathway, preventing adequate recognition of antigens(8,10). The augmented effectiveness of RAMT serum shortly after virus inoculation, when compared to later treatment, may in part be a result of delayed antigenic recognition. Once LCM recognition had occurred, and specifically immunocompetent cells had been produced, anti-

thymocyte serum action would be expected to be less effective.

It is of interest that anti-LCM antibody production proceeded in RAMT serum treated animals, despite suppression of cellular responsiveness, confirming the dissociation of humoral and cellular immunity in LCM infection noted by Rowe *et al*(2). The reduced intensity of clinical and histologic signs following discontinuation of prolonged RAMT serum therapy may have been a consequence of circulating anti-LCM antibody which had reached an effective titer prior to the appearance of immunologically competent and potentially damaging lymphocytes. The outcome of LCM infection in mice, thus, may depend intimately on the balance between cellular and humoral factors responding to virus invasion.

Summary. Rabbit anti-mouse thymocyte serum significantly modified the course of mouse LCM virus infection. Clinical and histologic signs of infection were absent so long as therapy was regularly maintained, and the severity of the host response to the virus was reduced following discontinuation of prolonged therapy. Treatment in the early period of incubation was more protective than at later times.

Addendum. This work was presented in part before Am. Assn. of Immunologists, April 1967, Chicago, Ill.(11). Since it was submitted, Gledhill has reported preliminary data describing similar protective effects of anti-thymocyte serum in LCM infection(12).

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Growth and Cationic Stabilization of Group A Coxsackieviruses.* (32255)

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Coxsackieviruses were first isolated by Dalldorf and Sickles in 1947 by inoculation of newborn mice with fecal specimens from two children in Coxsackie, N. Y., who were suspected of having poliomyelitis(1). The first strains isolated produced lesions limited to muscles of the inoculated animals. In 1948, Melnick *et al*(2) isolated, from aseptic meningitis patients, a number of new types which produced lesions in a variety of other tissues of the newborn mouse. Because of their characteristic histopathology, the new types were classed as representative of

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