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Effect of Inapparent Murine Hepatitis Virus Infections on Macrophages and Host Resistance

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Inapparent infections of mice with murine hepatitis virus (MHV) altered host resistance to experimental infection with a second virus, encephalomyocarditis virus (EMC), reduced the protective effects of exogeneously administered interferon against EMC infections, and it altered macrophage ectoenzyme phenotypes in two macrophage populations. Resident peritoneal macrophages from mice experimentally infected with one of two strains of MHV also demonstrated altered ectoenzyme phenotypes. These data demonstrate that inapparent infections with MHV alter several host resistance and macrophage parameters and directly demonstrate that effects of inapparent MHV infection on macrophage parameters can be reproduced experimentally.

Key words: viral infections, mononuclear phagocytes, ectoenzymes, bone marrow derived macrophages

INTRODUCTION

Inapparent viral infections of laboratory rodents have been reported to have a pronounced impact on various experimental results [4,6,9,10,11,20]. Nevertheless, although the immunomodulatory effects of many viruses are well established, the potential effects of inapparent viral infections on immunologic and host resistance studies are not fully appreciated. In the present study, we document that inapparent natural infection of mice with murine hepatitis virus (MHV), a ubiquitous group of coronaviruses, alters host resistance to a second virus infection and changes macrophage (MO) biochemical parameters. We further substantiate these findings by demonstrating phenotypic changes in murine peritoneal MO after experimental infection of mice with MHV.

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MATERIALS AND METHODS

Mice

The effects of inapparent infections with MHV were documented in female CD-1 (Charles River Breeding Labs, Kingston, NY) and B6C3 F1 (Simonsen Labs, Gilmore, CA). Specific pathogen-free mice were monitored routinely, including on the date of arrival, for seroconversion to MHV and Sendai viruses (Elisa method-Biocon Inc., Rockville, MD). Experimental infections of MHV were initiated in specific pathogen-free 4-wk-old female BALB/cByJ mice (Jackson Labs, Bar Harbor, ME). All mice were maintained on a 12-hr light/dark cycle, provided food and water ad libitum, and cared for according to National Institutes of Health (NIH) guidelines. Infected and control BALB/cByJ mice were housed in separate facilities within micro-isolator cages (Lab Products, Maywood, NJ), which were opened only in a class II biological safety cabinet.

Microbial Infections

Groups of seven to eight female CD-1 mice were injected intraperitoneally with one of four dilutions of encephalomycarditis virus (EMC) (ATCC strain VR129). Mortality was monitored daily, and the LD_{50} was calculated from the overall survival data by the Reed-Muench procedure [16]. BALB/cByJ mice were infected experimentally with one of two strains of MHV (MHV-RI or MHV-JHM). The low passage, enterotropic MHV-RI strain was inoculated orally, and the pantropic MHV-JHM strain was inoculated intranasally. Each mouse received 10^4 $TCID_{50}$. Successful infections were monitored by indirect immunofluorescence serology [5].

Preparation of Peritoneal MO

Resident peritoneal cells were obtained by lavage of the peritoneal cavity with 5 ml of heparinized (2 U/ml, Abbott Labs, Chicago, IL) phosphate-buffered saline, pH 7.2). The number of cells was determined by mechanical counting with a ZBM Coulter counter (Coulter Instruments, Hialeah, FL), and differential cell counts were determined by visual examination of Dif-Quik stained cytocentrifuge preparations of peritoneal cells. Resident MO were isolated by adherence for 2 hr as previously described [15].

Preparation of Bone Marrow Derived MO (BMDMO)

Bone marrow cells isolated were cultured for up to 7 days in alpha-minimal essential medium containing 10% fetal bovine serum, 10% horse serum, and 10% L-929 cell conditioned medium as a source of colony stimulating factor as previously described [21]. All assays were performed on the resulting adherent BMDMO population. The number of adherent cells was determined by counting the number of nuclei after lysis of the cells with 3% cetrimide as reported by Stewart [21].

Ectoenzyme Determination

Adherent cells were lysed with 0.05% Triton X-100 and aliquots of the solubilized cell membranes were analyzed for protein content, 5′ nucleotidase (5′N) activity, and alkaline phosphodiesterase-I (APD-I) activity. Protein content was assayed according to the BioRad procedure (Bio-Rad Labs, Rockville Center, NY). 5′N activity was determined by measuring the hydrolysis of ^{3}H-adenosine monophosphate, and

TABLE 1. Effect of Inapparent MHV Infection on 5′ Nucleotidase Activity in BMDMO

Cells	MHV	5′N (SA/10^6 cells)[ab]
5-day BMDMO	+	0.07 ± 0.01
	−	0.73 ± 0.16[c]
7-day BMDMO	+	0.05 ± 0.0
	−	0.06 ± 0.03

[a]5′N activity is expressed as specific activity (nmoles produced/min/10 cells). The specific activity was expressed per 10^6 cells because of variations in protein content among cells at different days in culture.
[b]Represents the mean ± 1 SD from 2–4 experiments.
[c]$p < 0.05$ as compared with uninfected 5-day samples by Student's t-test.

APD-I activity was determined by measuring the conversion of p-nitrophenyl thymidine-5′-monophosphate substrate (Sigma 104, Sigma Labs, St. Louis, MO) to p-nitrophenol as previously described [7,8,15].

RESULTS

Effect of Inapparent MHV Infections on Resistance to EMC Infection

During a 12-month period, we observed that mice that had seroconverted to MHV were at least tenfold more resistant to lethal infection with EMC virus than were MHV-free mice. In four experiments with mice that did not seroconvert, the $\log_{10}LD_{50}$ was 7.7 ± 0.2, while the mean $\log_{10}LD_{50}$ in two experiments in which mice seroconverted was 6.4 ± 0.6. Moreover, treatment with exogenous interferon (10,000 units daily for 6 days with either murine β (gift of Dr. D.M. Murasko) or recombinant human α A/D (courtesy of Hoffman LaRoche, Nutley, NJ) was only marginally protective in MHV-infected mice. In contrast, there was significant protection in uninfected mice; mice treated with mock interferon showed a median survival of 4.0 days, and mice treated with a or B interferon had an increase to more than 13.5 days.

Effect of Inapparent MHV Infections on MO

In separate experiments, it was observed that inapparent MHV infections also altered several MO parameters in specific pathogen-free B6C3 F1 female mice (Simonsen Labs, Gilmore, CA). Infection with MHV before isolation of bone marrow for culturing of stem cells to obtain bone marrow derived macrophages transiently altered the ectoenzyme phenotype expressed by BMDMO (Table 1). The 5′N specific activity was significantly depressed in 5-day cultured BMDMO obtained from MHV-infected mice (Table 1). 5′N activity generally peaks on day 5 of culture and drops dramatically by day 7 of culture (unpublished observations). No differences were detectable in the ectoenzyme levels in 7-day BMDMO obtained from MHV-free or MHV-infected mice.

The peritoneal MO compartment was also affected by MHV infection. Mice that had seroconverted to MHV demonstrated higher total peritoneal cell numbers than did uninfected mice (Table 2). The increase in cell number was reflected as increases in both the macrophage and lymphocyte compartments. Sporadic changes were also noted in the ectoenzyme phenotype of resident peritoneal MO.

TABLE 2. Effect of Inapparent MHV Infections on Peritoneal Cell Numbers

Cell population	Cell number × 10^6/mouse[a]	
	Infected	Uninfected
Total	8.09 ± 0.72[b]	5.80 ± 0.45
Lymphocyte	1.94 ± 0.28[c]	1.21 ± 0.15
Macrophages	5.84 ± 0.55[c]	4.34 ± 0.33

[a]Represents the mean ± 1 SEM from 2-3 experiments; n (infected) = 11, n (uninfected) = 23.
[b]$p < 0.005$ as compared with uninfected population by Student's t-test.
[c]$p < 0.01$ as compared with uninfected population by Student's t-test.

TABLE 3. Effect of Experimental Infections With Two MHV Strains on Ectoenzyme Phenotype of Resident Peritoneal MO

Day after infection	MHV-RI[a]		MHV-JHM[a]	
	5′N[b]	APD-I[b]	5′N[b]	APD-I[b]
3	20.3 ± 3.6	15.6 ± 1.6[d]	19.8 ± 8.8	14.1 ± 0.8[d]
5	24.6 ± 5.8	17.7 ± 0.9	13.3 ± 1.5[e]	10.0 ± 0.6[e]
7-8	16.8 ± 2.2	19.9 ± 1.1	10.9 ± 1.2[e]	20.8 ± 1.4
10	20.7 ± 2.4	23.9 ± 0.7	1.1 ± 1.0[e]	18.0 ± 1.5
Control[c]	18.2 ± 1.5	20.4 ± 0.7	21.5 ± 1.7	20.4 ± 1.3

[a]Balb/cByJ mice (5/group) were infected with either the RI strain or the JHM strain of murine hepatitis virus.
[b]Ectoenzyme levels are expressed as the specific activity per milligram protein. Protein levels do not vary significantly among resident peritoneal MO populations. Data are presented as the mean ± 1 SEM from two experiments.
[c]Resident peritoneal MO from uninfected mice.
[d]Represents a significant suppression as compared with uninfected controls; $p < 0.05$ by Student's t-test.
[e]Represents a significant suppression as compared with uninfected controls; $p < 0.01$ by Student's t-test.

Alteration of Ectoenzyme Phenotype of Peritoneal MO by Experimental MHV Infections

These data clearly demonstrate that inapparent or subclinical infection of mice with MHV can alter host resistance and phenotypic MO parameters. The sporadic and transient changes noted in the ectoenzyme patterns of two distinct MO populations were of particular interest and concern to us in interpretation of studies with immunomodulators. As cellular markers, the ectoenzymes, 5′N, and alkaline phosphodiesterase-I (APD-I), often reflect functional or activational changes occurring within an MO population [15]. Changes occurring owing to inapparent MHV infection lead to obvious problems in interpreting effects of immunomodulators that may activate MO. To determine directly if MHV infections could alter MO ectoenzymes, BALB/cByJ mice were experimentally infected with one of two strains of MHV (MHV-RI or MHV-JHM). BALB/cByJ mice infected with MHV-RI [3] exhibited no alteration in 5′N, but they showed a transient suppression in APD-I activity (Table 3). In contrast, peritoneal MO from mice infected with the pantropic MHV-JHM strain

exhibited decreased 5'N activity as the infection progressed. APD-I activity was suppressed early in the infection but returned to normal after a week (Table 3). These data emphasize two important factors in inapparent MHV infections. Effects on experimental results may be transient in nature, and results may differ with the infecting virus strain. We have also observed that there are differences among mouse strains (unpublished observations).

DISCUSSION

The present data provide additional evidence that inapparent, sporadic infections of mouse colonies with MHV can alter experimental results, particularly in immunological studies. In this regard, Li et al [13] recently demonstrated that the therapeutic effect of cyclophosphamide as an antitumor drug was augmented in MHV-infected B6D2F1 mice. Moreover, experimental error in animal survival time after transplantation with the P388 leukemia increased in MHV-infected mice thereby masking the therapeutic activity of combined treatment with cyclophosphamide and the immunomodulator, pyrimidinone. Effects of MHV infection on melanoma tumor and Moloney murine leukemia virus growth have also been noted [1,14]. Murasko (D.M. Murasko, personal communication) has observed that natural killer cell activity in aged mice could not be induced by poly I-C or interferon treatments after the mice had seroconverted to MHV. We have also found that splenic lymphocyte mitogen responses were markedly suppressed in BALB/cByJ mice after infection with the MHV-JHM strain and sporadically suppressed after infection with the MHV-RI strain (Smith A.L., unpublished observations). In contrast to these immunosuppressive effects, nude mice reportedly developed some T cell markers and produced IgG and secondary responses to immunization with sheep red blood cells [17,22].

MO parameters appear to be particularly sensitive to MHV infections. This sensitivity may result from the reported permissiveness of some macrophage populations for MHV infection [2,19]. The data presented here and by Boorman et al [4] indicate that the number of resident peritoneal MO increases after MHV infections. While Boorman et al [4] reported that resident peritoneal MO from infected mice exhibited increased cytostasis, we noticed no difference in macrophage cytostatic/cytolytic activity (unpublished observations). However, transient and sporadic differences in ectoenzyme levels, which are MO activation markers, did occur in two MO populations (peritoneal and BMDMO) in mice after inapparent infection with MHV. Moreover, permanent and transient changes were observed in 5'N and APD-I levels, respectively, in peritoneal MO from mice after experimental infection with the MHV-JHM strain. Enhanced phagocytic activity has also been reported for MO from MHV-infected nude mice [23], and Badger (A. Badger, personal communication) has observed altered MO oxidative metabolism after MHV infection. In addition, Kenyon [12] has reported delayed wound healing and reduced wound tensile strength, both of which are macrophage dependent, in MHV-infected mice.

In addition to changes reported here in host resistance to systemic viral infection, MHV infections can alter susceptibility to other respiratory viruses. Carrano et al [5] recently reported that pre-infection of mice with MHV increased resistance to lethal Sendai virus infections in normally susceptible genotypes and interfered with replication of pneumonia virus of mice in respiratory tract tissues. In the present study, inapparent infections with MHV increased natural resistance to EMC virus.

Concomitantly, administration of exogenous interferon did not confer additional protection against EMC in MHV-infected mice. Since some MHV infections induce interferon [18,24], it is likely that the increased natural resistance to EMC virus in our studies was due to endogenous interferon levels; the normally protective effects of exogenous interferon were thereby masked by the induced endogenous interferon levels.

In summary, the present data demonstrate that inapparent infections with MHV can alter host resistance to another virus infection, can alter experimental results with immunomodulators, and can result in measurable differences in various MO parameters. Moreover, these data are the first that demonstrate directly that effects of inapparent MHV infections on MO parameters can be reproduced by experimental MHV infection. Therefore, the potential effects that inapparent viral infections, particularly MHV infections, can have on immunologic research are multitudinous. These effects become increasingly important as experiments and experimental techniques become more sophisticated and subtle immunoregulatory changes are measured.

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