Suppression of Immune Response Induction in Peyer's Patch Lymphoid Cells from Mice Infected with Mouse Hepatitis Virus

DONALD B. CASEBOLT,[1] DAVID M. SPALDING, TRENTON R. SCHOEB, AND J. RUSSELL LINDSEY

Department of Comparative Medicine, Division of Rheumatology and Clinical Immunology, Department of Medicine, and Veteran's Administration Medical Center, University of Alabama at Birmingham, Birmingham, Alabama 35294

Multiple previous studies have demonstrated significant alterations of immunologic parameters associated with mouse hepatitis virus (MHV) infection, but effects of the virus on mucosal lymphoid cells have not been examined. Coincident with a natural outbreak of MHV at our institution, we noted alterations in immunoglobulin secretion by mature Peyer's patch B cells under an inductive stimulus provided by dendritic cells and mitogen-activated T cells (DC-T). MHV was isolated from mice affected during the outbreak, and experimental infection of mice with the isolate consistently resulted in failures of immunoglobulin secretion by cocultures of Peyer's patch DC-T and B cells. In subsequent experiments, MHV appeared to negatively affect DC-T more than B cells. Therefore, the effects of inapparent MHV infection on experimental mucosal immune responses can result from natural infection and can be experimentally reproduced. © 1987 Academic Press, Inc.

INTRODUCTION

Mouse hepatitis virus (MHV) is an aggregate term for a group of coronaviruses with a range of antigens, tissue tropisms, and pathogenic potential (1). Natural MHV infection recently has been reported to occur in over 70% of mouse populations in the United States (2, 3) and Canada (4). Multiple studies have demonstrated alterations in immunologic parameters associated with MHV infection of mice (5–15). However, the interactions between MHV and the mouse immune system are not well understood, and the effects of the virus on mucosal lymphoid cells have not been studied previously. Because mucosal cells of the upper respiratory and intestinal tracts are primary sites of MHV infection (1), it is likely that encounters with lymphoid tissue at these sites are important early events in natural MHV infection.

Our laboratory has studied mucosal immune responses using a novel system for the induction of immunoglobulin synthesis and secretion. This system is dependent on an inductive stimulus provided by mixtures of dendritic cells and mitogen-activated T cells. Cocultures of Peyer's patch (PP) dendritic cells and PP T cells with B-

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[1] To whom correspondence should be addressed at Department of Comparative Medicine, University of Alabama at Birmingham, Birmingham, AL 35294.

lineage cells results in the induction of synthesis and secretion of immunoglobulin by mature B cells (16), pre-B cells (17), and pre-pre-B cells (18). During studies of induction of mature B cells by DC-T from PP, alterations in immunoglobulin secretion were noted to coincide with a natural outbreak of MHV at our institution. This observation prompted us to investigate the potential effects of MHV on the mucosal immune response using a field isolate of virus obtained from these infected mice.

MATERIALS AND METHODS

Natural infection of mice. C3H/HeJ mice affected during the outbreak and found to be infected with MHV by serologic testing were housed in a Trexler-type flexible film isolator serviced by gnotobiotic techniques. Athymic BALB/cAnNCr-*nu/nu* mice (Frederick Cancer Research Facility, Frederick, MD) were placed in the isolator and co-caged with the infected mice. Eighteen to 141 days later, athymic mice with signs of weight loss and lethargy were euthanatized by sodium pentobarbital overdosage and exsanguinated. Liver sections were collected aseptically, placed in sterile tubes, and stored at −70°C.

Virology. Liver sections from infected athymic mice were homogenized in glass tissue grinders with 2 ml minimal essential medium containing 6 g/liter Hepes buffer (MEMH), 10% fetal bovine serum (FBS), and 70 mg/ml Cefobid (Roerig, New York, NY). Homogenates were centrifuged at 400g for 10 min, and 1 ml of the supernatants was inoculated onto monolayers of BALB 3T3 17C11 cells (19) with MEMH and 3% FBS. After 18 hr of incubation at 37°C and ambient humidity and CO_2 levels, monolayers were examined for cytopathic effects consisting of plaques containing multinucleate syncytial cells.

Virus isolated from a single liver sample was cloned according to a modified agar overlay method (20, 21). Briefly, virus stock was serially diluted on monolayers of 17C11 cells grown on 24-well plates and incubated for 24 hr in MEMH containing 3% FBS and 0.5% agarose. Wells at limiting dilutions contained single, discrete plaques which were removed with a sterile Pasteur pipet and inoculated onto monolayers of 17C11 cells. This procedure was repeated two times, followed by two passages on 17C11 cells with MEMH and 3% FBS to allow for amplification of virus titers. The resulting stock virus was stored at −70°C and was used for all inoculations described below.

The isolate was serially diluted in 24-well plates and incubated in MEMH containing 0.5% agarose as described for cloning. At limiting dilutions, individual plaques were counted and virus titers calculated as mean plaque-forming units per milliliter (22, 23).

Histology. Tissues for histologic examination were fixed in 10% Formalin:70% ethanol:20% distilled water, embedded in paraffin, sectioned 5 μm thick, and stained with hematoxylin and eosin. The organs examined included stomach, intestines, liver, spleen, kidneys, nasal passages, trachea, lungs, heart, brain, and skin.

Serology. Serum samples were tested by enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA) for antibodies to mouse hepatitis virus, minute virus of mice, pneumonia virus of mice, and reovirus 3; by ELISA for Sendai virus, mouse adenovirus, ectromelia virus, lymphocytic choriomeningitis virus, and mouse rotavirus; and by hemagglutination inhibition for polyoma virus (Charles River Biotechnical Services, Wilmington, MA).

Experimental inoculations. To determine whether a pure field isolate of MHV was obtained, a mouse antibody production test was performed. Euthymic BALB/cAn-NCr mice (Frederick Cancer Research Facility, Frederick, MD) were housed in poly-carbonate microisolator cages (24, 25) with all manipulations performed inside a class II laminar flow biological cabinet. Mice were inoculated with 4×10^3 tissue culture infectious units of MHV, half given intraperitoneally and half intranasally. Mice were euthanatized at 1, 2, 3, or 4 weeks after inoculation for histopathologic examination and serologic testing as described above.

Animals for collection of Peyer's patch cells were C3H/HeJ mice (Jackson Laboratory, Bar Harbor, ME) housed in microisolator cages (24, 25). At 7 weeks of age, mice were inoculated with MHV at the same dose and route of administration as described above. Control mice were sham inoculated with 200 µl sterile MEMH with 3% FBS.

Preparation of PP cells enriched in DC and Lyt-1$^+$ T cells. Peyer's patch DC-T were prepared as described previously (16). Briefly, B cells were removed from PP cell suspensions by panning (26). The remaining cells were treated with sodium metaper-iodate, washed twice in RPMI 1640, suspended in complete medium (RPMI 1640 with addition of 5% FBS, 20 µg/ml gentamicin, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, and 1 µg/ml indomethacin), and cultured at 10×10^6 cells/750 µl in 24-well plates. Cell clusters formed after 16 hr of incubation were separated on a continuous density gradient of bovine serum albumin (BSA). Clustered cells were cultured for an additional 72 hr, followed by separation of a low-density population on a discontinuous BSA gradient.

B-cell preparation. Peyer's patch B-cell populations were obtained from infected and control mice by panning (26) enzyme-digested PP cells on petri dishes coated with polyvalent goat anti-mouse immunoglobulin (Southern Biotechnology, Birmingham, AL).

Cocultivation of low-density cluster-derived cells and B cells. Low-density cluster-derived cells (DC-T) were cultured (1.25×10^5 cells in 0.5 ml) with B cells (1×10^6 cells in 0.5 ml) in complete medium with addition of 2 µg/ml concanavalin A in 24-well plates at 37°C in Mishell–Dutton gas mixture. At 7 days, culture supernatants were harvested and assayed by isotype-specific radioimmunoassays for IgA, IgG, and IgM (27).

RESULTS

Experimental Infections

One week after inoculation for the mouse antibody production test, BALB/c mice had multiple 1- to 2-mm pale foci on the liver surface. Histologic lesions consisted of mild multifocal necrotizing hepatitis and syncytial epithelial giant cells in the cecal mucosa. These lesions are consistent with acute MHV infection (1) and were absent in mice examined 2, 3, and 4 weeks after inoculation. No lesions were noted in any of the other organs examined. Serum samples taken 1 through 4 weeks after inoculation were positive for MHV by ELISA and IFA testing. The absence of other positive serologic test results or histologic lesions indicated that a pure field isolate of MHV was obtained.

In MHV-infected C3H/HeJ mice, there was mild edema of Peyer's patches noted at 1 week after inoculation. However, infected and control mice yielded comparable
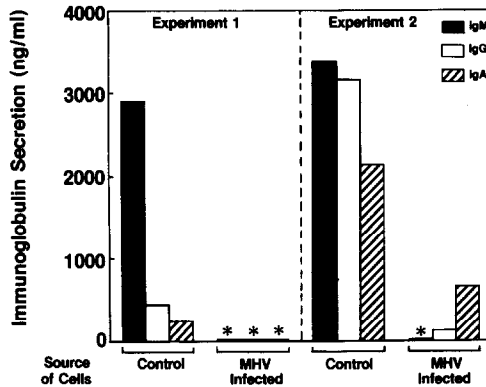
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FIG. 1. Suppression of immunoglobulin secretion in cultures of PP cells obtained from MHV-infected mice. Cocultures of DC-T and B cells obtained from MHV-infected mice were compared with cocultures of cells obtained from control mice. DC-T (1.25×10^5) were cultured with 1×10^6 B cells in 24-well plates. Supernatants were harvested at 7 days and assayed by isotype-specific radioimmunoassay (RIA). Values for nanograms of secreted immunoglobulin per milliliter are means of two to three replicate wells with backgrounds for DC-T and B cells alone subtracted. *, <12.5 ng/ml.

numbers of PP cells after enzymatic digestion, and cells from the two groups did not differ significantly in their viability in culture.

Immunoglobulin Secretion by Mixtures of PP DC-T and B Cells

We first examined immunoglobulin secretion by cultures of DC, Lyt 1^+ T cells, and B cells obtained from either MHV infected or uninfected mice (Fig. 1). In Experiment 1, cells obtained from MHV-infected mice did not secrete detectable immunoglobulin of any isotype. In the second experiment, cells from MHV-infected mice secreted some IgG and IgA, but in amounts 30- and 3-fold less, respectively, than control cultures. Isolated DC-T secreted small amounts of immunoglobulin (generally less than 15% of concentrations reached when cultured with B cells from uninfected mice). Isolated B cells secreted immunoglobulin concentrations less than 10% of those reached when cultured with control DC-T. These data demonstrate dramatic alterations in immunoglobulin secretion by mucosal lymphoid cells 1 week after experimental MHV infection.

We next sought to determine whether the alteration in immunoglobulin secretion was secondary to direct inhibition of B-cell function or indirect through alteration of the inductive ability of DC-T. Experiments were performed using all four combinations of infected and uninfected cells. Data for immunoglobulin secretion are shown in Figs. 2 (IgM), 3 (IgG), and 4 (IgA). Immunoglobulin secretion was generally lower in these crossed experiments. Although responses varied somewhat, MHV-infected DC-T, cultured with either infected or control B cells, generally secreted undetectable or very low concentrations of immunoglobulin of all three isotypes when compared with paired cultures of control DC-T for each experiment. However, infected B cells often produced significant immunoglobulin when cultured with control DC-T. In two cases (IgG in Experiment 3 and IgA in Experiment 4) infected B cells were induced to higher levels than paired control B cells, indicating that MHV infection did not impair B-cell function as consistently as it did DC-T function.
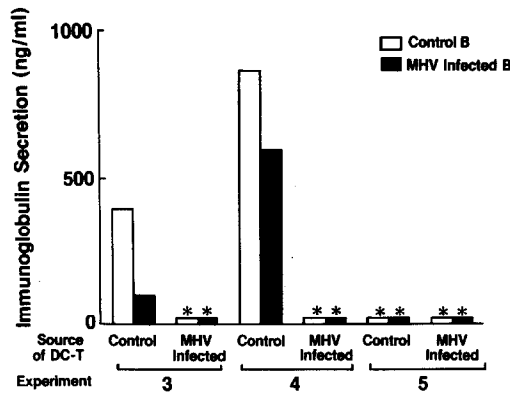
FIG. 2. Induction of IgM secretion by different combinations of DC-T and B cells. DC-T and B cells obtained from both MHV-infected and control mice were cocultivated in all combinations: control DC-T with control B; control DC-T with infected B; infected DC-T with control B; or infected DC-T with infected B. DC-T (1.25×10^5) were cultured with 1×10^6 B cells in 24-well plates. Supernatants were harvested at 7 days and assayed by isotype-specific RIA. Values for nanograms of secreted immunoglobulin per milliliter are means of two to three replicate wells with backgrounds for DC-T and B cells alone subtracted. *, <12.5 ng/ml.

In a single experiment, mice were inoculated with the isolate 3 weeks before PP collection. Immunoglobulin secretion (ng/ml) in cocultures from control mice was 200 IgM, 1780 IgG, and 780 IgA versus 160, 1340, and 490, respectively, for cocultures from MHV-infected mice. Therefore, pronounced suppression of immunoglobulin secretion did not occur in mice 3 weeks following MHV inoculation.

DISCUSSION

The results of this study clearly demonstrate that polyclonal immunoglobulin secretion by cultures of PP lymphoid cells is effectively abrogated when the cultured cells originate from MHV-infected mice. The mechanism of these alterations is not known, but in crossed experiments, the inductive stimulus provided by DC and
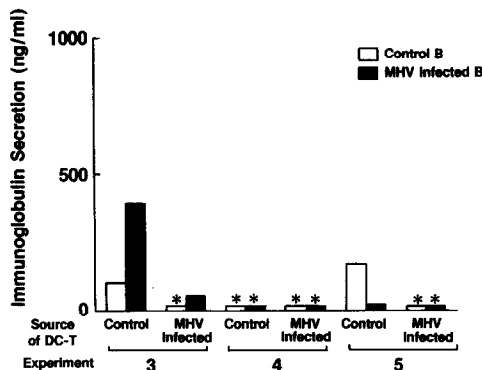

FIG. 3. Induction of IgG secretion by different combinations of DC-T and B cells. Conditions were identical to those in Fig. 2. *, <12.5 ng/ml.
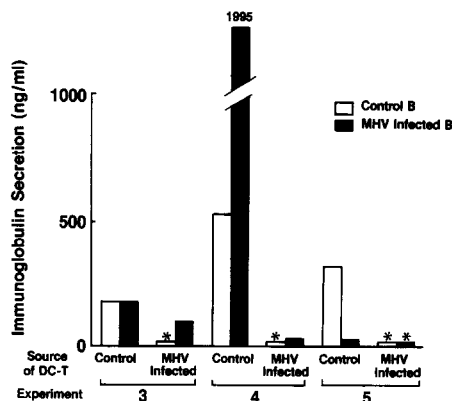
FIG. 4. Induction of IgA secretion by different combinations of DC-T and B cells. Conditions were identical to those in Fig. 2. *, <12.5 ng/ml.

helper–inducer T cells was consistently absent in MHV infection, whereas infected B cells could be induced to immunoglobulin production by DC-T from control uninfected mice.

Macrophages are permissive sites of MHV replication (28–30) and the number of peritoneal macrophages increases during MHV infection (12, 15). MHV infection can also cause functional alterations in macrophages, such as enhanced phagocytic activity (12) and altered ectoenzyme levels (15). Mixed-lymphocyte cultures produce interferon or other soluble factors which either protect macrophages against MHV infection (9) or convert genetically resistant macrophages to an MHV-susceptible phenotype (31). Accessory cells such as PP dendritic cells also may be sites of MHV replication. The relative importance of MHV replication in PP DC and Lyt 1^+ T cells, pathologic alteration of these cells, and production of soluble factors deserve further study.

The effects of MHV on this experimental system appeared to be limited to 1 week after MHV inoculation. In a study of MHV effects on macrophages, ectoenzyme levels were altered only during the period up to 1 week after infection (15). Subacute or chronic MHV infection may result in no changes or in changes in various systems different from those occurring in acute infection. In addition, differences in virus strain and host genotype could affect these responses. Further experiments will be required to address these complex issues.

These experiments duplicated, with a field isolate of virus obtained from affected mice, failures of immunoglobulin induction that occurred during a natural MHV outbreak. Therefore, results of mucosal immunity studies in mice can be altered by inapparent MHV infection. In light of reported effects of MHV on macrophages, it is reasonable that other immune system functions could be affected as well. For these reasons, investigators using mice for studies of mucosal immunity and other immunologic research should endeavor to obtain and maintain mice free of MHV infection.

ACKNOWLEDGMENTS

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