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Resistance of Naive Mice to Murine Hepatitis Virus Strain 3 Requires Development of a Th1, but not a Th2, Response, Whereas Pre-Existing Antibody Partially Protects Against Primary Infection[1]

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Murine hepatitis virus strain 3 (MHV-3) produces a strain-dependent spectrum of disease. The development of liver necrosis has been shown to be related to production of a unique macrophage procoagulant activity (PCA), encoded by the gene *fgl-2*, in susceptible mice. These studies were designed to examine the influence of Th1/Th2 cells on resistance/susceptibility and production of macrophage PCA in resistant (A/J) and susceptible (BALB/cJ) strains of mice following infection with MHV-3. Immunization of A/J mice with MHV-3 induced a Th1 cellular immune response, and one Th1 cell line (3E9.1) protected susceptible mice and inhibited PCA production by macrophages both in vitro and in vivo. In contrast, immunization of BALB/cJ mice with an attenuated variant of MHV-3 derived from passaging MHV-3 in YAC-1 cells resulted in a Th2 response. Transfer of spleen cells and T cell lines from immunized BALB/cJ mice failed to protect naive susceptible syngeneic mice from infection with MHV-3 and augmented macrophage PCA production to MHV-3 in vitro. However, serum from immunized BALB/cJ mice contained high titrated neutralizing Ab that protected naive BALB/cJ animals from lethal primary MHV-3 infection. These results demonstrate that susceptible BALB/cJ mice generate a Th2 response following MHV-3 infection and that these Th2 cells neither inhibit MHV-3-induced macrophage PCA production nor protect naive mice from MHV-3 infection. The results suggest that Ab protects against primary infection but cannot eradicate ongoing infection. Thus, these data define the differential role of Th1/Th2 lymphocytes in primary and secondary MHV-3 infection and emphasize the importance of PCA in the pathogenesis of MHV-3 infection. *The Journal of Immunology,* **1996, 156: 3342–3349.**

Fulminant viral hepatitis remains a severe disease, with a mortality rate approaching 80% (1). We have used a murine model of fulminant viral hepatitis caused by murine hepatitis virus strain 3 (MHV-3),[3] to study the pathogenesis of liver necrosis. MHV-3 is a single-stranded, positive-sense RNA virus belonging to the Coronaviridae family, which produces a strain-dependent pattern of disease in inbred laboratory mice (2). Fully susceptible strains, such as BALB/cJ, develop liver necrosis and die within 3 to 5 days following i.p. inoculation of as little as 1 plaque-forming unit (PFU) of MHV-3. Adult A/J mice are resistant and do not develop clinical or histologic evidence of hepatitis (3).

Viral replication is similar in both susceptible and resistant strains (4, 5), and we have suggested that differences in the host inflammatory response explain variations in susceptibility and resistance in inbred strains. Our laboratory has been involved in the characterization of a unique macrophage procoagulant activity (PCA) that is produced in MHV-3-infected susceptible and semi-susceptible, but not in resistant, mouse strains (4). This procoagulant cleaves prothrombin to thrombin, which leads to fibrin deposition, intravascular thromboses, and ischemic liver necrosis. The importance of this molecule during in vivo infection has been supported by the visualization of abnormalities in the microcirculation of the liver as early as 12 h preceding the detection of viral Ags by indirect immunofluorescence 24 h postinfection (3). A mAb against this prothrombinase, 3D4.3, which did not neutralize viral growth, prevented liver necrosis and mortality (6). This mAb was used to identify the gene encoding this MHV-3-induced prothrombinase as *fgl-2* (formerly known as mouse fibrinogen-like protein or musfiblp), which localizes to chromosome 5 of the mouse (7, 8). The immune system plays an essential role in the outcome of viral infection. One mechanism of regulation of the immune response in vivo involves $CD4^+$ Th cells, which, through the production of cytokines, control the development of immune effector mechanisms such as Ab production, generation of cytotoxic T cells, and macrophage activation. Following Ag exposure, Ag-specific Th cells differentiate along two pathways (9). Th1 cells, through the production of IL-2, IFN-γ, and lymphotoxin, mediate cellular immunity, which is essential for clearance of viral and other intracellular pathogens such as *Leishmania* (10). Th2 cells, conversely, produce IL-4, IL-10, and IL-13 and are most effective in providing help for B cell differentiation to plasma cells. IL-12 production by APCs has been shown to preferentially stimulate a Th1 response

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[3] Abbreviations used in this paper: MHV-3, mouse hepatitis virus strain 3; PCA, procoagulant activity; PFU, plaque-forming unit; MOI, multiplicity of infection; musfiblp, mouse fibrinogen-like protein; SMNC, splenic mononuclear cells; TCGF, rat spleen cell concanavalin A supernatant; CI, confidence interval.

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(11). IFN-γ production by Th1 cells inhibits the proliferation of Th2 cells (12, 13), whereas treatment with neutralizing Abs to IFN-γ promotes the development of a Th2 response (14). IL-4 and IL-10 are regulatory cytokines, favoring the development of a Th2 response. IL-4 directs the development of Th2-like helper effectors (15, 16). IL-10 has been shown to act on the APC to inhibit IFN-γ production by Th1 clones (17).

A Th1 response has been associated with host resistance, and a Th2 response with susceptibility in murine models of leishmaniasis (10), candidiasis (18, 19), and listeriosis (20). Immunomodulatory treatments that have converted a Th1 to a Th2 response have resulted in the loss of resistance (16, 21), and conversely, induction of a Th1 response has led to resolution of the infection (20, 22, 23). Th1 and Th2 cells have been documented in human diseases (24, 25) and have been implicated in a number of mycobacterial, protozoal, and parasitic infections.

Th lymphocyte collaboration plays an important role in the induction of macrophage procoagulant activity by MHV-3 (26), LPS (27), and alloantigens (28). Although there exists an absolute restriction in prothrombinase expression in response to MHV-3 at the level of the macrophage, lymphocytes from susceptible mice are necessary for maximal PCA expression, whereas lymphocytes from immunized resistant mice inhibit PCA production (26). We have previously examined the regulation of PCA in resistant A/J mice and demonstrated that Th1 cells inhibited PCA production (29), probably through a soluble mediator other than IFN-γ. We, therefore, wished to examine the cellular regulation of macrophage PCA production in susceptible mice. As previous attempts to immunize susceptible BALB/cJ mice with live or inactivated MHV-3 were unsuccessful (30), we used an attenuated variant of MHV-3 (YAC-1-MHV-3) (31) to immunize susceptible BALB/cJ mice. $CD4^+$ Th cell lines were isolated from immunized susceptible mice, and their effects on macrophage procoagulant production and on survival of MHV-3-infected naive susceptible BALB/cJ mice were compared with those of whole spleen cell populations from immunized mice.

Materials and Methods

Animals

Female A/J ($H\text{-}2^a$), C57BL/6J ($H\text{-}2^b$), and BALB/cJ ($H\text{-}2^d$) mice, 8 wk of age, were obtained from The Jackson Laboratory (Bar Harbor, ME). The recombinant inbred strain AXB8 ($H\text{-}2^a$) was obtained from the pathogen-free breeding colony at the Montreal General Hospital (Montreal, Canada). Recombinant inbred strains were derived by inbreeding mice from the F2 generation of the cross between A/J (A) and C57BL/6J (B) mice as described previously (32). All mice were kept in microisolator cages and fed standard lab chow and water ad libitum. Mice were tested at random and were seronegative for all routine viruses, including MHV. Lewis rats were obtained from Charles River Laboratories (Montreal, Canada).

Virus

MHV-3 was plaque-purified on monolayers of DBT cells. It was grown to a titer of 1×10^7 PFU/ml in 17CL1 cells. Viral titers were determined on monolayers of L2 cells. The attenuated variant of MHV-3, YAC-1-MHV-3, was grown by repeated passages of the virulent MHV-3 in YAC-1 cells, using the protocol of Lamontagne and Jolicoeur (31). After 15 passages, the virus was passaged once in 17CL1 cells and grown to a titer of 1×10^7 PFU/ml.

Cells

Mononuclear cells from spleen or lymph nodes of mice or rats were isolated at the interface on Ficoll-Hypaque gradients (density, 1.074; Pharmacia, Laval, Canada) after centrifugation at $1500 \times g$ for 15 min. Cells were recovered and washed three times in RPMI 1640 (ICN Biomedicals, Costa Mesa, CA). Macrophages were obtained from mice following i.p. injection of 2 ml of Brewer's thioglycolate, pH 6.9 (Difco Laboratories, Detroit, MI), and were >97% pure, as demonstrated by morphology and uptake of neutral red and latex. Cells were washed three times and resuspended at a concentration of 1×10^6 cells/ml in RPMI 1640 supplemented with 2 mM glutamine (Sigma Chemical Co., St. Louis, MO). Lymphocytes and macrophages recovered were >95% viable by trypan blue exclusion.

Serum

Susceptible BALB/cJ mice were immunized i.p. with 1000 PFU of attenuated MHV-3 (YAC-1-MHV-3) and boosted i.p. 14 days later with 1000 PFU of virulent MHV-3. Blood was collected in nonheparinized capillary tubules by axillary bleeding 3, 7, 11, 14, and 18 days following primary immunization. After incubation for 1 h at 4°C, blood samples were centrifuged at 14,000 rpm for 10 min at 4°C in an Eppendorf microcentrifuge. Serum was aspirated, pooled, and stored at −20°C until use. Pooled sera from BALB/cJ mice immunized with MHV-3 were tested by plaque for the presence of infectious virus in a standard plaque assay, and none was detected. Sera from naive nonimmunized BALB/cJ mice were similarly collected and used as a control.

The neutralizing Ab titer was determined as the reciprocal of the dilution that inhibited plaque formation in L2 cells by 50% following a 30-min incubation with MHV-3 at 37°C as described previously (33).

To study the effect of serum from immunized BALB/cJ mice on MHV-3 replication, we used the same standard lot of pooled serum used for adoptive transfer experiments (see above) collected 18 days after primary immunization and 4 days after secondary immunization with MHV-3. This lot contained a neutralizing Ab titer of 1/250 and was free of infectious MHV-3, determined as described above. Naive BALB/cJ mice received 200 μl of serum i.p. 1 h before infection with 10 PFU i.p. of virulent MHV-3. Groups of mice were killed at 24, 48, and 72 h postinfection, then liver homogenates were prepared, and viral titers were determined by plaque assay (6).

Liver tissue

Frozen liver tissue (−70°C) was homogenized in DMEM with 2% FCS and 4 mM glutamine as a 10% homogenate at 4°C, as previously described (3, 4). Serial dilutions were made, and viral titers were then determined on monolayers of L2 cells in a standard plaque assay. The assay has previously been shown to be sensitive to 1 PFU/g liver tissue (4).

Adoptive transfer experiments

To investigate whether susceptible BALB/cJ mice immunized with attenuated MHV-3 developed humoral or cellular immunity to MHV-3, we adoptively transferred 200 μl of serum i.v. or 5×10^7 splenic mononuclear cells from immunized to naive BALB/cJ mice and then infected them with 10 PFU of virulent MHV-3 i.p. 1 h later.

A standard lot of pooled sera collected 18 days following primary immunization and 4 days following secondary immunization was used for all experiments. This serum was free of infectious virus, as assessed by plaque assay.

Splenic mononuclear cells were also collected 4 days following secondary immunization from BALB/cJ and A/J mice that had been immunized with 1000 PFU of attenuated MHV-3 and boosted with 1000 PFU of virulent MHV-3 14 days later. A spleen cell suspension was prepared, and mononuclear cells were isolated on Ficoll-Hypaque gradients. Mononuclear cells were then counted, and 5×10^7 cells were infected i.p. with 500 μl of RPMI-1640. Cells were reinjected into a naive mouse within 2 h of collection from the immunized mouse. Splenic mononuclear cell suspensions were tested for the presence of infectious virions by plaque assay, and no infectious virus was detected.

Antigen specificity of serum from immunized BALB/cJ mice

To determine the MHV Ags to which Abs were directed, antisera were analyzed by radioimmunoprecipitation to a $[^{35}S]$methionine-labeled MHV-3 lysate as previously described (34).

Isolation of T cell lines

$CD4^+$ T cell lines were derived from susceptible mice as previously described (29). Briefly, BALB/cJ mice were immunized in the footpad with 1000 PFU of attenuated MHV-3 and boosted with 1000 PFU of attenuated MHV-3 2 wk later. The draining lymph nodes were removed 8 days later. Responder lymph node cells (5×10^7) were cocultured with 1×10^7 irradiated (2000 rad) SMNC that had been infected with MHV-3 at a multiplicity of infection (MOI) of 0.5. The culture medium used was RPMI 1640 supplemented with 10% FCS (Flow Laboratories, Mississauga, Canada), 0.05 mM 2-ME, 100 U/ml penicillin, 100 U/ml streptomycin, 2 mM L-glutamine, and 2 mM sodium pyruvate. Cells were incubated at 37°C in a humidified atmosphere with 5% CO_2. After 9 days of incubation, blast cells were incubated at limiting dilution with 5×10^5 irradiated, infected

SMNC in a 0.2-ml volume in 96-well U-bottom plates (Costar, Cambridge, MA). The medium was supplemented with 10% TCGF (rat spleen cell Con A supernatant, prepared as described previously (29)). Cells were fed weekly by adding fresh medium and freshly harvested syngeneic SMNC. MHV-3 at an MOI of 0.1 to 0.5 was added on alternate weeks, and TCGF was added 2 to 3 days following rechallenge with Ag. Wells showing proliferation were transferred to 96-well flat-bottom plates with 5×10^6 irradiated SMNC, fresh medium, and TCGF. The T cells were then expanded in 24-well plates before being transferred to T-25 tissue culture flasks. Fresh medium was added weekly, and irradiated SMNC and MHV-3 were added every 14 days. TCGF was added 2 to 3 days following Ag restimulation, and the T cell lines were passaged when they reached 1×10^6 cells/ml. The T cells were redistributed at limiting dilution under the same conditions, and colonies were taken from plates that had <33% positive wells.

Surface phenotype assessment

The T cells obtained as described above were subtyped into $CD3^+$, $CD4^+$, and $CD8^+$ populations based upon indirect immunofluorescence analysis of surface membrane markers. Cells (1×10^6) were incubated with 4 μl of rat anti-mouse Thy-1.2, Lyt-2, or L3T4 Abs (Becton Dickinson, Mountain View, CA) at 4°C for 45 min. After washing and resuspension, the cells were incubated with 10 μl of FITC-conjugated goat anti-rat Ab (Zymed Laboratories, San Francisco, CA) for 45 min at 4°C. After a final wash, cells were either transferred to a glass slide (5×10^4 cells/slide) and viewed on a Leitz phase/epifluorescence microscope (Willowdale, Canada) or analyzed by flow cytometry (FACScan, Becton Dickinson).

Cytokine production

To test for lymphokine production by the T cell lines, lines were cocultured in growth factor-free medium with MHV-3-infected, irradiated SMNC as APCs, and the supernatants were collected 48 h later. The supernatants were stored at −20°C until assayed for IL-2, IL-4, and IFN-γ. IL-2 and IL-4 activities were assessed by the ability of supernatants to support the proliferation of CTLL-2 (35) and CT4.S cells in the presence of anti-IL-2 (American Type Culture Collection (ATCC), Rockville, MD) and anti-IL-4 mAbs (ATCC), respectively. IFN-γ activity was determined by inhibition of plaque formation induced by MHV-3 on L2 cells as previously described (36). Results from the cytokine assays were expressed as nanograms per ml by comparison with standard curves of purified natural or recombinant cytokines.

Antigen specificity of T cell lines

The Ag specificity of T cell lines from susceptible BALB/cJ mice was determined, as previously described, by measuring proliferation in response to lysates from H-2-compatible cells transfected with recombinant vaccinia viruses expressing spike, matrix, and nucleocapsid proteins as well as cells infected with MHV-3 (29).

Macrophage PCA

Peritoneal macrophages were infected with virulent MHV-3 (MOI of 2) and incubated at 37°C in 5% CO_2 for 12 h. Following incubation, samples were assayed for their ability to shorten the spontaneous clotting time of normal citrated human platelet-poor plasma, as previously described (4). Millliunits of PCA were assigned by reference to a standard curve generated with serial log dilutions of a standard rabbit brain thromboplastin (Dade Division, American Hospital Supply Co., Miami, FL). Media and reagents were without activity.

Effects of T cell lines on PCA induction

Thioglycolate-elicited peritoneal macrophages (5×10^5) from susceptible recombinant inbred AXB8 mice were stimulated with 1×10^6 PFU of MHV-3 in the presence or the absence of 8×10^6 T cells from the 3E9.1 Th1 cell line established from immunized A/J mice (29). An equivalent number of peritoneal macrophages from susceptible BALB/cJ mice was similarly infected with 1×10^6 PFU of MHV-3 (MOI of 2) in the presence or the absence of 8×10^6 T cells from each of the Th cell lines established from BALB/cJ mice immunized with attenuated virus.

Effect of T cells on MHV-3 infection in vivo

To assess the course of T cells on the course of MHV-3 infection in vivo, susceptible recombinant inbred AXB8 or BALB/cJ mice received 5×10^6 T cells i.v. from the A/J-derived Th1 line 3E9.1 or the BALB/cJ-derived Th2 line 4B6.8, respectively. They were then infected i.p. with 10 PFU of virulent MHV-3. Control mice received 5×10^6 syngeneic spleen cells i.v. from nonimmunized mice.

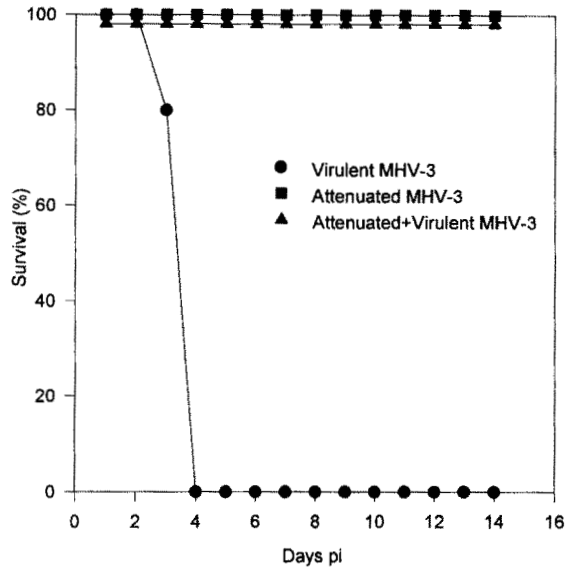

FIGURE 1. Attenuated YAC-1 MHV induces immunity to virulent MHV-3 in BALB/cJ mice. Groups of 10 naive BALB/cJ mice were infected with either 10 PFU of virulent MHV-3 i.p. or 1000 PFU of attenuated MHV-3 i.p. The mice that received the attenuated MHV-3 were reinfected 2 wk later with 1000 PFU of virulent MHV-3. pi indicates postinfection.

Statistics

Where applicable, data are expressed as the mean ± SD. Student's paired *t* test (two-tailed) was used to analyze the data. In adoptive transfer experiments, mean survival times ± 95% confidence intervals (CI) of experimental and control groups were compared by survival analysis using the Kaplan-Meier method.

Results

Immunization of susceptible BALB/cJ mice

Infection of susceptible BALB/cJ mice with 1 to 5 PFU of MHV-3 resulted in the rapid and uniformly fatal development of fulminant hepatitis (4). By 24 h postinfection, high viral titers ($3.3 \pm 1.6 \times 10^4$ PFU/g liver) were recovered from liver homogenates, which increased at 72 h to $4.6 \pm 3.7 \times 10^8$ PFU/g liver and persisted until the death of the animals.

In contrast, immunization of these mice with inactivated MHV-3 failed to elicit T cell activation either in vivo or in vitro (30, 37). Therefore, to overcome these difficulties in immunizing susceptible BALB/cJ mice with live or inactivated MHV-3, we used the protocol of Lamontagne and Jolicoeur (31) to develop an attenuated variant of MHV-3 by passaging the virus repeatedly in YAC-1 cells. Infection of BALB/cJ mice with 1000 PFU of attenuated MHV-3 induced mild acute hepatitis, characterized by the presence of small discrete foci of hepatic necrosis with a sparse polymorphonuclear leukocyte infiltrate 72 to 96 h postinfection coincident with peak viral titers of $1.0 \pm 0.5 \times 10^4$ PFU/g liver, which resolved with complete clearance of virus, as indicated by the inability to detect even 1 PFU of MHV-3 by day 10 postinfection as determined by plaque assay of liver and spleen homogenates. All BALB/cJ mice survived a subsequent lethal i.p. dose of 1000 PFU of virulent MHV-3 administered 2 wk following the primary immunization with attenuated MHV-3 (Fig. 1). No infectious virus was detected by plaque assay in liver, serum, or spleen homogenates 4 days after infection with virulent MHV-3.

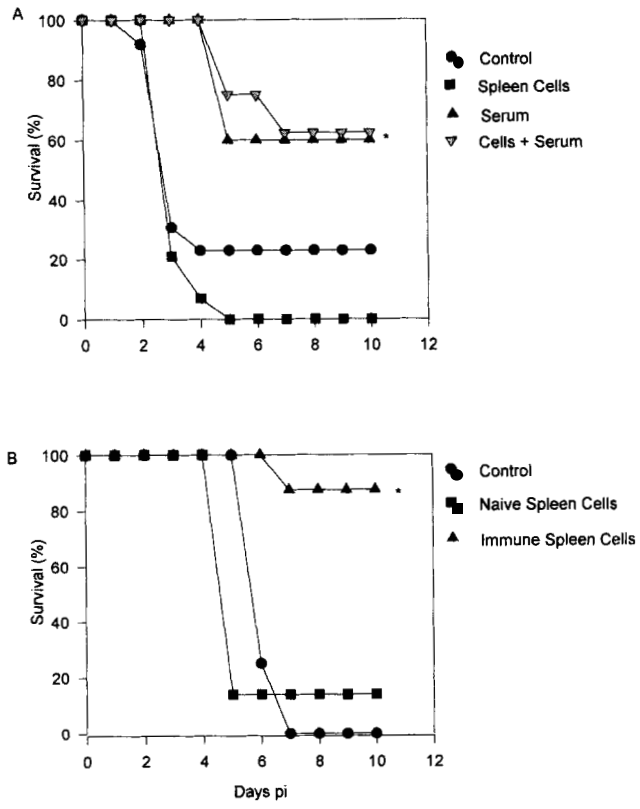

FIGURE 2. *A*, Transferring serum from immunized to naive BALB/cJ mice improves survival following MHV-3 infection, whereas transferring spleen cells does not. Groups of 15 naive BALB/cJ mice received 5×10^7 spleen cells i.v., 200 μl of serum i.v., or both from BALB/cJ mice that had been immunized with attenuated MHV-3, following which they were infected with 10 PFU of virulent MHV-3 i.p. Fifteen control mice received 10 PFU of virulent MHV-3 i.p. alone. The mean survival times and 95% CIs for each group were compared by survival analysis using the Kaplan-Meier method (* indicates $p < 0.05$). *B*, Transferring spleen cells from immunized to naive irradiated A/J mice improves survival following MHV-3 infection. Groups of 10 naive A/J mice were irradiated with 650 rad from a gamma source, following which they received 5×10^7 spleen cells i.v. from naive or immunized A/J mice before infection with virulent MHV-3 (10 PFU i.p.). Ten control mice received 10 PFU of virulent MHV-3 i.p. only. The mean survival times and 95% CIs for each group were compared by survival analysis using the Kaplan-Meier method. pi indicates postinfection.

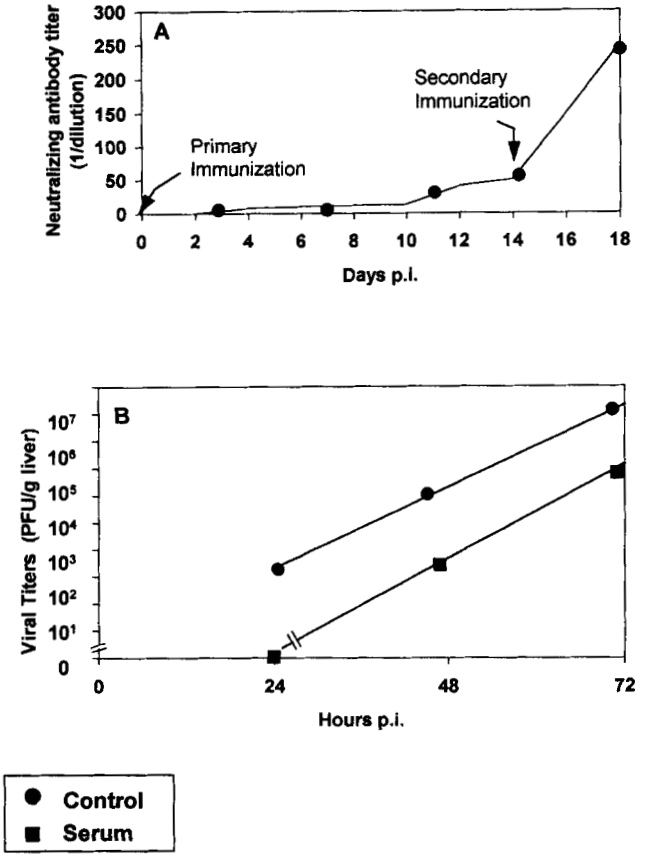

FIGURE 3. Serum from immunized BALB/cJ mice contains neutralizing Abs. *A*, BALB/cJ mice were immunized with 1000 PFU of attenuated MHV-3 dip on day 0 and boosted with virulent MHV-3 10 PFU i.p. on day 11. Groups of mice were killed at each time point, and sera were pooled. The neutralizing Ab titer was defined as the reciprocal of the dilution producing a 50% decrease in plaque formation following a 30-min incubation with MHV-3 at 37°C. Serum from naive mice did not affect MHV-3 infectivity in a plaque assay (not shown). *B*, Naive BALB/cJ received 200 μl of immune serum i.v. 1 h before infection with 10 PFU of virulent MHV-3 i.p. Control mice received MHV-3 alone. Groups of mice were killed 24, 48, and 72 h postinfection. Liver homogenates were prepared, and viral titers were determined by plaque assay. Values represent the mean ± SD for six separate determinations. p.i. indicates postinfection.

Adoptive transfer experiments

To investigate whether humoral or cell-mediated immunity conveyed resistance to BALB/cJ mice immunized with YAC-1-MHV, naive BALB/cJ received 200 μl of immune serum i.v. or 5×10^7 immune spleen cells i.v. 1 h before infection with 10 PFU of virulent MHV-3 i.p. MHV-immune serum and spleen cells were obtained from BALB/cJ mice immunized with attenuated MHV-3 and rechallenged with virulent MHV-3 2 wk later.

The transfer of spleen cells from immunized BALB/cJ mice failed to protect naive susceptible BALB/cJ mice from MHV-3 infection (Fig. 2*A*). The transfer of spleen cells from naive mice to susceptible mice subsequently infected with MHV-3 similarly did not demonstrate any protective effect (data not shown). To exclude the possibility that an insufficient number of spleen cells was administered, the following experiment was performed. Spleen cells from A/J mice immunized with MHV-3 were injected into naive A/J mice rendered susceptible to MHV-3 infection by irradiation (650 rad). Adoptive transfer of these spleen cells prevented mortality following MHV-3 infection (Fig. 2*B*), thus demonstrating that the number and viability of spleen cells used for the adoptive transfer experiments were adequate to protect naive susceptible mice against MHV-3 infection.

In contrast, when susceptible mice received a single dose of serum from immunized, but not from naive, mice, survival was significantly prolonged compared with that of untreated mice (Fig. 2*A*; $p < 0.05$). Serum from immunized BALB/cJ mice was found to contain neutralizing Abs at a titer of 1/32 following a primary immunization, increasing to a titer of 1/250 following a secondary immunization (Fig. 3*A*). A single i.v. dose of immune serum significantly decreased viral titers in the livers of MHV-3-infected, naive BALB/cJ mice (Fig. 3*B*). Analysis of the serum by radioimmunoprecipitation demonstrated reactivity to all three MHV structural proteins (nucleocapsid, spike, and matrix proteins; Fig. 4).

Characterization of T cell lines

To examine the T cell response in BALB/cJ mice infected with attenuated MHV-3, we stimulated bulk cultures of spleen-derived

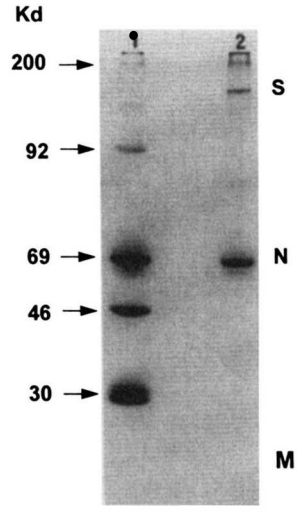

FIGURE 4. Characterization of Ab response to MHV-3 immunization in serum from BALB/cJ mice on a 10% polyacrylamide-SDS gel. *Lane 1,* m.w. standards; *lane 2,* radioimmunoprecipitation of MHV-3 [^{35}S]methionine-labeled lysate by serum from immunized BALB/cJ mice demonstrating spike glycoprotein (S; M_r = 180,000 daltons), nucleocapsid protein (N; M_r = 60,000 daltons), and matrix glycoprotein (M; M_r = 24,000 daltons).

mononuclear cells with the attenuated virus. In these studies, analysis of cytokine production demonstrated significant levels of IL-4 (data not shown). To investigate these findings further, we established 12 Th cell lines from BALB/cJ mice immunized with attenuated MHV-3, all of which had cytokine profiles consistent with a Th2 phenotype. Four of these were studied in detail (Table I). By FACS analysis, all T cell lines were $CD3^+$ and $CD4^+$. These Th cell lines proliferated in response to cells transfected with nucleocapsid protein or infected with MHV-3 (Table II).

All four $CD4^+$ Th cell lines isolated from BALB/cJ mice immunized with attenuated MHV-3 produced IL-4 and low amounts of IFN-γ or IL-2 in response to Ag stimulation, consistent with a Th2 cytokine secretion profile (Table I). This was in marked contrast to the T cell lines from immunized A/J mice (29), which produced IL-2 and IFN-γ, but only low amounts of IL-4, consistent with a Th1 profile.

Modulation of PCA by T cell lines

Previous studies from our lab have demonstrated cellular regulation of macrophage PCA in response to MHV-3 infection. Spleen cells and cloned Th1 cells from resistant A/J mice immunized with MHV-3 inhibited macrophage procoagulant production in response to MHV-3, whereas lymphocytes from susceptible mice were required for maximal production of macrophage PCA (26, 29). Therefore, we wished to examine the effects of T cells from MHV-3-immunized susceptible BALB/cJ mice on macrophage PCA induction. In this study, spleen cells from BALB/cJ mice immunized with attenuated MHV-3 augmented macrophage PCA production, while spleen cells from naive BALB/cJ mice had no effect on PCA (data not shown). All four Th2 cell lines from immunized susceptible BALB/cJ mice also augmented MHV-3-induced macrophage procoagulant production (Table III), consistent with the effects seen using bulk spleen cell cultures. No PCA was measured when Th2 cells were incubated with macrophages in the absence of MHV-3 (data not shown). These findings are in contrast to those using the Th1 cell line from resistant A/J mice, 3E9.1, which was able to inhibit PCA (29). Although we have observed

Table I. *Characterization of T cell lines*[a]

Source of T Cell Line	T Cell Line	Cytokine Profile IL-2 (ng/ml)	IFN-γ (ng/ml)	IL-4 (ng/ml)
A/J	3E9.1	9.3 ± 1.1	114 ± 12	0.31 ± 0.16
BALB/cJ	4B6.8	0.21 ± 0.15	5.6 ± 2.8	18.3 ± 1.6
	2F4.1	0.08 ± 0.11	9.2 ± 1.1	12.4 ± 3.2
	1A1.6	0.25 ± 0.14	7.2 ± 2.8	14.6 ± 2.8
	8V4.3T	0.16 ± 0.09	8.1 ± 2.1	17.3 ± 4.1

[a] T cell lines were cocultured in growth factor-free media with MHV-3-infected, irradiated SMNC for 48 h. The supernatants were assayed for IL-2, IL-4, and IFN-γ as described in *Materials and Methods.* IL-2 and IL-4 activities were assessed by the ability of supernatants to support the proliferation of CTLL-2 and CT4.S cells, respectively, and IFN-γ activity was determined by inhibition of plaque formation induced by MHV-3 on L2 cells. Results from the cytokine assays were expressed as ng/ml by comparison with standard curves of purified natural or recombinant cytokines. Values represent the mean ± SD for three experiments, each done in triplicate.

PCA inhibition only by Th1 clones, it should be noted that not all Th1 clones inhibit PCA production, and the failure of BALB/cJ Th2 clones to inhibit may be due to either strain differences or cytokine patterns.

Effects of T cell lines on the course of MHV-3 infection in vivo

To assess the effects of Th cell lines from susceptible BALB/cJ mice on the course of MHV-3 infection in vivo, T cells were collected 8 days following Ag restimulation, and 5×10^6 cells were injected i.v. into naive BALB/cJ mice. Control mice received 5×10^6 syngeneic spleen cells from naive mice. The Th2 cell line 4B6.8 from immunized susceptible BALB/cJ mice, which increased PCA, did not protect naive BALB/cJ mice following MHV-3 infection (Fig. 5*B*). In contrast, as previously reported (29), the Th1, PCA-inhibitory 3E9.1 cell line from A/J mice prevented mortality in naive MHC-compatible and susceptible AXB8 mice (Fig. 5*A*).

Discussion

The outcome of viral infection represents a balance between replication of the virus and the host immune response, which consists of an early innate nonspecific inflammatory response and a later acquired Ag-specific immune response. Studies on the pathogenesis of MHV-3 infection in our laboratory as well as by others have shown that A/J mice that are resistant generate a protective cellular immune response following MHV-3 infection (37–39). Furthermore, spleen cells from resistant A/J mice can adoptively transfer resistance from adult to susceptible neonatal mice (39), and the resistance can be overcome following implementation of various immunosuppressive regimens that inhibit cell-mediated immunity (38). The principal effector cells responsible for resistance to MHV-3 infection have been shown to be $CD4^+$ Th cells (40), although $CD8^+$ cytotoxic T cells and the production of neutralizing Abs have also been implicated in immunity to other strains of MHV, such as the neurotropic strain, MHV-JHM (41).

In contrast, few data exist that assess acquired resistance in susceptible BALB/cJ mice, at least in part because of an inability to successfully immunize animals with virulent MHV-3 (30, 37). Infection of naive susceptible BALB/cJ mice with virulent MHV-3 is rapidly fatal and associated with the induction of macrophage PCA, recently identified as musfiblp, encoded by the *fgl-2* gene location on chromosome 5, which results in microvascular thrombosis and hepatocellular necrosis, with all mice dying within 5

Table II. *Antigen specificity of T cell lines*[a]

T Cell Line	MHV-3	MHV-JHM	[³H]TdR Incorporation in the Presence of Antigen			Vaccinia	Control (Naive SMNC)
			M	S	N		
3E9.1	19,209 ± 3,738	26,194 ± 6,824	4,725 ± 3,037	5,367 ± 1,371	4,568 ± 1,412	5,406 ± 1,458	4,647 ± 331
4B6.8	15,704 ± 1,429	21,461 ± 1,845	3,400 ± 1,296	4,748 ± 844	12,049 ± 945	4,975 ± 845	3,324 ± 295
2F4.1	11,770 ± 1,027	22,308 ± 4,845	5,202 ± 992	3,718 ± 700	12,698 ± 3,341	4,146 ± 777	4,067 ± 702
1A1.6	13,804 ± 1,414	12,440 ± 736	4,271 ± 531	5,468 ± 672	3,913 ± 1,607	3,973 ± 202	2,597 ± 1,424
8V4.3T	37,075 ± 8,563	24,450 ± 3,842	4,577 ± 1,162	5,613 ± 708	20,788 ± 5,397	5,928 ± 939	4,648 ± 1,318

[a] T cell clones (1×10^5) were cocultured with irradiated (2000 rad) splenocytes in the presence or absence of MHV-3, M (matrix protein), S (spike protein), N (nucleocapsid protein), or vaccinia virus in 96-well plates for 72 h. Sixteen hours before harvesting the cells, 1 mCi of [³H]TdR was added to each well, and incorporation was measured using a liquid scintillation beta counter. Data are expressed as cpm.

Table III. *Modulation of PCA by T cell lines*[a]

Source of Macrophages	Source of T cells	T Cell Line	Stimulus	PCA (mU/10^6 Macrophages)
AXB8	None	None	None	95 ± 4
AXB8	None	None	MHV-3	4,500 ± 1,244
AXB8	A/J	3E9.1	MHV-3	434 ± 267*
BALB/cJ	None	None	None	120 ± 18
BALB/cJ	None	None	MHV-3	7,840 ± 440
BALB/cJ	BALB/cJ	4B6.8	MHV-3	9,850 ± 860*
BALB/cJ	BALB/cJ	2F4.1	MHV-3	12,160 ± 880*
BALB/cJ	BALB/cJ	1A1.6	MHV-3	11,680 ± 380*
BALB/cJ	BALB/cJ	8V4.3T	MHV-3	9,240 ± 220*

[a] To determine the effect of T cell lines on PCA induction, 8×10^6 T cells from A/J or BALB/cJ mice were added to 5×10^5 thioglycolate-elicited peritoneal macrophages from susceptible AXB8 or BALB/cJ mice, respectively, in the presence of 1×10^6 PFU of MHV-3. Following incubation for 12 h, PCA was measured in a one-stage clotting assay as described in *Materials and Methods*. Values represent the mean ± SD for three experiments, each done in triplicate. (* signifies a two-tailed p value of <0.05, compared with macrophages incubated with MHV-3 only).

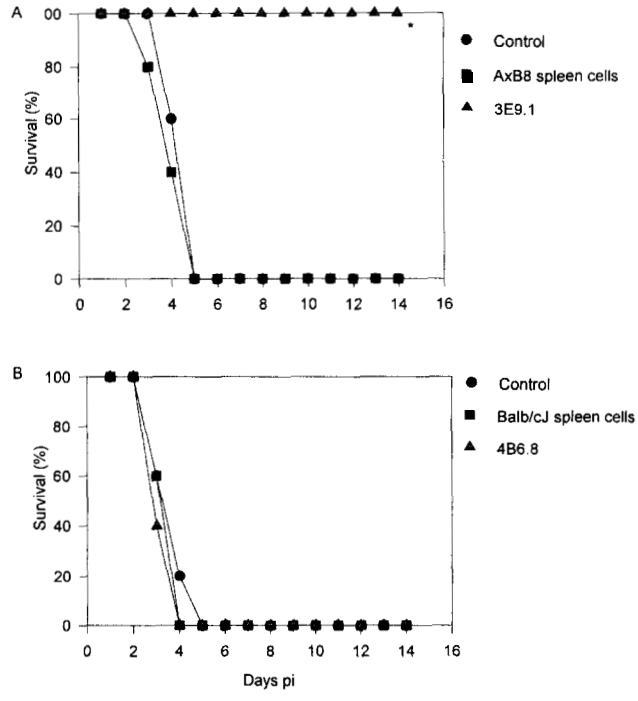

FIGURE 5. *A*, The Th1, PCA-inhibitory, T cell line 3E9.1 from A/J mice prevents mortality in MHV-3 infected, H-2-compatible, susceptible AXB8 mice. Groups of 10 AXB8 mice received either 5×10^6 T cells i.v. or 5×10^6 naive AXB8 spleen cells i.v., follwoing which they were infected with 10 PFU of virulent MHV-3 i.p. Ten control mice received 10 PFU of virulent MHV-3 alone.The mean survival times and 95% CI for each group were compared by survival analysis using the Kaplan-Meier method. *B*, The Th2, PCA-augmenting T cell line 4B6.8 from BALB/cJ mice does not prevent mortality in MHV-3-infected naive BALB/cJ mice. Groups of 15 naive BALB/cJ mice received either 5×10^6 T cells i.v. or 5×10^6 naive BALB/cJ spleen cells i.v., following which they were infected with 10 PFU of virulent MHV-3 i.p. Fifteen control mice received 10 PFU of virulent MHV-3 alone. The mean survival times and 95% CI for each group were compared by survival analysis using the Kaplan-Meier method. pi indicates postinfection.

days (7, 8). It has been shown that induction of musfiblp is regulated by T cells in both susceptible and resistant mice (26, 29).

Lamontagne et al. suggested that susceptibility is associated with the loss of both peripheral and thymic T cells shortly after viral infection (42). Furthermore, she demonstrated that infection of susceptible mice with an attenuated variant of MHV-3 derived by passaging virulent MHV-3 in YAC-1 cells (YAC-1-MHV-3) imparted resistance to subsequent challenge with virulent MHV-3 (31). We used YAC-1-MHV-3 to study the nature of the immune response to MHV-3 in susceptible mice. The attenuated variant of MHV-3 is antigenically similar to the virulent MHV-3 and, in the present study, induced neutralizing Abs that protected susceptible mice against infection with a lethal dose of virulent MHV-3. These Abs reacted against all three structural proteins of MHV-3 (nucleocapsid, spike, and matrix proteins), as demonstrated by radioimmunoprecipitation. While neutralizing Abs were produced in the susceptible BALB/cJ mice following infection with the attenuated variant of MHV-3, no neutralizing Abs were detected following infection with virulent MHV-3 as previously described (30, 34).

Our data demonstrate that neutralizing Abs confer immunity by preventing initial viral entry and replication. Thus, even though T cell lines derived from immunized BALB/cJ mice augment PCA production in vitro, in vivo circulating Abs block initial viral replication and the subsequent production of PCA and other pro-inflammatory mediators by macrophages. As shown in Figure 3*B*, viral replication is not completely prevented by Ab treatment; consistent with this observation, we observed small foci of liver necrosis 3 days following challenge of YAC-MHV-3-immunized BALB/cJ mice with virulent MHV-3. In contrast, nonimmunized mice, which do not have neutralizing Ab and are unable to contain initial viral entry and replication, produce PCA and develop lethal MHV-3 infection. We have previously shown that the administration of neutralizing Abs once viral infection becomes established is ineffective in preventing mortality associated with MHV-3 (34).

Adoptive transfer of spleen cells from YAC-1-MHV-3-immunized BALB/cJ mice failed to protect naive mice from MHV-3 infection. This was in contrast to the protection afforded by transfer of spleen cells from immunized resistant A/J mice, suggesting differences in cell-mediated immunity in resistant and susceptible

mice. This led us to undertake studies to determine the nature of the cellular immune response in both resistant and susceptible mice. MHV-3-specific Th cell lines isolated from resistant A/J mice had a cytokine profile consistent with a Th1 phenotype, as evidenced by IL-2 and IFN-γ production (29). In contrast, all MHV-3-specific Th cell lines isolated from susceptible BALB/cJ mice were of the Th2 phenotype, as demonstrated by the production of IL-4. These latter T cell lines augmented MHV-3-induced macrophage PCA production, whereas a Th1 line from the A/J inhibited PCA production.

Despite the fact that the T cell lines proliferated in vitro in response to MHV-3, adoptive transfer of the T cell lines derived from immunized BALB/cJ mice failed to protect naive susceptible BALB/cJ mice against lethal MHV-3 disease. This lack of protection is consistent with the ability of these T cell lines to augment MHV-3-induced macrophage PCA production, which is a critical event, leading to sinusoidal thrombosis and fulminant ischemic liver necrosis (4, 6). We, therefore, undertook a series of experiments to determine whether we could alter resistance/susceptibility to MHV-3 by blocking the Th2 cytokine IL-4 in susceptible mice and the Th1 cytokine IFN-γ in resistant mice. In a first series of experiments, susceptible BALB/cJ mice ($n = 8$) were treated 24 h before infection with 1000 PFU of MHV-3 with either 1 mg/mouse of a mAb to IL-4 (11B11) or an isotype control Ab. Unlike in *Leishmania* infection, treatment of naive susceptible BALB/cJ mice with the neutralizing anti-IL-4 mAb failed to improve survival following MHV-3 infection, and all mice died within 8 days of infection with a similar time course. Several possibilities could explain this result. The treatment may have failed to block the induction of a Th2 response because the site of the anti-MHV Th2 response may have been less accessibile than that in other systems. Furthermore, although we may have blocked the Th2 response, a Th1 response may not have developed. Alternatively, even if we were able to induce a Th1 response, it may have been inadequate in rapidity of onset or magnitude. Thus, the observation that treatment with an anti-IL-4 mAb did not confer protection against MHV-3 to nonimmunized susceptible mice does not discount a possible causative role of Th2 cells in the pathogenesis of MHV-3 infection. In contrast, treatment of resistant A/J mice ($n = 8$) with 1 mg of a rat monoclonal anti-murine antibody to IFN-γ (R4–6A2) given i.p. 24 h before infection with MHV-3 resulted in the loss of resistance to MHV-3, with all mice dying within 5 days of infection, demonstrating the importance of the Th1 response to resistance to MHV-3, as previously described (43).

We were unable to obtain Th2 cell lines from A/J mice or Th1 cells from BALB/cJ mice immunized with MHV-3. This may be for several reasons. The differential maturation of Th1 or Th2 phenotypes from resistant and susceptible mice may be explained by their responses to specific epitopes on the nucleocapsid protein (44) consistent with a recent observation described by Mougneau et al. in parasitic infections (45). Alternatively, differences in Ag presentation or the presence or the absence of costimulatory signals in the resistant or susceptible mice may be responsible for the differential Th1/Th2 maturation process (42, 46, 47). Genetic factors may also be involved, as it has been suggested that strains of mice, such as BALB/cJ, may be predisposed toward a "default" Th2 response in the absence of immunomodulatory treatment (48).

Thus, these results demonstrate significant differences in the cellular immune responses of resistant and susceptible mice following MHV-3 infection. Resistant A/J mice generate a protective Th1 cellular immune response. In contrast, while susceptible BALB/cJ mice are able to produce neutralizing anti-viral Abs following immunization with an attenuated variant of MHV-3, all anti-MHV-3 Th cell lines are of theTh2 phenotype and do not protect naive animals from the lethality of MHV-3 infection. Their failure to protect naive mice is consistent with the fact they augment PCA production. This may be an important factor in the pathogenesis of liver necrosis, and may have implications for our understanding of the pathogenesis of fulminant viral hepatitis. These studies provide new insights into our understanding of the pathogenesis of fulminant viral hepatitis by MHV-3, demonstrating a protective role for Th1 cells and a role for Th2 cells in the pathogenesis of MHV-3-induced liver failure.

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References

1. Lee, W. J. 1993. Acute liver failure. *N. Engl. J. Med. 329:1862.*
2. Spaan, W., D. Cavanagh, and M. Horzinek. 1990. Coronaviruses. In *Immunochemistry of Viruses. II. The Basis for Serodiagnosis and Vaccines.* M. H. V. Van Regenmortel and A. R. Neurath, eds. Elsevier Science Publishers, Amsterdam, p. 359.
3. MacPhee, P. J., V. J. Dindzans, L. S. Fung, and G. A. Levy. 1985. Acute and chronic changes in the microcirculation of the liver in inbred strains of mice following infection with mouse hepatitis virus type 3. *Hepatology 5:649.*
4. Levy, G. A., J. L. Leibowitz, and T. S. Edgington. 1981. Induction of monocyte procoagulant activity by murine hepatitis virus type 3 parallels disease susceptibility in mice. *J. Exp. Med. 154:1150.*
5. MacNaughton, M. R., and S. Patterson. 1980. Mouse hepatitis virus strain 3 infection of C57,A/Sn and A/J strain mice and their macrophages. *Arch. Virol. 66:71.*
6. Li, C., L. S. Fung, A. Crow, N. Myers-Mason, J. L. Leibowitz, E. Cole, and G. A. Levy. 1992. Monoclonal anti-prothrombinase (3D4.3) prevents mortality from murine hepatitis virus infection (MHV-3). *J. Exp. Med. 1763:689.*
7. Parr, R., J. L. Leibowitz, L. S. Fung, S. J. Reneker, N. Myers-Mason, and G. A. Levy. 1995. The association of mouse fibrinogen-like protein (Musfiblp) with murine hepatitis virus induced prothrombinase activity. *J. Virol. 69:5033.*
8. Qureshie, S. T., S. Clermont, J. Leibowitz, L. S. Fung, G. A. Levy, and D. Malo. 1995. Murine hepatitis virus-3 induced prothrombinase (Musfiblp) maps to proximal chromosome 5. *Genomics 29:307.*
9. Mosmann, T. R., and R. L. Coffman. 1989. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol. 7:145.*
10. Heinzel, F. P., M. D. Sadick, B. J. Holaday, R. L. Coffman, and R. M. Locksley. 1989. Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis: evidence for expansion of distinct helper T cell subsets. *J. Exp. Med. 169:59.*
11. Sypek, J. P., C. L. Chung, S. H. Mayor, J. M. Subramanyam, S. J. Goldman, D. S. Sieburth, S. F. Wolf, and R. G. Schaub. 1993. Resolution of cutaneous leishmaniasis: interleukin-12 initiates a protective T helper type 1 immune response. *J. Exp. Med. 177:1797.*
12. Scott, P. 1991. IFN-gamma modulates the early development of Th1 and Th2 responses in a murine model of cutaneous leishmaniasis. *J. Immunol. 147:3149.*
13. Gajewski, T., and F. W. Fitch. 1988. Anti-proliferative effect of IFN-gamma in immune regulation. I. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. *J. Immunol. 140:4245.*
14. Belosevic, M., D. S. Finbloom, P. H. Van der Meide, M. V. Slayter, and C. Nacy. 1989. Administration of monoclonal anti-IFN-gamma antibodies in vivo abrogates natural resistance of C3H/HeN mice to infection with *Leishmania major. J. Immunol. 143:266.*
15. Swain, S., A. D. Weinberg, M. English, and G. Huston. 1990. IL-4 directs the development of Th2-like helper effectors. *J. Immunol. 145:3796.*
16. Chatelain, R., K. Varkila, and R. L. Coffman. 1992. IL-4 induces a Th2 response in *Leishmania major*-infected mice. *J. Immunol. 148:11827.*
17. Fiorentino, D. F., A. Zlotnik, P. Vieira, T. R. Mosmann, M. Howard, K. W. Moore, and A. O'Garra. 1991. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J. Immunol. 146:3444.*
18. Romani, L., S. Mocci, C. Bietta, L. Lanfaloni, P. Puccetti, and F. Bistoni. 1991. Th1 and Th2 cytokine secretion patterns in murine candidiasis: association of Th1 responses with acquired resistance. *Infect. Immun. 59:4647.*
19. Cenci, E., L. Romani, A. Vecchiarelli, P. Puccetti, and F. Bistoni. 1990. T cell subsets and IFN-gamma production in resistance to systemic candidosis in immunized mice. *J. Immunol. 144:4333.*
20. Haak-Frendscho, M., J. F. Brown, Y. Iizawa, D. R. Wagner, and C. J. Czuprynski. 1992. Administration of anti-IL-4 monoclonal antibody 11B11 increases the resistance of mice to *Listeria monocytogenes* infection. *J. Immunol. 148:3978.*
21. Liew, F. Y., S. Millott, Y. Li, R. Lelchuk, W. L. Chan, and H. Ziltener. 1989. Macrophage activation by interferon-gamma from host-protective T cells is inhibited by interleukin (IL) 3 and IL-4 produced by disease-promoting T cells in leishmaniasis. *Eur. J. Immunol. 19:1227.*

22. Romani, L., A. Mencacci, U. Grohmann, S. Mocci, P. Mosci, P. Puccetti, and F. Bistoni. 1992. Neutralizing antibody to interleukin 4 induces systemic protection and T helper type 1-associated immunity in murine candidiasis. *J. Exp. Med. 176:19.*
23. Sadick, M. D., F. P. Heinzel, B. J. Holaday, R. T. Pu, R. S. Dawkins, and R. M. Locksley. 1990. Cure of murine leishmaniasis with anti-interleukin 4 monoclonal antibody: evidence for a T cell-dependent, interferon gamma-independent mechanism. *J. Exp. Med. 171:115.*
24. Romagnani, S. 1991. Human Th1 and Th2 subsets: doubt no more. *Immunol. Today 12:256.*
25. Modlin, R. L., and T. B. Nutman. 1993. Type 2 cytokines and negative immune regulation in human infections. *Curr. Opin. Immunol. 5:511.*
26. Chung, S., S. Sinclair, J. Leibowitz, E. Skamene, L. S. Fung, and G. Levy. 1991. Cellular and metabolic requirements for induction of macrophage procoagulant activity by murine hepatitis virus strain 3 in vitro. *J. Immunol. 146:271.*
27. Levy, G. A., and T. S. Edgington. 1980. Lymphocyte cooperation is required for amplification of macrophage procoagulant activity. *J. Exp. Med. 151:1232.*
28. Fan, S., A. L. Glasebrook, and T. S. Edgington. 1990. Clonal analysis of $CD4^+$ T helper cell subsets that induce the monocyte procoagulant response. *Cell. Immunol. 128:52.*
29. Chung, S., R. Gorczynski, B. Cruz, R. Fingerote, E. Skamene, S. Perlman, J. L. Leibowitz, L. S. Fung, M. Flowers, and G. A. Levy. 1994. A TH1 helper cell line (3E9.1) from resistant A/J mice inhibits induction of macrophage procoagulant activity (PCA) in vitro and protects against MHV-3 mortality in vivo. *Immunology 83:353.*
30. Dindzans, V. J., B. Zimmerman, A. Sherker, and G. A. Levy. 1987. Susceptibility to mouse hepatitis virus strain 3 in BALB/cJ mice: failure of immune cell proliferation and interleukin 2 production. *Adv. Exp. Med. Biol. 218:411.*
31. Lamontagne, L., and P. Jolicoeur. 1991. Mouse hepatitis virus 3-thymic cell interactions correlating with viral pathogenecity. *J. Immunol. 146:3152.*
32. Nesbitt, M. N., and E. Skamene. 1984. Recombinant inbred mouse strains derived from A/J and C57BL/6J: a tool for the study of genetic mechanisms in host resistance to infection and malignancy. *J. Leukocyte Biol. 36:357.*
33. Dindzans, V. J., P. J. MacPhee, L. S. Fung, J. L. Leibowitz, and G. A. Levy. 1985. The immune response to mouse hepatitis virus: expression of monocyte procoagulant activity and plasminogen activator during infection in vivo. *J. Immunol. 135:4189.*
34. Levy, G. A., R. Shaw, J. L. Leibowitz, and E. Cole. 1984. The immune response to mouse hepatitis virus: genetic variation in antibody response and disease. *Adv. Exp. Med. Biol. 173:345.*
35. Gillis, S., M. M. Fern, W. Ou, and K. A. Smith. 1978. T cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol. 120:2027.*
36. Vilcek, J., and I. C. Oliveira. 1994. Recent progress in the elucidation of interferon-gamma actions: molecular biology and biological functions. *Int. Arch. Allergy Immunol. 104:311.*
37. Le Prevost, C., E. Levy-Leblond, J. L. Virelizier, and J. M. Dupuy. 1975. Immunopathology of mouse hepatitis virus type 3 infection. I. Role of humoral and cell-mediated immunity in resistance mechanisms. *J. Immunol. 114:221.*
38. Dupuy, J. M., E. Levy-Leblond, and C. Le Prevost. 1975. Immunopathology of mouse hepatitis virus type 3 infection. II. Effect of immunosuppression in resistant mice. *J. Immunol. 114:226.*
39. Levy-Leblond, E., and J. M. Dupuy. 1977. Neonatal susceptibility to MHV-3 infection in mice. I. Transfer of resistance. *J. Immunol. 118:1219.*
40. Korner, H., A. Schliephake, J. Winter, F. Zimprich, H. Lassmann, J. Sedgwick, S. Siddell, and H. Wege. 1991. Nucleocapsid or spike protein-specific $CD4^+$ T lymphocytes protect against coronavirus-induced encephalomyelitis in the absence of $CD8^+$ cells. *J. Immunol. 147:2317.*
41. Stohlman, S. A., G. K. Matsushima, N. Casteel, and L. P. Weiner. 1986. In vivo effects of coronavirus-specific T cell clones: DTH inducer cells prevent a lethal infection but do not inhibit virus replication. *J. Immunol. 136:3052.*
42. Lamontagne, L., J. P. Descoteaux, and P. Jolicoeur. 1989. Mouse hepatitis virus 3 replication in T and B lymphocytes correlate with viral pathogenicity. *J. Immunol. 142:4458.*
43. Virelizier, J. L., and I. Gressor. 1978. Role of interferon in the pathogenesis of viral disease in mice as demonstrated by the use of anti-interferon serum. V. Protective role in mouse hepatitis virus type 3 infection of susceptible and resistant strains of mice. *J. Immunol. 120:1616.*
44. Stohlman, S., C. Bergmann, D. Cua, H. Wege, and R. Van der Veen. 1994. Location of antibody epitopes within the mouse hepatitis virus nucleocapsid protein. *Virology 202:146.*
45. Mougneau, E., F. Altare, A. E. Wakil, S. Zheng, T. Coppola, Z. Wang, R. Waldmann, R. M. Locksley, and N. Glaichenhaus. 1995. Expression cloning of a protective *Leishmania* antigen. *Science 268:563.*
46. Bretscher, P. A., G. Wei, J. N. Menon, and H. Bielefeldt-Ohmann. 1992. Establishment of stable, cell-mediated immunity that makes "susceptible" mice resistant to *Leishmania major*. *Science 257:539.*
47. Corry, D. B., S. L. Reiner, P. S. Linsley, and R. M. Locksley. 1994. Differential effects of blockade of CD28-B7 on the development of Th1 or Th2 effector cells in experimental leishmaniasis. *J. Immunol. 153:4142.*
48. Hsieh, C., S. E. Macatonia, A. O'Garra, and K. M. Murphy. 1995. T cell genetic background determines default T helper phenotype development in vitro. *J. Exp. Med. 181:713.*