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Mouse hepatitis virus infection of mice causes long-term depletion of lactate dehydrogenase-elevating virus-permissive macrophages and T lymphocyte alterations

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Abstract

Intraperitoneal injection of pathogen-free B10.A mice with mouse hepatitis virus (MHV)-A59 resulted in a short subclinical infection which was terminated by a rapid antiviral immune response. The infection resulted in a rapid, but transient, about 10-fold increase in the number of macrophages and total cells in the peritoneum of the mice. This increase was preceded by a complete depletion of the peritoneum of the subpopulation of macrophages that supports a productive infection by lactate dehydrogenase-elevating virus (LDV). The depletion of LDV-permissive macrophages was a long-term effect; at 50 days post-infection with MHV, the proportion of LDV-permissive macrophages in the peritoneum had reached only 20% of that observed in the peritoneum of uninfected mice, whereas the total number of macrophages in the peritoneum had returned to normal. Furthermore, MHV infection resulted in a long-term alteration in the proliferative response of spleen T cells to concanavalin A (ConA) and in their ability to produce interferon γ; several times higher concentrations of ConA were required to induce a maximum proliferative response in spleen T cell populations from 5-week MHV-infected B10.A mice than in spleen T cell populations from infected companion mice but the former produced 5 times more interferon γ than the T cells from uninfected mice.

Keywords: Mouse; Hepatitis virus; Lactate dehydrogenase-elevating virus; Macrophage; T lymphocyte

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Effects of inapparent virus infections of mice can compromise studies with other viruses, transplantable tumors and noninfectious agents. For example, inapparent infections by contaminating lactate dehydrogenase-elevating virus (LDV) causes various host effects that were erroneously attributed to transplantable tumors or other viruses (Riley et al., 1978; Rowson and Mahy, 1985; Plagemann and Moennig, 1992; Plagemann, 1996). A recent survey showed that 49/81 of transplantable mouse tumors maintained by mouse passage, 4/45 human tumors passaged in nude mice, and 2/58 monoclonal antibodies (mAbs) in ascites fluid were contaminated with LDV (Nicklas et al., 1993). Similar situations hold for mouse hepatitis virus (MHV) (Barthold, 1986; Compton et al., 1993; Nicklas et al., 1993). Furthermore, it has been estimated that a majority of mouse colonies might be infected with MHV (Lindsey et al., 1986). In a previous study we found that practically all mice raised in our animal facilities possessed significant titers of anti-MHV antibodies at 4–6 weeks of age without ever showing any clinical symptoms (Even and Plagemann, 1995). Since LDV replication in mice is restricted to a subpopulation of macrophages (Plagemann and Moennig, 1992) and MHV also replicates in these cells (Smith et al., 1984), we suspected that inapparent MHV infections may lead to a loss of LDV-permissive macrophages in mice and be responsible for the great variations (0–25%) in the proportion of LDV-permissive macrophages that have been observed in different populations of adult mice over the last 20 years (Plagemann and Moennig, 1992). This hypothesis seemed supported by our recent observation that the proportion of LDV-permissive macrophages in cultures of peritoneal cells from adult pathogen-free B10.A mice ranged from 25 to 50% (see bar at 0 time, Fig. 1), whereas it was only 6–10% for peritoneal macrophages from FVB mice that were raised in our facilities and possessed anti-MHV antibodies

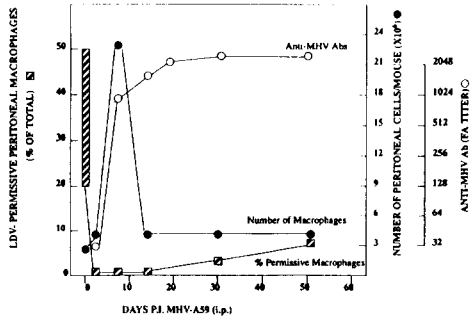

Fig. 1. Effect of MHV-A59 infection of mice on LDV-permissive peritoneal macrophages. A group of B10.A mice about 5 weeks of age was infected with about 10^5 PFU of MHV-A59. At various times p.i. the mice were bled by the retroorbital method and their plasma titrated for anti-MHV antibodies (Abs) by an indirect fluorescent antibody staining assay as described previously (Even and Plagemann, 1995). Peritoneal macrophages were harvested from mice before infection and at various times p.i. with MHV, enumerated, cultured for one day and then assayed for susceptibility to LDV infection (LDV permissiveness). The striped rectangle indicates a range of values observed in a number of experiments for the proportion of LDV-permissive peritoneal macrophages in B10.A mice before MHV infection; the values for mice p.i. with MHV are for peritoneal macrophages from one to three individual mice.

(Even and Plagemann, 1995). Our present results directly demonstrate that MHV infection of mice causes a long-term depletion of peritoneal macrophages that are permissive for LDV infection as well as an alteration in concanavalin A (ConA)-responsiveness of spleen T lymphocytes. These effects persist a long time after infectious MHV appears to have been cleared from the mice.

A group of pathogen-free, 5-week-old B10.A mice (kindly supplied by Drs. Torseth and Gregerson) was injected intraperitoneally (i.p.) with 10^5 plaque-forming units (PFU) of MHV-A59 (provided by Dr. K. Holmes; Holmes, 1990). The mice were bled at various times post-infection (p.i.) and their plasma assayed for infectious MHV and anti-MHV antibodies as described previously (Even and Plagemann, 1995). Peritoneal cells were harvested from individual mice, counted, cultured and assayed for LDV permissiveness as described previously (Cafruny et al., 1986; Even and Plagemann, 1995). In brief, the cells were cultured for one day and infected with about 500 50% infectious dose (ID_{50}) of LDV/cell. At 8 h p.i. the cultures were fixed in acetone. The fixed slides were incubated sequentially with mAb 159-12 which recognizes the envelope glycoprotein of LDV (Harty et al., 1990) or with polyclonal anti-LDV which consisted of plasma from 5-month LDV-infected mice and then with fluorescein-isothionate-conjugated goat F(ab')2 anti-mouse IgG. The cultures were examined with a fluorescence microscope and the proportion of stained cells estimated by counting about 500 cells.

The results in Fig. 1 show that within 2 days p.i. with MHV-A59 all LDV-permissive peritoneal cells had disappeared from the peritoneum in spite of the fact that the total number of peritoneal macrophages at that time was little altered. Lack of LDV-permissive cells persisted for at least 14 days (Fig. 1). However, by 30 days p.i., LDV-permissive cells started to return; about 2% of total peritoneal macrophages were LDV-permissive (Fig. 1). By 50 days p.i, about 5% of the peritoneal macrophages of the mice were LDV-permissive (Fig. 1), a proportion similar to that observed in cultures of macrophages from mice raised in our facilities that seemed naturally infected with MHV. These observations have been confirmed in another independent experiment (data not shown).

Within a few days of the initial MHV infection, the total number of cells in the peritoneum increased transiently about 10-fold (Fig. 1). This increase largely reflected an increase in macrophages since the number of cultured adherent cells had similarly increased about 10-fold. A comparable maximum increase was apparent in three individual mice at 7 days p.i. in the absence of any overt clinical symptoms. A similar transient increase in peritoneal macrophages has been previously observed after natural MHV infection of mice in the absence of clinical disease (Boorman et al., 1982).

The reason for the transient marked increase in peritoneal macrophages associated with acute MHV infection is not entirely clear and neither is the reason for the disappearance of LDV-permissive macrophages. However, both are probably related to an extensive replication of MHV in peritoneal macrophages during the first day p.i. and the destruction of these cells. This view is supported by the finding that many peritoneal macrophages were MHV-infected when harvested at 1 day p.i. When cultured, these cells rapidly formed syncytia and complete

destruction of the culture was apparent within one day of culture (data not shown). The reason why the massive destruction of peritoneal macrophages was not reflected in a decrease in total number of peritoneal cells was probably that it was compensated by an influx of macrophages that ultimately resulted in the transient massive accumulation of these cells in the peritoneum (Fig. 1). Most of these cells seemed to be highly activated (Boorman et al., 1982) and therefore probably did not support LDV replication as activated macrophages are nonpermissive (Kowalchyk et al., 1985). Anti-MHV immune responses became rapidly generated as measured by anti-MHV antibody formation (Fig. 1). They probably suppressed MHV replication and thus terminated the accumulation of macrophages into the peritoneum (Fig. 1). No MHV-infected macrophages were detected by indirect fluorescent antibody staining (Even and Plagemann, 1995) in cultures of macrophages harvested from mice at 7, 14, 30 and 50 days p.i. with MHV (data not shown). At no time were we able to detect infectious MHV in the plasma of the infected mice. The mice also exhibited minimal signs of disease. These consisted of mild hind limb tremors in some of the mice only. However, by 30 days p.i. with MHV, the mice seemed to have completely recovered and possessed relatively high levels of anti-MHV antibodies (Fig. 1). In other words, they appeared comparable to the 5- to 6-week-old and older mice raised in our facilities (Even and Plagemann, 1995), except that their anti-MHV titers were 2- to 4-fold higher (see later). Also, the cultured peritoneal macrophages obtained from mice 30 or 50 days p.i. with MHV were morphologically indistinguishable from the resident macrophages present in the pathogen-free B10.A mice before MHV infection or from other adult mice raised in our facilities that possess anti-MHV antibodies (see Onyekaba et al., 1989; Even and Plagemann, 1995). They appeared morphologically normal and their NO production was normal (see later). However, the population remained largely depleted of LDV-permissive macrophages. A disappearance of LDV-permissive macrophages from the peritoneum was also observed in mice infected with MHV-A59 intranasally (i.n.; data not shown). However, mice infected i.n. generally died 5–7 days p.i. with and without hind limb paralysis.

The long-term depletion of LDV-permissive peritoneal macrophages in MHV-infected mice seems to coincide with long-term alterations in T lymphocyte properties in MHV-infected mice. For example, skin graft rejections were impaired in MHV-infected mice as long as 102 days p.i., even though the virus appeared to have been cleared 2–3 weeks p.i. (Cray et al., 1993). The proliferative response of spleen T cells to concanavalin A (ConA) has been found to be greatly reduced during the acute phase of infection with MHV (reviewed in Compton et al., 1993) but was still impaired at 35 days p.i., though not at 102 days p.i. (Cray et al., 1993). Since the long-term effects of MHV infection on macrophage and T lymphocyte populations could have a common cause, we have further investigated the long-term effect of MHV infection on T cell properties. In addition, we have explored the combined effects of MHV and LDV infection on the proliferation of T lymphocytes since previous studies have shown that LDV infection also causes a transient impairment of the proliferative response of T cells to ConA (Li et al., 1990; Plagemann et al., 1995). The effect is maximal at 3 days p.i. and has dissipated by 1

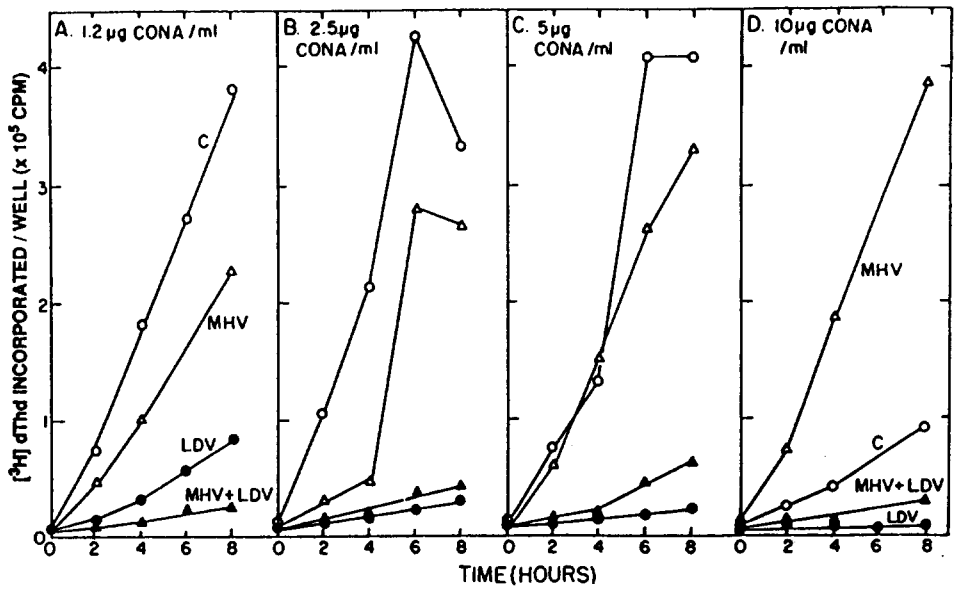

Fig. 2. Effects of LDV and MHV infection of mice on the ConA-induced proliferative response of their spleen cells. One group of 4 B10.A mice was infected with MHV-A59 and one group remained uninfected. Five weeks later, two mice of each group were infected with LDV. After another 3 days, the mice were bled and their plasma was titrated for anti-MHV antibodies and infectious LDV. Their spleen cells were harvested, samples of the spleen cell suspensions were incubated with the indicated concentrations of ConA for 2 days and then the time course of incorporation of [^{3}H]dThd by the cells into DNA was measured as described previously (Li et al., 1990; Rowland et al., 1994).

week p.i. In the present experiment, therefore, groups of pathogen-free B10.A mice were infected with MHV or remained uninfected. About 5 weeks later, one group of the MHV-infected mice and one group of uninfected mice were infected with LDV. Three days later, spleen cells were isolated from each group of mice and samples thereof incubated with various concentrations of ConA for 2 days and then the time course of incorporation of [^{3}H]thymidine (dThd) was measured (Fig. 2) or the cultures were analyzed for cytokine production (Fig. 3).

The results in Fig. 2 illustrate the drastic reduction in the proliferative response of spleen cells from 3-day LDV-infected mice (filled circles). The results also show that the proliferative response to 1.2 and 2.5 μg ConA/ml of spleen cells from 5-week MHV-infected mice was reduced about 50% (empty triangles), similarly as reported by Cray et al. (1993). However, we found that at 5 μg ConA/ml the proliferative responses of the spleen cells from uninfected and 5-week MHV-infected mice were about the same. Moreover, the proliferative response of the spleen cells from the MHV-infected mice to 10 μg ConA/ml was undiminished, whereas that of spleen cells from uninfected mice was diminished as is normally observed with spleen cells from uninfected mice at above optimal ConA concentrations. In fact, the proliferative response of the spleen cells from MHV-infected

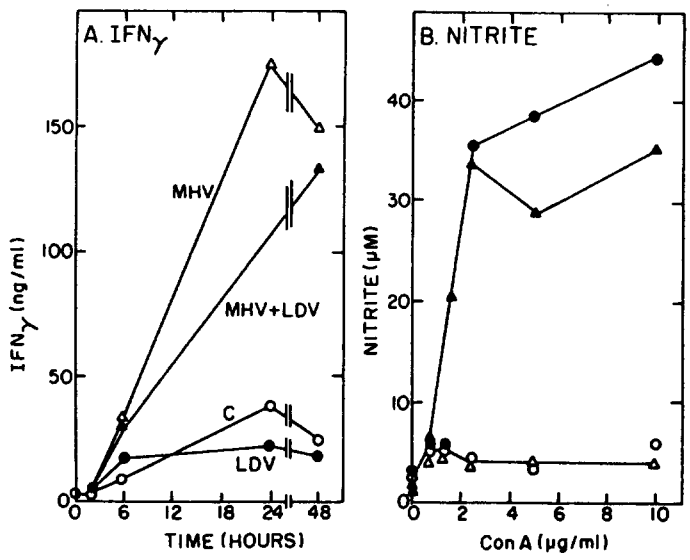

Fig. 3. IFNγ (A) and NO (B) production by ConA-stimulated spleen cells from uninfected, 5-week MHV-infected, 3-day LDV-infected and dually infected B10.A mice. The details of the experiment are described in the legend to Fig. 2. (A) Cultures were incubated with 2.5 μg ConA/ml and at the indicated times of incubation samples of the culture fluid were assayed for IFNγ by ELISA as described previously (Rowland et al., 1994). (B) Cultures were incubated with the indicated concentrations of ConA for 48 h and then their culture fluid was assayed for nitrite as described previously (Rowland et al., 1994).

mice increased progressively with the ConA concentration (Fig. 2). These results were confirmed in a second independent experiment in which spleen cells from the four groups of mice were labeled with [^{3}H]dThd for 4 h after 1, 2 and 3 days of incubation with 0.05, 2.5 and 10 μg ConA/ml (data not shown). The results suggest that the T cells from MHV-infected mice possessed the ability to proliferate but that higher concentrations of ConA were required to induce maximum proliferation. This was not the case for the spleen cells from 3-day LDV-infected mice (Fig. 2). Furthermore, LDV infection of the MHV-infected mice caused a drastic reduction in the proliferative response of the spleen cells to ConA (filled triangles), but to a slightly lesser extent than infection of previously uninfected mice, at least at the higher concentrations of ConA (Fig. 2).

The impaired proliferative responses correlated with reduced IL-2 production by the cells as measured by a bioassay (Rowland et al., 1994). For example, when the spleen cells were incubated with 10 μg ConA/ml for 24 h, the culture fluid of spleen cells from 3-day LDV-infected and the dually infected mice contained only 5 and 6 units/ml of IL-2, respectively, whereas the spleen cells from uninfected and 5-week MHV-A59-infected mice similarly produced about 3 times higher amounts (19.0 and 17.5 units/ml, respectively). Furthermore, we did not observe a significant spontaneous production of IL-2 by the spleen cells from the MHV-A59-infected mice that were incubated in the absence of ConA (1.1 units IL-2/ml)

as has been reported for spleen cells from mice infected with MHV-4 for 5–30 days (Kyuwa et al., 1988).

On the other hand, the T cell population from MHV-A59-infected mice produced increased amounts of IFNγ in response to ConA. The spleen cells from the 5-week MHV-infected mice produced about 5 times more IFNγ when incubated with 2.5 μg ConA/ml than spleen cells from uninfected mice (Fig. 3A), even though their proliferative response was reduced about 50% (Fig. 2B). Most striking was the IFNγ production by the spleen cells from the dually infected mice; they produced almost as much IFNγ as the spleen cells from the mice infected with MHV alone (Fig. 3A), whereas their proliferation was reduced 80% as the result of the LDV infection (Fig. 2B).

Activated macrophages produce increased amounts of nitric oxide (NO) and IFNγ is the major cytokine inducing NO production by these cells (Mills, 1991; Farrar and Schreiber, 1993). The spleen macrophages from 5-week MHV-infected mice produced little NO (measured as nitrite; Fig. 3B). Some NO production was induced by IFNγ generated by the T cells in the spleen cell population in response to ConA, but not more than produced by spleen macrophages from uninfected mice under the same condition (Fig. 3B). In contrast, as reported previously (Rowland et al., 1994), spleen macrophages from 3-day LDV-infected mice produced about 10 times more NO than the spleen macrophages from uninfected mice (Fig. 3B). The same was the case whether or not the mice had been infected with MHV-A59 5 weeks before LDV infection. We have demonstrated previously that the NO production was induced by the IFNγ produced by the ConA-activated T cells in the spleen cell population (Rowland et al., 1994). The results confirm that the spleen macrophages from 3-day LDV-infected mice are partially activated. In contrast, those from 5-week MHV-infected mice behaved like normal resident macrophages. Serological tests proved that the uninfected mice and the mice only infected with LDV were free of anti-MHV antibodies at the time of removal of their spleens, whereas the plasma titer of anti-MHV antibodies of the 5-week MHV-infected mice was 4096.

In summary, our results indicate that MHV-A59 infection of pathogen-free mice results in long-term alterations in the hosts' macrophage and T lymphocyte populations. This effect is reflected in a long-term depletion of LDV-permissive macrophages in the peritoneum and an alteration in the ConA responsiveness of the spleen T lymphocytes. The effect on LDV-permissive macrophages seems to explain the low and variable proportion of LDV-permissive cells we have observed in peritoneal macrophage populations harvested from adult mice raised in our facilities since our colony is infected with MHV. In fact, on two occasions over the last 20 years we were unable to detect any LDV-permissive peritoneal macrophages in such mice over periods of several months (unpublished data).

The MHV-A59-induced alterations in spleen T cell populations that are the basis of the observed changes in their properties are not understood. The T cells proliferate normally in response to ConA, but only when exposed to higher ConA concentrations than that maximally stimulating T cells from uninfected mice. The T cells from MHV-A59-infected mice do not produce IL-2 or IFNγ spontaneously,

but produce normal amounts of IL-2 in response to ConA, whereas IFNγ production is increased about 5-fold over that observed in populations of spleen cells from uninfected mice. A 3-day superinfection by LDV drastically suppresses the ConA-stimulated proliferation and IL-2 production, while little affecting the IFNγ production

One interpretation of the data is that the proliferating and IFNγ-producing spleen cells from the MHV-infected mice represent two different cell populations, a major and a minor one, respectively. The major population may represent T cells that proliferate in response to ConA and produce IL-2 and whose response is suppressed by LDV superinfection, whereas the IFNγ production in response to ConA by the minor population is little affected by LDV infection. Further work is required to investigate this postulation and to characterize these cells.

The mechanism by which MHV infection induces these long-term immunological effects is unknown. Cray et al. (1993) have suggested that a massive replication of MHV in bone marrow, thymus and perhaps other tissues during the first week p.i. may cause a permanent alteration in the generation of lymphoidal cells from stem cells in bone marrow. Such effect could explain alterations in both macrophage and T lymphocyte populations. Another possibility might have been that MHV replication persists in lymphoidal tissues at a level too low to be detectable by isolation of infectious virus and that such persistent infection is responsible for the prolonged immunological effects. However, recent results from in situ hybridizations in our laboratory speak against this possibility. We have failed to detect any MHV-infected cells in sections of spleen, brain and spinal cord of three 30-day MHV-A59-infected mice using procedures described previously (Anderson et al., 1995a, b). The probe was a ^{32}P-labelled 1.8 kb cDNA containing ORF 7 of MHV-JHM (Lai, 1990, kindly supplied by Dr. M. Lai) which hybridizes both to genomic RNA and all subgenomic mRNAs. We found only a single MHV-infected cell in many liver sections that were examined (data not shown). Thus it seems unlikely that the long-term immunological effects are caused by a low-level persistent infection in lymphoidal tissues.

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