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Mouse Hepatitis Virus (MHV) Infection in Thymectomized C_3H Mice (40278)

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The macrophage plays a crucial role in the genetic susceptibility of mice to develop fatal hepatitis when infected with mouse hepatitis virus (MHV) (1). The adult C_3H mice do not die after inoculation of MHV grown in Princeton mice (MHV(PRI)) and their macrophages do not support MHV(PRI) multiplication. On the other hand, infant C_3H and infant and adult Princeton (PRI) mice develop a fatal infection after inoculation with MHV(PRI) and their macrophages support the multiplication of this virus and are destroyed by it (2). Intermediate susceptibility of macrophages *in vitro* is associated with virus persistence *in vivo* (3). A variety of treatments which depress the cell mediated immune response makes the MHV infection pathogenic for the genetically resistant mouse. Adult C_3H mice develop fatal MHV infection after neonatal thymectomy (4) or after treatment with cortisone (5), cytoxan (6) or preinfection with *Eperythrozoon coccoides* (7) and adult A strain mice are rendered susceptible to fatal MHV infection by a variety of treatments such as x-irradiation, administration of antilymphocyte serum and neonatal thymectomy (8, 9).

We report here that although the outcome of MHV(PRI) inoculation in adult PRI and C_3H mice is very different, both strains are infected by the same minimal infectious dose of MHV(PRI). In addition, although MHV(PRI) infection is fatal both for adult neonatally thymectomized C_3H mice and the genetically susceptible PRI mice, the course of infection in these two strains is quite dissimilar. These findings suggest that the routine recovery of adult C_3H mice from MHV(PRI) infection requires both virus resistant macrophages and normal thymic function.

Materials and methods. Virus. MHV-2 strain of virus originally obtained from Dr. John Nelson (10) was maintained in our laboratory by intraperitoneal (ip) inoculation of 4 week old PRI mice. This strain is referred to as MHV(PRI). A variant strain MHV(C_3H) which was derived from MHV(PRI) but which is lethal for both adult C_3H and adult PRI mice (11) was maintained by ip inoculation of 4-week old C_3H mice. The stock virus preparations were 10% homogenates of livers from virus infected moribund mice. Titrations were performed by inoculation of 0.2 ml of serial tenfold dilutions in each of three tubes of cultured peritoneal macrophages from PRI mice prepared as described previously (12), but maintained in Eagle's minimum essential medium (Earle's salts) supplemented with 20% fetal calf serum (FCS). The cultures were observed for viral cytopathic effect (CPE) for 8 days. The 50% tissue culture infectious dose ($TCID_{50}$) was calculated by the method of Reed and Muench (13).

Mice. Three strains of inbred mice, PRI, C_3H and C_3Hss were used (12, 14, 15). The C_3Hss strain is congenic with the C_3H strain but is susceptible to fatal infection with MHV(PRI) and its macrophages support MHV (PRI) multiplication. It was developed by introducing the PRI gene for susceptibility to MHV(PRI) into C_3H mice (15). Mice were infected by the ip route. Thymectomy was performed on C_3H mice within 24 hr of birth. Both left and right sections of the thymus were removed by gentle suction. Thymectomized mice were infected at 4–6 weeks of age. Sham operated mice served as controls.

Immunofluorescence. Anti-serum was prepared in vaccinated PRI mice. PRI mice were inoculated for four successive weeks with β-propiolacetone inactivated vaccine (6), then challenged with live virus and bled one week later. The serum was stored at $-20°$. Liver sections were cut on a cryostat at 4 microns. Fluorescein conjugated goat anti-mouse IgG, concentration 1:10 (Meloy Laboratories Inc., Springfield, VA) was used with a counterstain of Evans Blue prepared as a 0.5% stock so-

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lution and used at a 1:8 concentration.

Histology. Sections of liver were placed in neutral buffered formalin, cut and stained with hematoxylin and eosin for histopathology.

Results. Infectivity and pathogenicity of MHV(PRI) for PRI and C3H mice. MHV-(PRI) was titrated in 4–6 week old PRI and C_3H mice using four mice per dilution and the inoculated mice were observed for 7 days for mortality. On day 7, the surviving mice of both titrations were challenged ip with 2.6 $\log_{10}$ units of MHV(C_3H) virus. Ability of a mouse to resist MHV(C_3H) challenge was taken as evidence that it was previously infected with MHV(PRI).

The MHV(PRI) had a LD_{50} titer of $10^{8.3}$ for PRI mice (Table I). None of the PRI survivors of the titration resisted MHV(C_3H) challenge indicating that MHV(PRI) did not produce a nonfatal immunizing infection in PRI mice. In contrast MHV(PRI) produced no mortality in C_3H mice inoculated with $10^{-2.0}$–$10^{-8.0}$ dilutions of the virus and all but two of the survivors of this titration resisted challenge with MHV(C_3H). The immunizing titer of the virus was $10^{8.0}$ for C_3H mice. These results indicate that PRI and C_3H mice were equally susceptible to infection with MHV(PRI) but that the infection was uniformly fatal in PRI and uniformly nonfatal in C_3H mice.

Course of MHV(PRI) infection in thymectomized C3H mice. Neonatally thymectomized or sham operated C_3H mice were inoculated ip with $10^{5.0}$ $TCID_{50}$ of MHV(PRI) and observed for mortality, virus titers in liver and liver pathology. All thymectomized mice were checked for the completeness of operation at the time they died or were sacrificed and animals with thymus remnants were excluded from the study.

In the 13 mice infected with MHV(PRI) and also completely thymectomized, the mortality was 100% (Fig. 1). This was in contrast

TABLE I. INFECTIVITY AND PATHOGENICITY OF MHV(PRI) FOR 4–6 WEEK OLD PRI AND C_3H MICE.

	MHV(PRI) virus	
Mouse	Infectious $dose_{50}$	Lethal $dose_{50}$
C_3H	$10^{8.0}$	$<10^{2.0}$
Princeton	$10^{8.2}$	$10^{8.2}$

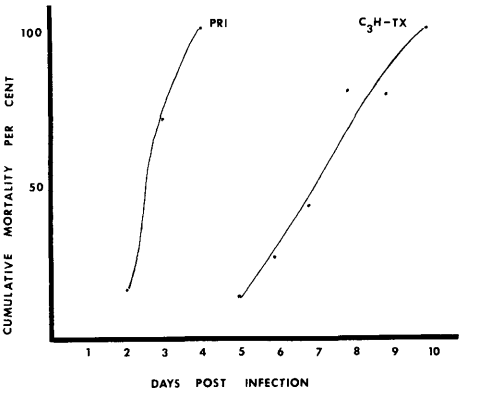

FIG. 1. Cumulative mortality of PRI and C_3H thymectomized mice infected with MHV(PRI) virus. Data on PRI mortality were pooled from several experiments and include over 100 mice which were inoculated with similar virus dilutions. Data on C_3H-thymectomized mice are based on 13 completely thymectomized mice.

to the 0% mortality in intact C_3H mice which were inoculated with MHV(PRI). Deaths occurred between day 5 and 10 after inoculation of virus with an average survival time of between 7 and 8 days. This timing of mortality was very different from that in PRI mice which, while they also have a mortality of 100%, survive only 2–3 days postinfection (Fig. 1).

Virus titers in livers of thymectomized and sham operated C_3H mice are shown in Fig. 2. Until day 7 postinfection, the titers in the two groups were comparable and ranged between $10^{5.0}$ and $10^{7.5}$. Exceptions to this were two sham operated mice which had titers between $10^{2.0}$ and $10^{3.0}$ on day 6. After day 7, there was a marked reduction in liver titers of sham operated mice. Of eight livers titrated between days 8 and 12, seven were negative for virus and the eighth had a titer of $<10^{1.0}$. In contrast, virus titers in completely thymectomized mice continued to remain high; all of four livers harvested from this group between days 8 and 10 had titers between $10^{5.0}$ and $10^{7.0}$.

The pathologic lesions in sham operated and thymectomized C_3H mice were very similar until day 5. By day 4 the livers showed general architectural disruption with coagulative change with diffuse and focal inflammation in which polymorphonuclear leukocytes were most prominent. Eosinophilic bodies as described by Ruebner and Miyai (16) could be seen in areas of necrosis. The liver

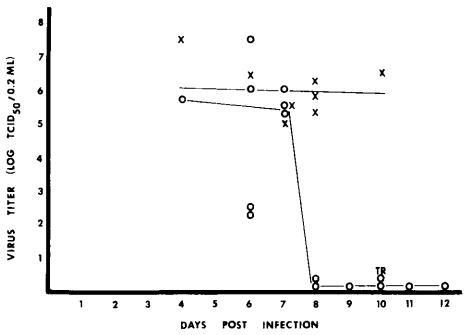

FIG. 2. Virus titers in livers from virus infected sham operated C_3H and thymectomized C_3H mice on PRI macrophage cultures. This graph includes the results of several experiments. O = liver from one C_3H sham operated mouse; × = liver from one C_3H thymectomized mouse, TR = Trace of virus, only one of three cultures inoculated with lowest dilution affected.

sections from sham operated C_3H mice taken on day 6 showed perivascular infiltration of mainly mononuclear cells with small foci of mononuclear cells on top of necrotic parenchymal cells. The livers of C_3H thymectomized mice of the same time period had large areas of acute fulminating lesions with tissue hemorrhage, necrotic debris, and the presence of polymorphonuclear leukocytes and mononuclear cells. On day 8, liver sections of sham operated C_3H mice showed a few focal areas of resolving lesions, while liver sections from C_3H thymectomized mice showed foci of degeneration with larger foci showing centers of liquefaction. Sections of livers of sham operated C_3H mice from 9 through 14 days were normal with the exception of two mice whose livers showed ocassional resolving lesions. In summary, liver sections from sham operated C_3H controls showed focal hepatitis with subsequent recovery, whereas liver sections form thymectomized C_3H mice showed focal hepatitis progressing to diffuse hepatitis with no recovery. The sham operated mice described above had more severe pathologic lesions and higher virus titers in livers than what is ordinarily found in normal C_3H mice infected with MHV(PRI). The reason for this was not clear.

We also compared the pattern of viral multiplication in livers of PRI, C_3H and C_3Hss mice by histopathology, immunofluorescence and viral titrations. Mice were infected ip with $10^{5.0}$ $TCID_{50}$ of MHV(PRI) and were sacrificed at 3, 6, 10, 24, 48 and 72 hr postinfection. Liver sections from PRI mice showed increased cellular infiltration by 10 hr which progressed to necrosis of parenchymal cells with eosinophilic bodies by 48 hr and extensive tissue destruction with hemorrhage by 72 hr. Immunofluorescence was detected and observed to spread as the lesions and cellular destruction grew. In C_3H mice an infiltration of mononuclear cells was detected as early as 6 hr postinfection. Small necrotic foci were observed by 48 hr. In these liver sections very little fluorescence was noted in the first 10 hr. Small fluorescent foci of necrosis containing eosinophilic bodies and Kupffer cells were apparent by 48 hr. There was a striking difference in viral growth between the susceptible (PRI, C_3Hss) and resistant (C_3H) mice (Fig. 3). Virus titers in PRI livers were higher than those in C_3H livers by 2 $\log_{10}$ units by 24 hr and this difference increased to 6 $\log_{10}$ units by 72 hr. The C_3Hss mice resembled PRI mice with respect to both virus titers in liver and pattern of mortality.

Growth of MHV(PRI) in macrophage cultures from thymectomized and nonthymectomized C_3H mice. Earlier work has shown that the pathogenic effect of MHV(PRI) on the mouse was closely correlated with the ability of the peritoneal macrophage of that mouse to support multiplication of the virus (2, 12). It was therefore of interest to see if cultures of macrophages derived from thymectomized C_3H mice supported growth of MHV(PRI).

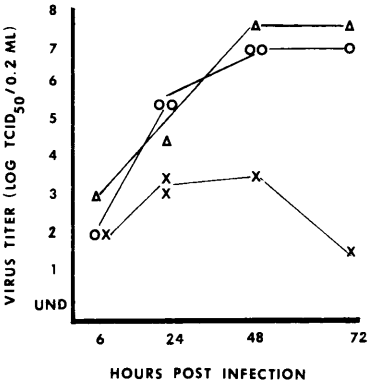

FIG. 3. Virus titers in livers of PRI, C_3H and C_3Hss mice in the first 3 days after infection with MHV(PRI). O = PRI mice. × = C_3H mice. Δ = C_3Hss mice.

Macrophages were removed from 4 to 6 week old thymectomized and nonthymectomized mice. Only those thymectomized mice which clearly had no grossly visible thymus remnants were used as macrophage donors. Inoculum containing $10^{8.0}$ infective units of virus was allowed to adsorb for 30 min and the cultures were then washed and fresh medium added. The tubes of cultures were observed for viral CPE and harvested at various time periods after infection. They were stored frozen at −70° until they were titrated on macrophages from PRI mice.

The virus did not produce CPE in either C_3H or C_3H thymectomized macrophage cultures and there was no difference between these macrophages in their ability to support MHV(PRI) multiplication (Fig. 4). Low titers of virus were recovered from both kinds of macrophages through the observation period of 8 days. This pattern of virus growth in C_3H macrophages was markedly different from that in PRI macrophages (Fig. 4), in which the virus grows rapidly with complete lysis of the cells in 48 hr.

Discussion. Our studies confirm the previous reports of Stutman and Yunis (4) and of Le Provost and his colleagues (8, 9) that thymectomy increases the pathogenicity of MHV in genetically resistant mice. MHV(PRI) produced no mortality in the intact C_3H mouse but it infected this strain as readily as it did the PRI mouse. Thymectomy increased the mortality in the C_3H mouse from 0 to 100%. The pathologic studies as well as virus titers in the liver clearly indicated that the death of the thymectomized C_3H mouse was due to its inability to resolve the early hepatic lesions which occurred in both thymectomized and nonthymectomized animals. These lesions progressed to fulminant hepatitis in the thymectomized C_3H mouse resulting in death about 6–10 days after inoculation of virus, but were completely resolved in the intact C_3H mouse. This requirement of thymic function for the recovery of C_3H mice from MHV hepatitis appears to be similar to that described by Blanden (17) for the resolution of ectromelia infection of mice.

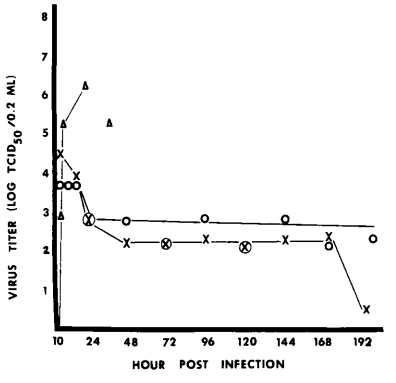

FIG. 4. Growth of MHV(PRI) in PRI, C_3H and thymectomized C_3H macrophage cultures. Data for PRI macrophages taken from Shif and Bang 1970 (11). △ = PRI macrophages. ○ = C_3H macrophages. × = thymectomized C_3H macrophages.

Although the MHV(PRI) infection was uniformly fatal in thymectomized C_3H mice as well as in PRI mice, the course of the disease was very different in these two strains. In PRI mice the virus multiplies very rapidly leading to death in 2–3 days whereas in the thymectomized C_3H mice mortality occurred later and over a longer period. This difference very likely reflects the fact that the PRI macrophages support very well the multiplication of MHV(PRI) while the macrophages of thymectomized C_3H mice do not. The C_3Hss mouse resembled the PRI mouse in its susceptibility to MHV(PRI). Differences in survival time after MHV infection has been shown even among susceptible strains of mice (18). This difference in susceptibility was related to the varying ability of the macrophages as the primary targets of the virus to support viral growth. These observations indicate that resistance to MHV(PRI) infection, as in the intact adult C_3H mouse, requires *both* a resistant macrophage which limits the spread and multiplication of the virus and an intact thymic function which is necessary for the resolution of focal hepatic lesions. In mice that have susceptible macrophages, namely, infant C_3H, infant and adult PRI, and the congenic C_3Hss strain (15), the infection is so overwhelming that it kills the mouse before it has had a chance to develop an effective T cell response. In experiments not described here, transfer of immune C_3H spleen cells to C_3Hss animals with susceptible macrophages failed to confer resistance to challenge with MHV(PRI) (19). MHV(PRI) infection of C_3H mice can also be made more pathogenic

by treatment of these mice with cortisone (5) or cytoxan (6). The mechanism by which these drugs bring about this effect is not clear but it could be by their destruction of T cells *per se*, or as suggested by Weiser and Bang (20), by release of lymphokines which alter macrophage susceptibility. LeBlond et al (21) have shown that both macrophages and T cells are necessary in the transfer of resistance to MHV to infant mice.

Summary. MHV(PRI) virus produced a non-fatal immunizing infection in adult C_3H mice over a greater than 6.0 $\log_{10}$ unit range but a uniformly fatal infection in adult Princeton (PRI) mice. Neonatally thymectomized 4–6 week old C_3H mice died by day 10 after inoculation with MHV(PRI). Intact and thymectomized C_3H mice had comparable virus titers in their livers until day 7 postinfection after which time virus was undetectable in intact C_3H mice but remained at high titers in thymectomized C_3H mice. The liver pathology was similar in both groups until day 6 post infection after which time resolving lesions were seen in livers of intact C_3H mice whereas thymectomized C_3H mice developed fulminant fatal hepatitis. In *in vitro* tests, the macrophages of the thymectomized C_3H mice did not support growth of MHV(PRI) virus to any greater extent than the macrophages of nonthymectomized C_3H mice.

Although infection with MHV(PRI) was fatal for both PRI and thymectomized C_3H mice, the course of infection was much more rapid in PRI mice. C_3Hss mice which are congenic with C_3H mice but have macrophages which support growth of MHV(PRI) responded to MHV(PRI) infection with a rapidly fatal illness in the same way as PRI mice. These data suggest that macrophages resistant to viral multiplication and intact thymic function are both necessary for resistance to the lethal effects of MHV(PRI) virus.

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