THE IMMUNE RESPONSE TO MOUSE HEPATITIS VIRUS: GENETIC VARIATION IN ANTIBODY RESPONSE AND DISEASE

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Coronaviruses are a group of enveloped RNA viruses which can produce a broad spectrum of diseases in their natural hosts (1,2). These diseases include encephalitis, hepatitis, interstitial pneumonitis, nephritis and enteritis (3). The nature and the severity of the resultant disease varies with the age and the genetic background of the host, the route of infection and the size of the virus inoculum.

Murine hepatitis virus type 3 (MHV-3) produces three distinct patterns of disease in genetically dissimilar inbred strains of mice. Normal adult mice of the A strain are totally resistant to MHV-3, whereas Balb/cJ, NZB, C57 and DBA/2 mice are all fully susceptible to the virus and die of fulminant hepatic necrosis (4). C3H/ebFeJ mice develop an acute hepatitis which then progresses to chronic viral persistence with focal hepatic inflammation and granulomatous formation (5).

It has recently been recognized that cellular elements of the immune system are extremely important to the host's survival and the elimination of virus (6-8). Cells of the monocyte/macrophage series play a vital role in the resistance to a number of viral infections (9). For example, the age dependent resistance to herpes simplex virus is probably due to the inability of the virus to replicate in the adherent cells of the adult mice which prevents the initial multiplication and dissemination of the virus (10). H-2 restricted

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cytotoxic T-lymphocytes appear 4 days after the initial infection and their actions peak at one week after the primary infection (11,12). Natural killer cells have been reported to be involved in antiviral activity (13), interferons have been shown capable of inhibiting herpes simplex virus multiplication in-vitro (14) and injections of anti-interferon serum increases the mortality (15). Observations made in murine hepatitis infection have led to the conclusion that resistence is dependant upon cellular immunity and specifically on the presence of a suitable number of functionally capable macrophages and T lymphocytes (16).

Viruses are strongly antigenic and elicit the sequential production of IgM, IgG and IgA antibodies during infection (17). These antibodies may inactivate and participate in the clearance of viruses either directly or in concert with complement and/or lymphoid cells. The formation of antibody-virus complexes and the resulting viral neutralization have been reported in conjunction with a number of viral systems (18). Antibody, by binding to the surface of a virion may neutralize viral infectivity by several mechanisms: (1) antibodies may bind to viral structures involved in the adherence of the virus to a potentially susceptible cell and thus prevent adsorption; (2) antibody may bind to virions in a way that allows adsorption but prevents penetration and uncoating; (3) antibody coated virions may be taken up and degraded by macrophages which might be otherwise permissive to the infection and (4) antibody on the surface of the virion may activate the complement system and or cellular elements of the lymphoid system with resultant elimination of virus particles.

Little attention has been directed towards the potential role of the humoral response to MHV infection. The studies presented here were designed to measure the primary antibody response to MHV infection, to determine both class and subclass specificities of antibody response and to determine whether the passive administration of high titered antibodies had any effect on the course of the disease in both semi-susceptible and fully susceptible mice.

MATERIALS AND METHODS

Cells

17 CL 1, DBT and L2 cells were grown as previously

described (17). The cells were propagated in Dulbecco°s modified Eagles medium (DMEM) (Flow Laboratories Inc., Rockville, Md.) supplemented with 10% new born calf serum (Flow Laboratories) and 25 µg/ml chlortetracycline hydrochloride grade II (Sigma Chemical Co., St. Louis, Mo) and buffered with 15 mM Hepes, 3-(N-morpholine-3-propanesulfonic acid) N-tris (hydroxymethyl)-3-methyl-2-aminoethane sulfonic acid and 4 mM glutamine (Sigma Chemical Co.)

Virus

The origin and growth of MHV-3 has previously been described (17). MHV-3 was obtained from the American Tissue Type Culture Collection (Rockville, Md) and was plaque purified twice on monolayers of DBT cells and seed stocks prepared.
Working stocks were grown in 17CL1 cells and the virus was assayed on monolayers of L2 cells in a standard plaque assay as previously described (17).

Viral Purification

Following growth to high titers on monolayers of 17 CL1 cells, the virus was harvested by one cycle of freeze-thawing and clarified by centrifugation at 4,500 X g for 1 hour at 4°C as previously described (18). The supernatant was recovered and the virus was precipated by adding to each 10 ml., 1.1 ml. of 5 M NaCl and 5.5 ml. 30% (W/V) PEG-6000 in MOPS-saline EDTA, pH 6.8 (10mM MOPS, 1mM EDTA, 15 M NaCl, Baker Scientific Company, Philipsburg, N.J.) (18). The solution was stirred on ice for 30 minutes and the virus pelleted by centrifugation at 9,250 x g at 4°C for 30 minutes as previously described (18). The pellet was resuspended in a small volume of MOPS-saline-EDTA and purified in sequential 5-25% and 10-40% K-tartrate gradients (18). The fractions containing virus were pooled, diluted to 5 ml. with MOPS-saline EDTA and pelleted at 45,000 RPM for 30 minutes at 4°C in a SW50.1 rotor (Beckman Scientific Co., Toronto, Canada). The virus pellet was resuspended in 0.5 ml carbonate buffer by three bursts of sonication at 4°C and viral protein concentration was determined in a modified Lowry assay as previously described (17).

Plaque Neutralization Assay

Virus was assayed on monolayers of L2 cells in a standard plaque assay as previously described (17). For the determination of neutralizing antibody, heat inactivated sera was incubated with 100 PFU of MHV-3 for 30 minutes at 4°C. 200 µl of the mixture were then layered onto confluent monolayers of L2 cells in 6 well plastic culture dishes (Linbro Plastics, McLean, Va.) for 30 minutes at 22°C and then overlayed with 1% agarose in DMEM supplemented with 2% FCS. The plates were incubated for 48 hours at 37°C in a 5% CO_2 incubator and then stained with 0.1 gm % crystal violet in 20% ethanol for enumeration of plaques. The end point of the assay was taken as the dilution of antibody which reduced the number of plaques to 50% of the control value.

Solid Phase Radioimmunoassay

Flexible 96 well microtiter plates were coated with 25 ng of purified MHV-3 which was diluted in 100 µl of carbonate buffer pH 9.6. The plates were then incubated overnight at 4°C in a humid chamber. The antigen was crosslinked using 10 µl of a 10 mg/ml solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl. The next day the plate was washed repeatedly with 200 µl aliquots of 1% BSA, 0.05 % Tween-20 and 0.02% sodium azide in phosphate buffered saline (PBS pH 7.4) (SPRIA buffer). The carbodiimide was inactivated with 0.1 M NH_4Cl for 1 hour and then the plate was repeatedly washed with SPRIA buffer. Mouse antisera at a dilution of 1/200 was added for 4 hours at room temperature, removed and the plates were washed repeatedly with SPRIA buffer. ^{125}I-labelled affinity purified goat anti-mouse immunoglobulin (Spec. Act. 3.2 x 10^{10} cpm/mg) was added and following an hour incubation the plates washed and individual wells counted in a gamma counter. Appropriate positive and negative control sera were included in all assays as controls.

Radioimmunoprecipitation

Confluent monolayers of DBT cells were grown on 6 well-plastic tissue culture plates. The cells were infected

at a high multiplicity of infection (M.O.I. 10) with MHV-3 and were then incubated at 22°C for 60-90 minutes. Unabsorbed virus was removed and the monolayers were covered with 2 ml of ADME-2 containing 5 μg/ml of actinomycin D. The plates were left to incubate at 37°C until 30% syncytia were present. The media was then removed, the cells washed twice with methionine free medium and 0.5 ml of methionine free ADME-2 containing 5 μg/ml actinomycin D and 250 μCi of ^{35}S-methionine (Amersham Scientific, Toronto, Canada) were added. The plates were left to incubate until completely covered with syncytia. The plates were then rinsed gently twice with PBS and then once with reticulocyte standard buffer (RSB 10 mM Tris, 10mM NaCl, 1.5 mM $MgCl_2$) and then the cells were lysed with 200 ul of RSB containing 0.5% NP-40, 0.1% SDS and 1% aprotinin.

An aliquot of lysate (5 μl) was added to 10 μl of mouse serum for 30 minutes on ice, then 100 μl of formalin fixed 10% (V/V) protein A bearing staphylococcus aureus (Cowan I) was added for 30 minutes and the mixtures brought to 400 μl with RSB. The sample was centrifuged at 12,000 RPM x 4 minutes and the pellet recovered and washed an additional 4 times. The pellet was then dissolved in 50 μl of tracking buffer and applied to a lane of a 10% sodium dodecyl sulfate (SDS) polyacrylamide gel. Following electrophoresis, the gels were developed for fluorography and autoradiography.

Antibody Subclass Determination

In order to determine antibody class and subtype responses, a standard commercially available enzyme linked immunosorbent assay system (ELISA) was utilized. Microtiter plates were coated with 20-50 ng of purified MHV-3 as described previously (18). Following post coating with a standard bovine albumin solution, 50 μl of a dilution of a mouse serum was added. Following a 2 hour incubation, the excess serum was removed, the plates were washed and 50 μl of rabbit anti- mouse IgA (Fc), IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 and IgM (Fc) were added and left for 2 hours. The plates were washed and 50 μl of peroxidase labelled goat anti-rabbit IgG antibody was added to each well and left to incubate at room temperature for 1 hour. 100 μl of freshly prepared ABTS-H-2 was added to each well and left for 30 minutes. The results were quantitated by measuring the optical density of each well at 415 nm in an automated

spectrophotometer (Flow Laboratories, Mississauga, Ontario).

RESULTS

Immune Response To Infection With MHV-3

The immune response of inbred strains of mice infected intra-peritoneally (i.p.) with 10^3 plaque forming units (PFU) of MHV-3 were determined by four different assay procedures; radioimmunoassay (RIA), plaque neutralization assay, an enzyme linked immunosorbent assay (ELISA) and by radioimmunoprecipitation (RIP).
We first examined the development of antibody during the course of primary infection with MHV-3. Balb/cJ, C3H/ebFeJ and A/J male mice, 6-8 weeks of age were infected with 10^3 PFU of MHV-3 i.p.. Blood was obtained daily for up to three months following infection or until death. All mice were pre-bled and tested for antibody to MHV and positive mice were eliminated from the study. Antibody levels were determined in a standard RIA as described previously (18). No antibody was detected in the serum of Balb/cJ mice throughout the course of the infection. In contrast, antibody was detected as early as 4 days p.i. in the A/J mice and the levels increased to maximal titers by day 5-7. The antibody titers remained high for up to three months (fig 1). In the semisusceptible C3H mice, there was a marked delay in the appearance of antibody as compared to the A/J mice with no antibody detected until day 10. The titer rose to high levels by day 11-12 and remained at these high levels throughout the course of the infection (fig 1).

Nature of the Antibody Response

Neutralizing antibody was determined in a standard plaque reduction assay (17). No neutralizing antibodies were detected in sera from MHV infected Balb/cJ mice while high titers of neutralizing antibody to MHV were detected in the A/J mice by day 2-3 and this antibody persisted for 60-90 days p.i. at high levels. These results correlated well with the data obtained by RIA (fig 1). In contrast, despite the detection by RIA of high titered antibody in the C3H mice, there was only weak neutralizing antibody detected at day 12 with no increase in titer until death at 3-4 months p.i. (fig 2).

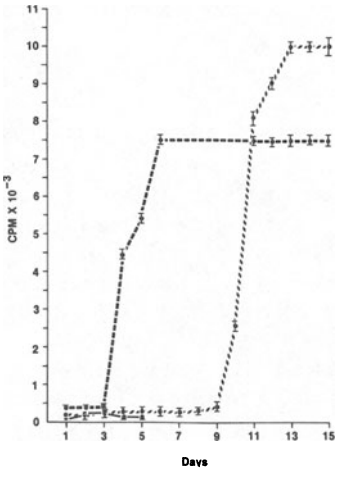

Figure 1. Antibody responses to MHV-3 infection in inbred strains of mice by radioimmunoassay (RIA). Sera at a dilution of 1/200 from Balb/cJ mice (——), A/J mice (▪▪▪▪▪▪▪▪▪▪) and C3H mice (▰▰▰▰) were assayed in a standard RIA for the presence of antibody to MHV-3.

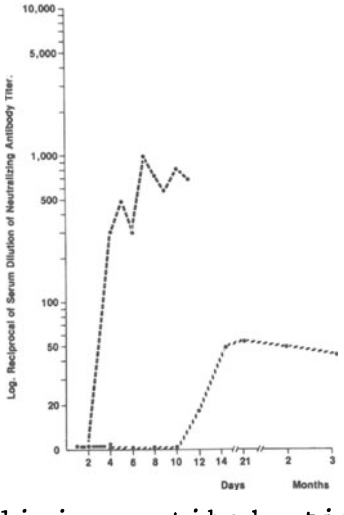

Figure 2. Neutralizing antibody titers to MHV-3 in inbred strains of mice. Heat inactivated sera from Balb/cJ (——), A/J (▪▪▪▪▪▪▪▪▪▪▪▪) and C3H mice (▰▰▰▰) were analysed for the presence of neutralizing antibody in a standard plaque reduction assay.

The Specificity of the Response to Viral Structural Proteins

To determine the antigenic site to which the antibodies were directed, antisera were analysed by radioimmunoprecipitation (RIP) to a ^{35}S-methionine labelled MHV lysate as previously described (18). DBT cells were infected with MHV-3 and labelled from 6-18 hours p.i. with ^{35}S-methionine. The cell lysate was prepared as described previously and the lysate electrophoresed on a 10% polyacrylamide gel and fluorographs prepared as described previously (18). E2 glycoprotein (180,000 daltons), nucleocapsid protein (50-60,000 daltons) and E1 glycoprotein (20-24,000 daltons) were easily distinguished in the MHV-3 lysates as compared to the mock infected lysates (fig 3,4).
When A/J sera were analysed, reactivity to nucleocapsid and E2 glycoprotein were seen as early as 2 days p.i (fig 3). This correlated with the finding of neutralizing antibody seen in the plaque reduction assay on day 2-3. Increased reactivity especially to the E2 glycoprotein was seen by day 5 and by day 13-15 p.i., antibodies to E2, E1 and nucleocapsid proteins were found in high quantities (fig 3).
In contrast, in sera from the C3H mice, antibody could only be detected at day 14 by RIP and was only directed to the nucleocapsid protein (fig 4). By day 21 a strong anti-nucleocapsid response was seen with a weak response to E2 glycoprotein (fig 4). This correlated well with the results both from the RIA and the plaque neutralization assays in which a strong antibody response was observed by day 14 but only a very weak titer of neutralizing antibody was detected. Reactivity to nucleocapsid protein increased by day 34 and persisted until death of the animals at 90-120 days p.i. At day 34 a weak E2 glycoprotein response was found and by day 90 weak E2 and E1 responses could be seen on the RIP (fig 4).

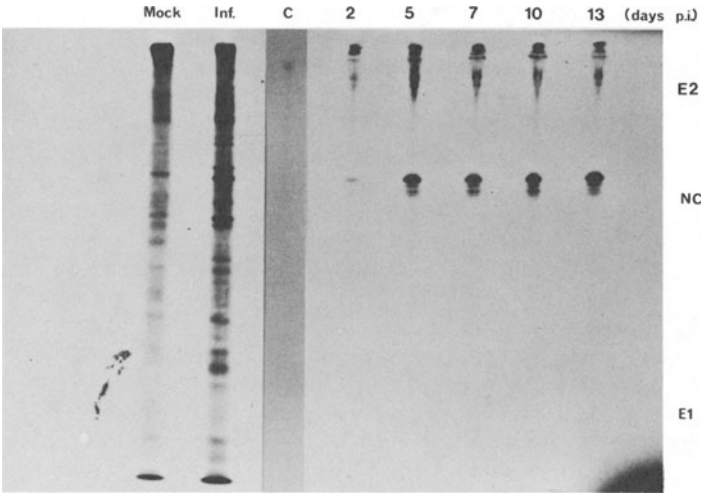

Figure 3. Polyacrylamide gel analysis of antibody response of A/J mice to MHV-3 infection. Lane 1, mock infected lysate. Lane 2 MHV-3 infected lysate demonstrating E2 glycoprotein (mw 180,000 daltons), nucleocapsid protein (nc) (mw 60,000 daltons) and E1 glycoprotein (mw 20-24,000 daltons). Lane 3-7 are radioimmunoprecipitations of sera from control, 2, 5, 7, 10 and 14 days post MHV-3 infected A/J mice.

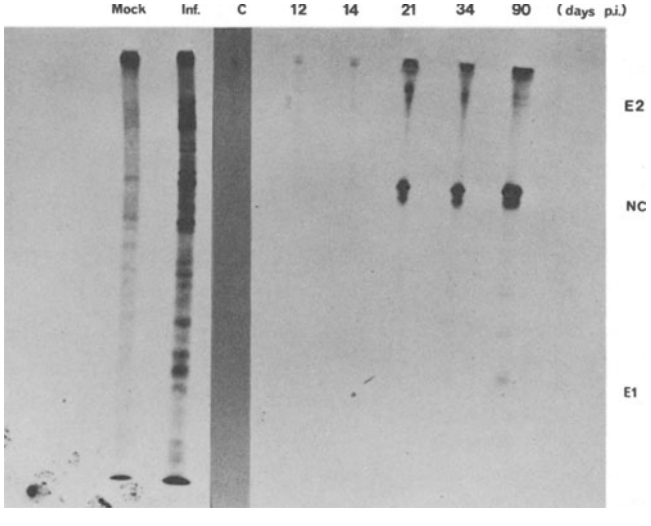

Figure 4. Polyacrylamide gel analysis of antibody response of C3H mice to viral proteins following MHV-3 infection. Lane 1 mock infected lysate, Lane 2 MHV-3 infected lysate demonstrating E2 glycoprotein (mw 18,000 daltons), nucleocapsid protein (nc) (mw 60,000 daltons and E1 glycoprotein (mw 20-24,000 daltons). Lane 3-7 are radioimmunoprecipations of sera from control (C), 12, 14, 21,34 and 90 days post MHV-3 infected C3H mice.

Immunoglobulin Class Determination of Antibodies

Specificity of the immunoglobulin response in the sera from A/J and C3H mice was determined in an enzyme linked immunosorbent assay (ELISA) using affinity purified rabbit anti mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgA (Fc) and IgM (Fc) probes as previously described. Individual wells of microtiter plates were coated with 25 ng of purified MHV-3 and sera from infected animals was added at a dilution of 1/50. Identification of the specific immunoglobulin response was determined as described above. A positive response was defined as an optical density of greater than 0.2 at 415 nm.
In the A/J mice, an early rise in IgA anti MHV-3 antibody was detected 2 days p.i. and this persisted until 7 day p.i. This correlated both with the acute phase of the infection and the ability to recover virus from livers and serum of A/J mice. IgM anti-MHV-3 was also detected at day 2 and this persisted until 13 days p.i (fig 5). By day 25, IgM was no longer detected (data not shown). A strong IgG_{2a} response was observed by day 7 and by day 13 an increase in IgG_1 was noted (fig 5).

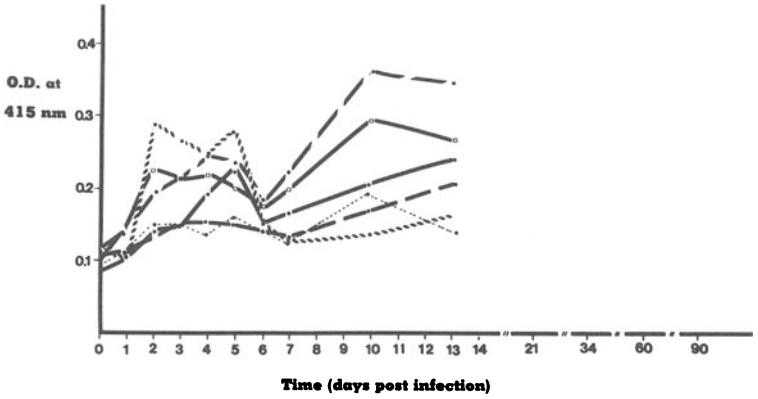

Figure 5. Immunoglobulin subclass determinations in sera from MHV-3 infected A/J mice by an enzyme linked immunosorbent assay (ELISA). Individual wells of 96 well microtiter plates were coated with 25 ng of purified MHV-3 and sera from A/J mice at a dilution of 1/50 added. Rabbit anti mouse IgG_1 (▬▬▬▬▬), IgG_{2a} (▬ ▬ ▬), IgG_{2b} (▬▬▬▬), IgG_3 (▬ ▬ ▬ ▬ ▬) IgM (▬ ▬ ▬) and IgA (▬▬▬) was added and the optical density (O.D.) determined at 415 nm.

When sera from C3H mice was analysed, an increase in IgA anti-MHV-3 was observed during the acute infection (days 2-7) which returned to normal by day 10 (fig 6). By day 3, a marked increase in IgG_1 antibody was found that remained elevated until the death of the animal at 3-4 months p.i. The IgM anti-MHV response was delayed as compared to the A/J mice and was first detected at 11 days p.i. (fig 6). The IgM antibody level remained elevated until the animals died at 3-4 months p.i. No significant amounts of IgG antibody could be detected in the sera from C3H mice, although on day 34 a questionable rise in IgG_{2a} and IgG_{2b} was noted. By day 90 the titers of these antibodies had not increased and was only barely detectable (fig 6).

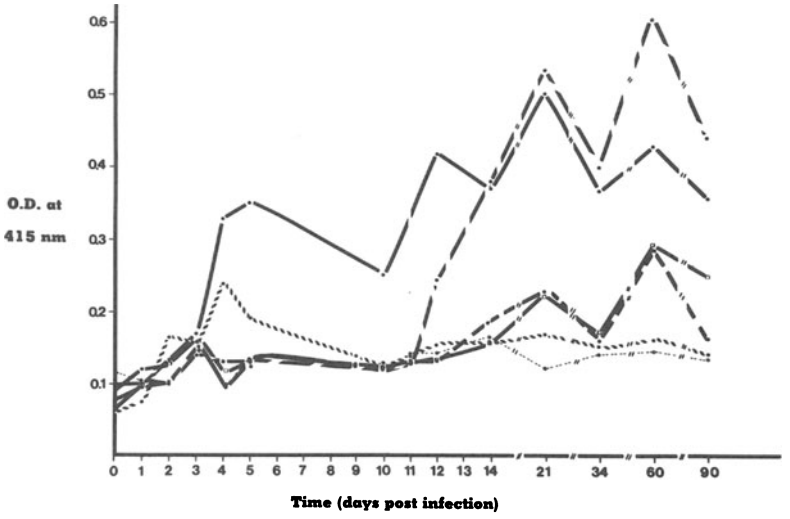

Figure 6. Immunoglobulin subclass determinations in sera from MHV-3 infected C3H mice by ELISA. Individual wells were coated with 25 ng of MHV-3, sera from C3H mice added at a dilution of 1/50 and rabbit anti mouse IgG_1 (— — —), IgG_{2a} (——— ———), IgG_{2b} (———————), IgG_3 (-- -- --), IgA (— — —) and IgM (———— ————) was added and the optical density (O.D.) determined at 415 nm.

No antibody was detected in the sera from the Balb/cJ mice in the ELISA at any time during the infection (data not shown).

Effect of Transfer of Immune Serum on MHV Infection

To determine the effects of the transfer of high titered neutralizing antisera on the course of MHV infection in the susceptible Balb/cJ mice and the semi-susceptible C3H/ebFeJ mice, antisera from A/J mice that had been infected with 10^3 PFU of MHV-3 10-14 days earlier was collected and pooled. Balb/cJ and C3H mice (6-8 weeks of age) were infected with 10^3 PFU of MHV-3 i.p. and divided into three groups of animals with 10 mice in each group. Mice in group 1 were given 100 µl of normal saline as controls; mice in group 2 were given 100 µl of sera from A/J mice 2-4 hours p.i. and mice in the third group were given 100 µl of A/J sera 2-4 hours p.i. and a second dose 24 hours p.i. Serum was given by an intravenous injection through the tail vein. Mortality was determined and the livers were removed from the animals and viral titers determined in a standard plaque assay.

Balb/cJ mice infected with 10^3 PFU of MHV-3 were given 2 hours p.i. 100 µl of normal saline I.V. All died within 5 days of infection (fig 7). In contrast, mice that were given 1 injection of 100 µl of sera from A/J mice (group 2) showed an increased survival (fig 7). At 5 days p.i. when all control Balb/cJ mice had died, only 2 of the group 2 mice had died (20%) (fig 7). However, with no further treatment, only one of the mice survived longer than 10 days and died 14 days p.i. In the animals in group 3, no animals died by day 5 p.i. but by 10 days 50% of the animals (5 mice) had died and all of the animals were dead by 21 days p.i. (fig 7).

In studies on viral recovery and growth from the livers of infected animals, a 2 log decrease in maximal virus growth was found in livers from group 2 mice as compared to the livers from control Balb/cJ mice (fig 8); whereas the livers from animals that had received 2 doses of sera from A/J mice (group 3) had a 3 log decrease in viral titers as compared to the livers of the control mice (fig 8).

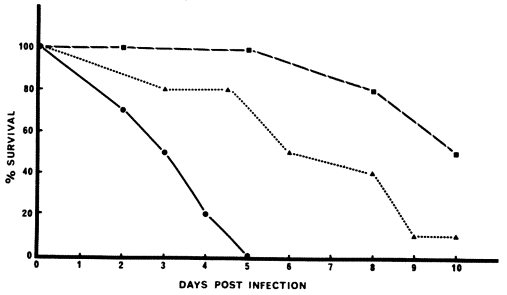

Figure 7. Effect of transfer of serum from A/J mice on the survival of susceptible Balb/cJ mice following MHV-3 infection. Balb/cJ mice were infected with 10^3 PFU of MHV-3 and then injected I.V. with 100 µl of normal saline (——) 2 hours p.i., 100 µl of sera from A/J mice (·····) 2 hours p.i. or 2 injections of 100 µl of A/J sera (— —) 2 hours and 24 hours p.i.

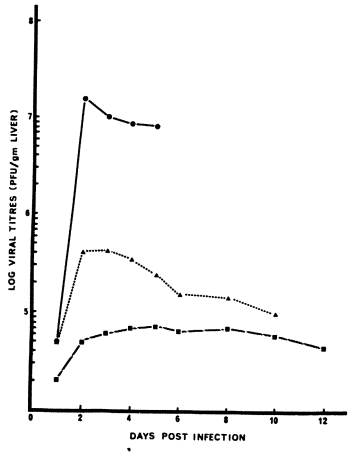

Figure 8. Effect of transfer of immune serum from A/J mice on recovery and growth of virus from the livers of MHV-3 infected Balb/cJ mice. Viral titers were determined in a standard plaque assay in homogenized livers from Balb/cJ mice which had been infected with 10^3 PFU of MHV-3 and were immunized with 100 µl of normal saline (——), 100 µl of serum from A/J mice (·····) or 2 injections of 100 µl of serum from A/J mice (— —).

Control C3H mice (group 1) that were given 100 µl of saline 2 hours p.i. had a 30% mortality during the acute phase of the MHV-3 infection (Day 1-10) and all of these animals developed viral persistence, chronic liver disease and died within 3-4 months of infection. In contrast, there were no deaths in any of the C3H mice that had received 1 injection (group 2) or 2 injections of A/J sera p.i. All of the animals survived and none of them developed chronic disease (fig 9).

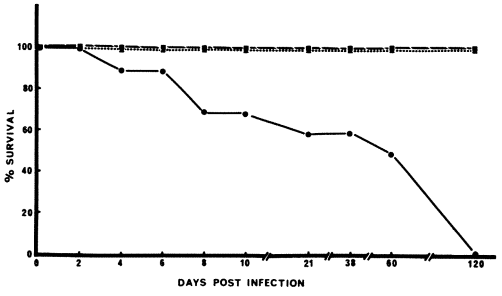

Figure 9. Effect of transfer of immune serum from A/J mice on survival of semisusceptible C3H mice following MHV-3 infection. C3H mice were infected with 10^3 PFU of MHV-3 i.p. and immunized with 100 µl of normal saline 2 hours p.i. (——), 100 µl of serum from A/J mice 2 hours p.i. (▪▪▪▪▪▪▪▪▪▪) or 2 injections of serum from A/J mice 2 hours and 24 hours p.i. (—·—·—).

Furthermore, we were unable to recover any virus from the livers of the C3H mice that had received sera from A/J mice in contrast to the high viral titers found in the livers of control C3H mice (fig 10).

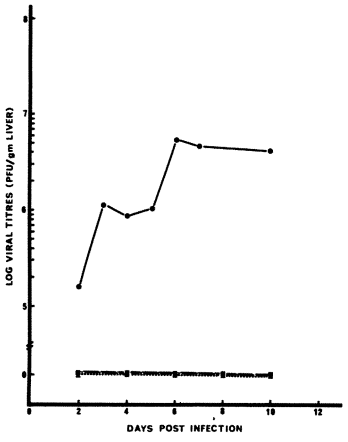

Figure 10. Effect of transfer of immune serum from A/J mice on viral titers from the livers of semisusceptible C3H mice. Viral titers were determined in livers from C3H mice infected with 10^3 PFU MHV-3 i.p. in a standard plaque assay which were immunized with 100 µl of normal saline 2 hours p.i. (——), 100 µl of serum from A/J mice 2 hours p.i. (▪▪▪▪▪▪▪▪▪▪▪▪▪▪) and 100 µl of serum from A/J mice 2 hours and 24 hours p.i. (——▪——▪——)

DISCUSSION

The results of these experiments indicate that there are major differences in the humoral response to MHV-3 in inbred strains of mice that differ in their suceptibility to MHV-3 infection. In the fully resistant A/J mice there is an early IgM response directed primarily to nucleocapsid protein followed by the production of IgG antibodies primarily of the IgG_{2a}, IgG_1 and IgG_{2b} classes. High titers of neutralizing antibody directed at E2 and E1 glycoprotein could be detected as early as 3-4 days p.i. and reached maximal titers by 7-10 days p.i. In contrast, no detectable antibody could be detected in the fully susceptible Balb/cJ mice, all of whom died within 5 days of infection. Furthermore, in the C3H mice, a strain in which there is viral persistence and chronic disease, antibody response was delayed and was primarily directed against nucleocapsid protein. Antibody appeared to be of the IgG_1 and IgM class. By 60 days, IgM remained predominant and there was very little production of IgG. Even late in the infection, the response was largely anti-nucleocapsid with very

low titer of anti E2 and E1 detected.

The IgG subclass concentrations of an individual are determined at least in part by genetic factors (19). The four subclasses of murine IgG listed in order of concentration are IgG_{2a}, IgG_1, IgG_{2b} and IgG_3 (20). Furthermore only certain IgG subclasses are produced in response to specific antigens (21). In rodents, immunization with protein antigens results in the synthesis of IgG_1 antibodies whereas immunization with carbohydrates or mixtures of proteins and carbohydrates results in the production of predominantly IgG_{2a} and IgG_{2b} (19). Mouse IgG_{2a} and IgM activate the classic complement pathway, whereas IgG_1 fails to do so (19). Furthermore, only IgG_{2a} is cytophilic for monocyte/macrophages and the other classes of antibody fail to bind to these cells (19). Therefore, the failure of Balb/cJ mice to produce any antibody and the failure of C3H mice to maintain an appropriate IgG response may explain the lack of resistence to MHV-3 exhibited by these strains.

It is now recognized that for in-vivo B cell responses to T-dependent antigens, both thymus matured T cells as well as monocyte/macrophages are necessary for the production of antibody (22). Even with the class of antigens designated T-independent, the presence of thymus-matured T cells and antigen presenting monocyte/macrophages results in an augmented production of circulating antibodies (23,24). Furthermore the influence of T lymphocytes over B cell responses with T independent antigens is restricted to only some of the Ig isotypes namely the IgG_{2a} and IgG_{2b} subclasses.

Resistance to infection with MHV is genetically restricted and is dependent upon the ability of macrophages to control MHV infection and upon T cells and T cell factors (1,2,16). The abnormalities in synthesis in antibody could reflect accessory cell dysfunction with deficiencies in processing of viral antigens by macrophages and/or T cells resulting in both failure of cellular and humoral immunity as is seen in the fully susceptible Balb/cJ mice or the production of abnormal subclasses of antibodies and the failure of production of specific neutralizing antibodies as is seen in the C3H mice. Furthermore, the persistence of IgM antibodies and the failure of the conversion from IgM to the production of IgG antibodies may reflect abnormalities in processing and antigen handling by T cells and monocyte/macrophages or a primary B cell defect.

Similar abnormalities have been observed in patients with viral hepatitis B infection. Patients who survive

and clear the virus produce large amounts of specific antibodies to both the core and surface antigens, whereas those patients who either die of acute infection or go on to a chronic disease state continue to produce high titers of anti-core IgM antibodies but fail to produce specific IgG anti-surface neutralizing antibodies (25). Chisari and co-workers have shown that the production of antibodies to the surface antigen of hepatitis B virus in inbred strains of mice is genetically controlled within the H-2 locus (26). A number of strains are high responders whereas some strains fail to generate any humoral response. This suggests that abnormalities in the immune response in MHV infection in some strains of mice may also be operative in man, resulting in the susceptibility of some patients to hepatitis B infection.

The presence of high titers of IgA early in the course of the infection in both A/J and C3H mice correlates with abnormal liver cell function. Delacroix et al have recently reported that patients with acute and chronic liver disease have abnormally high levels of polymeric and monomeric IgA in the serum (27). As the liver is believed to be the major site of removal of IgA from the circulation, he postulated that the increased levels of IgA found in these patients were due to abnormalities in binding of IgA to its liver cell receptor (secretory component) and decreased clearance through the liver (27). The presence of abnormally high levels of IgA even in the fully resistant A/J mice suggests that even in these animals there may be disruption of normal liver cell function.

We have shown here that passive transfer of high titered antisera containing neutralizing antibodies leads to the elimination of virus from semi-susceptible C3H mice and prevents the chronic disease state. Furthermore, this antibody at least partially protects the Balb/cJ mice resulting in lower viral titers in affected organs and increased survival. It is possible that the presence of high titered neutralizing antibody results in the activation of macrophages and T cells enabling a normal cellular immune response to occur. This could result in the elimination of the virus and the production of long lasting neutralizing antibodies. Future studies are required to determine the genetic requirements for both the cellular and humoral basis of the immune response to MHV infection. These studies are essential in order to determine those factors that contribute to either resistance or susceptibility in the particular host.

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