## *Brief Definitive Report*

# BLOCKING OF IN VITRO AND IN VIVO SUSCEPTIBILITY TO MOUSE HEPATITIS VIRUS

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During the last 15 yr, an increasing number of experiments has shown that genetic susceptibility to mouse hepatitis virus (MHV) and its phenotypic alteration induced by drugs and immune responses respond in a parallel fashion in the intact mouse and in cultures of macrophages. Thus the in vitro macrophage mirrors the susceptibility of the host. In a recent series of experiments it was shown that lymphokines given to resistant macrophages convert them to susceptibility (1). Thus it may be relevant to concentrate on the numerous interactions of macrophages and lymphocytes in the study of susceptibility.

No significant attempts to change susceptible cells into resistant ones have been reported. When investigating whether concanavalin A (Con A) might stimulate lymphocytes to change the susceptibility of the macrophages, we found that genetically susceptible macrophages were converted into resistant cells by Con A administration, and that a significant percentage of susceptible mice were saved from death by Con A.

## Materials and Methods

*Mice.* 4- to 6-wk-old inbred Princeton (PRI) mice were used. This strain of mice has been maintained in our laboratory through brother-sister mating since 1955.

*Virus.* The MHV-2 strain of mouse hepatitis virus was originally obtained from Dr. John Nelson of the Rockefeller Institute in 1952 and has since been passed in our laboratory by intraperitoneal inoculation and 1 mo old PRI mice. This is the MHV-PRI virus and here it will be referred to simply as MHV. Methods of preparing stock virus have been previously described (1).

*Macrophage Cultures.* Methods of harvesting and preparing cultures of mouse peritoneal macrophages have been previously described in detail (2).

*Con A.* Bacto-Con A 50 mg in 1 M NaCl was obtained from Difco Laboratories, Detroit, Mich. It was kept at 4°C and rehydrated with distilled water.

*Preparation of Supernatant Fluid from Con A-Treated Spleen Cell Cultures.* 1 mg/mouse of Con A was injected intraperitoneally in PRI mice. Spleens were removed 3 days after injection and passed through a 60-mesh stainless steel wire cloth (Small Parts, Inc., Miami, Fla.). $10^7$ cells/ml were suspended in a medium consisting of 2 $\mu$g/ml of Con A, 80% RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.), 20% Chang's medium (2), and 100 $\mu$g/mg per ml of penicillin and streptomycin, and cultivated in 2 × 15-cm centrifuge tubes in a 37°C incubator supplemented with 5% $CO_2$. After 3 days incubation, supernatant fluid was obtained by centrifuging the cell suspension at 1,000 rpm for 30 min.

## Results

*Effect of Con A on Survival among Genetically Susceptible PRI Mice after MHV Infection.* Each experimental mouse was injected i.p. with 1.0 mg/0.1 ml of Con A. 3 days later, 3 $\log_{10}$ mean lethal dose ($LD_{50}$) of MHV was inoculated

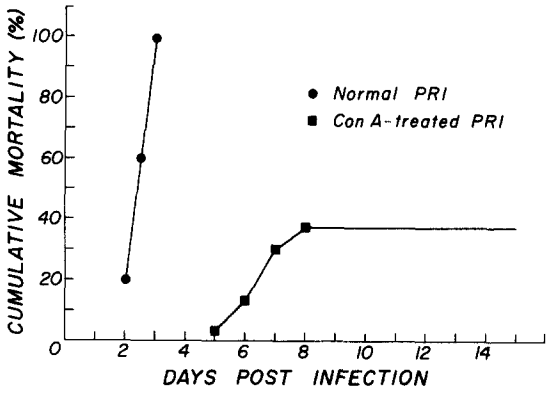


FIG. 1. Cumulative mortality of normal and Con A-treated PRI mice infected with MHV. Data were pooled from several experiments. Sample size: 50 mice (circles); 30 mice (squares).

i.p. into both experimental and control mice. Lethargy and ruffled fur became noticeable 36 h after MHV infection and the disease rapidly progressed to death in the untreated mice, while the onset of these symptoms was delayed among Con A-treated mice. Mortality among control mice commenced on day 2 and reached 100% by day 3. Some of the Con A-treated mice began to die on day 5, mortality increased on days 6 and 7, and ceased on day 8. The survival rate was more than 60% (Fig. 1). However, when phytohemagglutinin (Grand Island Biological Co.), another T-cell mitogen at a dose between 0.1 ~ 1.5 mg, or thioglycollate (Difco Laboratories) which increases the number of peritoneal macrophages, was used instead of Con A, no significant prolongation of survival time nor protection from death was obtained. Intraperitoneal injections of Con A and MHV simultaneously at adjacent sites, or injection of a mixture of Con A and MHV failed to prevent death of PRI mice.

At autopsy, the liver of every untreated moribund mouse was very pale and full of necrotic foci, whereas livers from 10 Con A-treated mice sacrificed at the same time showed only a few necrotic foci. The microscopic difference between Con A-treated and untreated mice was striking (Fig. 2). In the Con A-treated mice, MHV infection was accompanied by infiltration of mononuclear cells and polymorphonuclear cells. They surrounded the small necrotic foci, suggesting that the destructive effect of the virus to the liver parenchymal cells was arrested by inflammatory cells, whereas in the untreated controls the inflammatory response was absent or minimal, the necrotic foci were large, and little normal tissue remained.

*Susceptibility of Peritoneal Macrophages from Con A-Treated Mice to MHV.* Since genetic susceptibility to MHV resides largely in the macrophages of susceptible PRI mice (3), and cultured macrophages derived from peritoneal washings also reflect the genetic MHV trait of the host (2), the susceptibility of cells from the peritoneum of Con A-treated mice was determined. Peritoneal washings from thioglycollate-stimulated mice were used as controls. They were harvested 3 days after i.p. injection of thioglycollate. Experimental cultures were derived from peritoneal washings of day 3 Con A-treated mice (1.0 mg/mouse). After attachment, 100 tissue culture mean infectious dose ($TCID_{50}$)/0.1 ml of MHV was inoculated into each tube. All thioglycollate-stimulated PRI peritoneal macrophages were destroyed by the virus within 48 h, while macro-

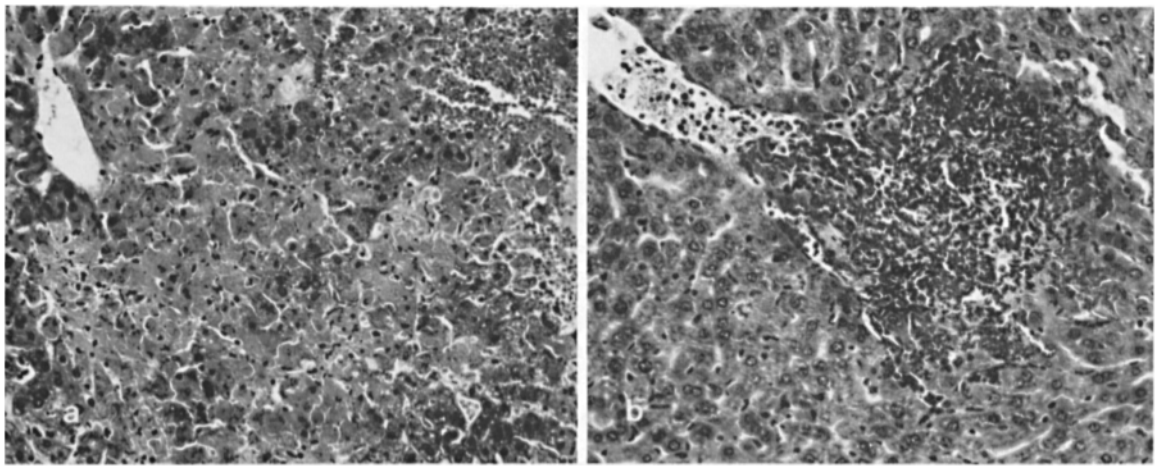

FIG. 2. H & E section of liver. (a) MHV-infected control liver, 3 days postinfection: absence of inflammatory response and large necrotic foci. × 250. (b) Liver from Con A-treated mouse, 3 days postinfection: prominent infiltration of inflammatory cells. × 250.

phages from Con A-treated mice were not uniformly susceptible to the virus (Fig. 3). In other words, there was phenotypic variation among the genetically susceptible cells.

*Effect of Supernatant Fluid Derived from Con A-Treated Spleen Cells on Syngeneic MHV-Susceptible Macrophages.* $^1/_{10}$ ml of supernatant fluid derived from Con A-treated spleen cells was added to each normal macrophage culture. After overnight incubation, 2 $\log_{10}TCID_{50}$ U of MHV were inoculated. Fig. 4 shows that macrophages incubated with supernatant fluid derived from Con A-activated spleen cells were protected against the cytopathic effect of the virus during 5 days of observation, while in the untreated controls, cultured macrophages were all destroyed 2 days after MHV infection. Supernatant fluid from normal PRI spleen cells or medium containing 2 $\mu$g/ml of Con A alone conferred no protection against MHV when incubated with syngeneic macrophages before infection. Therefore, supernatant fluid from Con A-treated spleen cells contained a factor or factors which made genetically susceptible macrophages resistant to MHV. This factor(s) was found to be acid (pH 2)-labile, but was effective after freezing at −70°C or heating at 56°C for 30 min.

## Discussion

It is well known that lymphokines can be produced via a variety of stimuli. In previous work we demonstrated a susceptibility factor obtained from supernatant fluid of allogeneic mixed lymphocyte cultures which phenotypically altered the resistance of genetically resistant C3H macrophages and rendered them susceptible to MHV (1). In searching for other susceptibility factors, Con A was used as a nonspecific polyclonal stimulator. However, it produced a factor(s) which conferred resistance on genetically susceptible cells and mice.

The first of the present experiments (Fig. 1) showed that i.p. injection of Con A at 1.0 mg/mouse prolonged the median survival time and increased the chance for survival from 0 to greater than 60% among genetically susceptible

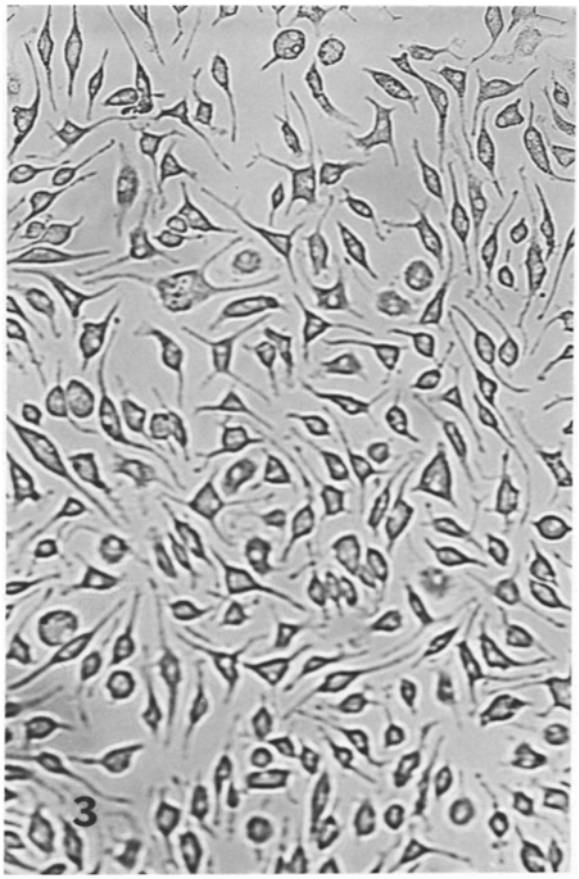

FIG. 3. Macrophages from Con A-treated PRI mice, infected with 100 $TCID_{50}$ of MHV, 3 days postinfection. Though many cells have detached from culture tube, the majority of cells remains intact. × 150.

PRI mice. Infected controls developed a fulminating necrosis of the liver with little or no inflammation, while Con A-treated mice developed small necrotic foci surrounded by a wide zone of inflammatory cells (Fig. 2). Since mice treated with Con A alone showed no cellular infiltration, it is possible that after infection with MHV, Con A activated the macrophages and lymphocytes so that they secreted a variety of biologically active mediators which brought about the inflammatory response and slowed down virus multiplication. The fact that adding virus to the Con A solution or injecting Con A and virus concomitantly at adjacent sites produced no protective effect also supports the idea that the action of Con A was via mediators and was not due to direct inactivation of the virus. Moreover, survivors were found to be immune to MHV upon challenge, which indicates that there was extensive viral multiplication in the Con A-treated mice. The importance of local (peritoneal) changes in the macrophage population, which may be bypassed by intravenous inoculation of the virus, is under study.

The role of mediators was also demonstrated in vitro by culturing normal

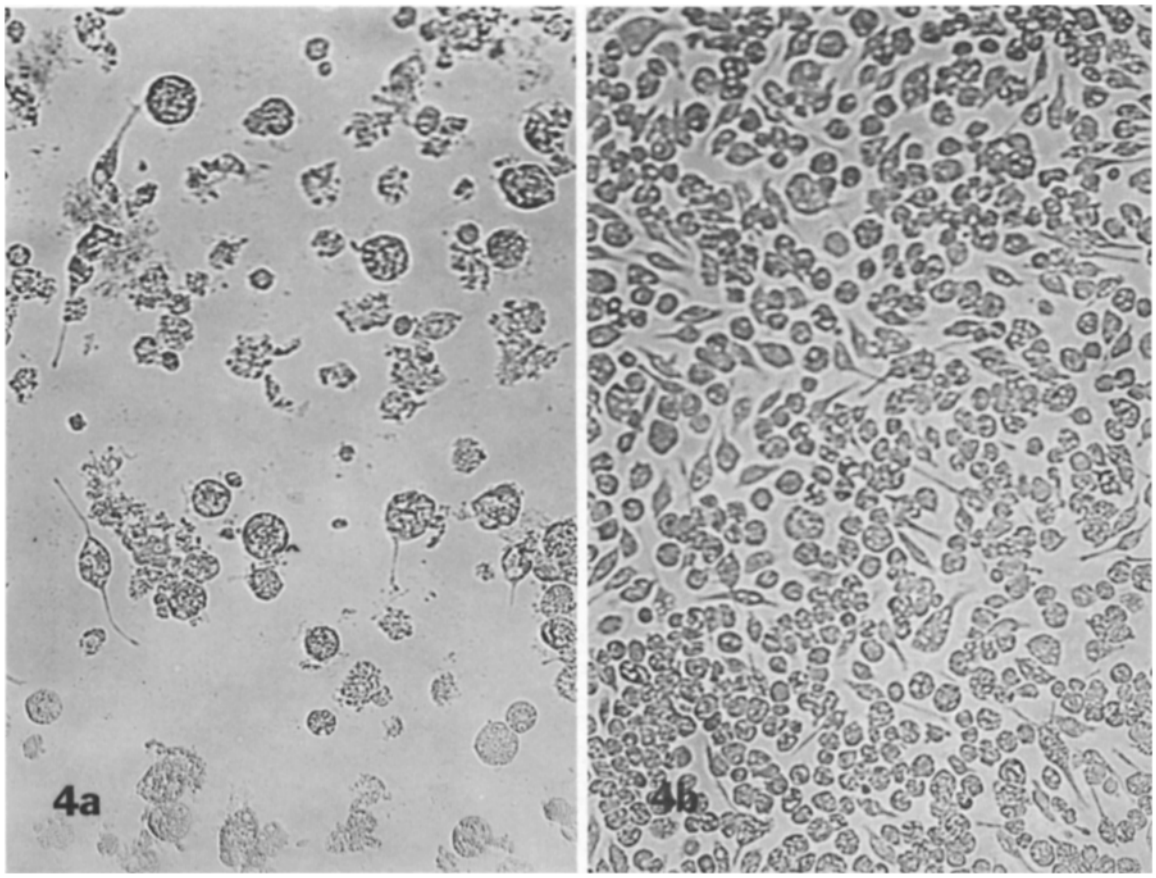


FIG. 4. PRI macrophages. (a) Normal PRI macrophages infected with 100 $TCID_{50}$ of MHV, 2 days after infection: severe destruction and loss of cells from culture tube. × 150. (b) Normal PRI macrophages with 0.1 ml of supernatant fluid from Con A-activated spleen cells added before addition of 100 $TCID_{50}$ of MHV, 5 days after infection: no apparent destruction. × 150.

susceptible macrophages in the presence of 0.1 ml of supernatant fluid from cultures of spleen cells which had been activated in vivo by Con A for 72 h. This prevented death of the cells (Fig. 4 b). Fluid from control cultures, or culture medium containing Con A alone, did not protect the susceptible macroophages (Fig. 4 a). Because lectins can stimulate the production of interferon by lymphocytes (3, 4), it was necessary to test whether the factor present in the supernatant fluid was a type of interferon. We have not yet been able to rule out the possibility that type II interferon (5) is responsible. It is known that Con A exerts multiple effects on lymphocytes, depending on the experimental conditions. Pierce et al. and Tadakuma and Pierce studied the production of a soluble immune response suppressor (SIRS) stimulated in lymphocytes by Con A and showed that SIRS mediated its suppression via macrophages (6, 7). However, our system produced results which were opposite to an expected depression in immunity. Con A stimulated immunity to MHV, presumably suggesting a very different mechanism.

## Summary

By pretreatment with concanavalin A (Con A) both in vivo and in vitro genetically susceptible mice and their cultured macrophages have been converted to animals and cells which are phenotypically resistant to mouse hepatitus virus (MHV). Con A at 1.0 mg/mouse decreased the mortality from 100% to less than 40% by inducing a prominent inflammatory response, increasing the number of macrophages in the virus inoculation site, and producing a population of macrophages not uniformly susceptible to the virus. In addition, mediators derived from Con A-treated spleen cells conferred resistance to normally susceptible syngeneic macrophages to 100 $TCID_{50}$ of MHV.

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