

INDUCTION OF MONOCYTE PROCOAGULANT ACTIVITY BY MURINE HEPATITIS VIRUS TYPE 3 PARALLELS DISEASE SUSCEPTIBILITY IN MICE*

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Macrophages play a central role in the pathogenesis of many chronic inflammatory lesions (1). By virtue of their functions, strategic location, and wide tissue distribution, these cells can determine susceptibility and resistance to infection. It appears that macrophages can delay and prevent the spread of infection to susceptible cells. However, where infectious agents with cytopathic properties can replicate freely within macrophages, widespread destruction of tissues may follow. Furthermore, infected circulating macrophages can disseminate infection by migration through the vascular compartment, with distribution of an infectious agent to susceptible organs. Inflammatory lesions may also result from a series of events starting with the direct interaction of infectious agents with the macrophage. This results in the production of monokines, which stimulate T cells to release lymphokines, which stimulate the macrophage bearing the infectious agent, e.g., *listeria monocytogenes* (2, 3). Macrophages, in addition to their phagocyte functions, can secrete substantial quantities of hydrolytic enzymes as well as other biologically active products in response to inflammatory stimuli. Such products may contribute to the pathogenetic process (4).

A second host defense system that may be activated in response to infection is the coagulation system. The consumption of proteins of the coagulation pathways coupled with the local or disseminated intravascular formation or deposition of fibrin is of diagnostic significance in a number of lesions, including acute proliferative glomerulonephritis (5), Henoch-Schonlein purpura (6), hyperacute renal allograft rejection (7), acute tubular necrosis (8), lupus erythematosus (9), and the Shwartzmann reaction (10). The perivascular deposition of fibrin is one of the earliest features of experimental allergic encephalomyelitis (11). Activation of the coagulation system is frequently associated with active rheumatoid arthritis and other connective tissue diseases (12).

Cells of the lymphoreticular system possess low basal procoagulant activity of a poorly defined character; however, significant procoagulant activity (PCA)¹ can be

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¹ *Abbreviations used in this paper:* DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; LD₅₀, lethal dose - 50%; LPS, lipopolysaccharide; MHV-3, murine hepatitis virus type 3; MOI, multiplicity of infection; PBM, peripheral blood mononuclear cells; PCA, procoagulant activity; PFU, plaque-forming units.

induced by a variety of stimuli, including lipopolysaccharides (LPS), antigen-antibody complexes, and lectins (13-20). We have recently established (14, 16) that the monocyte and macrophage represent the cellular sources of LPS and antigen:antibody induced PCA and that lymphocyte/macrophage collaboration is a necessary requirement for the full induction of PCA response. Current evidence suggests this is an immune response that functions via unidirectional lymphocyte induction of procoagulant monokines.

The murine hepatitis viruses (MHV) are a group of serologically related pathogenic coronaviruses (21, 22). Infection of mice by MHV may produce a broad spectrum of diseases, including encephalitis, hepatitis, interstitial pneumonitis, nephritis, and enteritis, depending on the serotype of MHV, the age and strain of the mouse, and the route of infection (23). Murine hepatitis virus type 3 (MHV-3) is a primarily hepatotropic serotype and causes hepatitis when administered intraperitoneally, intracerebrally, intravenously, or orally in susceptible strains of mice (24, 25). Balb/c and DBA/2 mice develop acute fulminant hepatitis, C3H mice develop a mild chronic disease, and mice of the A strain develop no evidence of hepatitis (26). The mechanism of resistance to MHV infection in animals is both complex and poorly understood. It has been demonstrated that resistance to fatal MHV infection may be associated with a resistance gene that inhibits MHV replication in macrophages and is also influenced by a susceptibility factor that is related to lymphocytes (26-30).

In this study, we demonstrate that the induction by lymphocytes of monocyte PCA in response to MHV-3 infection reflects the susceptibility of the host to infection by MHV-3. We further indicate that there exists a genetic restriction in the MHV-3-induced pathway of lymphoreticular PCA. This is characterized by the lack of generation of PCA in the resistant animal, modest induction in mice that develop mild and chronic disease, and a profound PCA response by the fully susceptible animal who develops fatal hepatitis. The induction of lymphoreticular PCA antedates viral replication and appearance of histologic lesions in susceptible organs. Furthermore, we demonstrate that the monocyte is the cellular source of this activity but cellular collaboration between lymphocytes and monocytes is required for the generation of the PCA response.

Materials and Methods

Cells. The origin and growth of 17 CL1, DBT, and L2 cells has been described previously (31-33). Cells were routinely propagated in Dulbecco's modified Eagle's medium (DMEM) (Flow Laboratories Inc., Rockville, Md.) supplemented with 10% newborn calf serum (Biocell Laboratories, Carson City, Calif.) and 25 μ g/ml chlortetracycline hydrochloride grade II (Sigma Chemical Co., St. Louis, Mo.) and buffered with 15 mM HEPES, 3-[*N*-morpholino]-propanesulfonic acid, *N*-tris [hydroxymethyl] 3 methyl-2 aminoethane sulfonic acid, and 4 mM glutamine (Sigma Chemical Co.).

Peripheral blood mononuclear cells (PBM) were isolated from mice as previously described (14). The cells were separated over Ficoll-Hypaque (density, 1.074 g/ml) at 22°C for 12 min at 1,400 *g* and were recovered at the interface. The cells were adjusted to 1×10^6 PBM/ml. Lymphocytes and monocytes were separated by adherence to plastic, as previously described (14). The recovery of cells was >86% and viability was >98%, as evidenced by trypan blue exclusion. Lymphocytes were defined by failure of adherence, morphology, and failure of uptake of neutral red and were <2% esterase positive. Monocytes were defined by morphology and uptake of neutral red and were >96% esterase positive, as previously described (14).

Mice. C3H/St, A/J, and BALB/c mice (Research Institute of Scripps Clinic breeding

colony) were used at 4–6 wk of age. After infection with MHV-3, they were maintained in strict isolation.

Virus. MHV-3 was obtained from the American Type Culture Collection, Rockville, Md. (ATCC VR-262), and the lyophilized infected liver homogenate was reconstituted with 1 ml sterile phosphate-buffered saline (PBS), pH 7.4. The virus was plaque purified twice on monolayers of DBT cells. The virus was subsequently passaged once in DBT cells to prepare a seed stock. A working stock of virus was grown in 17 CL1 cells infected at a multiplicity of infection (MOI) of 10^{-4} plaque-forming units (PFU)/cell. This stock virus had a titer of 1.2×10^7 PFU/ml and was used for all subsequent experiments. Virus was assayed on monolayers of L2 cells in a standard plaque assay, as previously described (31–34). Lethal dose—50% (LD_{50}) was determined in BALB/c, C3H/St, and A/J mice and was calculated according to the method of Reed and Muench (35), using an endpoint of 10 d.

PCA Assay. PCA was determined in a one-stage recalcified clotting assay, as previously described (16). Samples of frozen-thawed and sonicated cells in RPMI 1640 were assayed for the capacity to shorten the spontaneous clotting of human plasma. The assay consisted of 50 μ l of test sample, 50 μ l of 25 mM $CaCl_2$, and 50 μ l of citrated normal-fasting platelet-poor human plasma. Units were assigned from a log-log plot and standardized with dilutions of rabbit brain thromboplastin (Dade Div., American Hospital Supply Corp., Miami, Fla.), which at 36 μ g/ml was assigned a value of 100,000 mU. There was no activity present in DMEM, RPMI 1640, PBS, complete media, or the virus preparations.

Endotoxin Contamination. DMEM, RPMI 1640, PBS, and virus preparations were assayed for endotoxin contamination by standard limulus assay (*E. toxate*; Sigma Chemical Co.) and contained <0.1 ng/ml of endotoxin in the lower limits of this test (36, 37).

Results

Resistance of Mice to Lethal Infection with MHV-3. 28 BALB/c mice were infected in groups of seven with 1, 10, 100, or 1,000 PFU of MHV-3 by intraperitoneal injection and were observed for 10 d (Table I). Using the method of Reed and Muench (35), an LD_{50} for BALB/c of $<1-5$ PFU was established. In similar experiments, the LD_{50} for C3H/St was $\sim 10^6$ PFU, and A/J mice were resistant to $>10^7$ PFU.

MHV-3 Replication In Vitro. The one-step growth curve of MHV-3 in 17 CL1 cells was determined after inoculation of cultures at an MOI of 0.1 PFU/cell, and the virus titers in replicate cultures were determined at various subsequent time intervals (Fig. 1). MHV-3 had an eclipse phase of 4- to 5 h, reaching a maximum virus titer at 10 h postinfection.

One-step growth curves were then determined in monocytes harvested from the peripheral blood of A/J, BALB/c and C3H/St mice. For these experiments mice were killed, and PBM were isolated aseptically over Ficoll-Hypaque. Monocytes were separated from lymphocytes by adherence to plastic for 48 h and were $>92\%$ esterase positive (38). Monocytes at 3×10^5 were seeded in 24-well cluster plates (Costar Data

TABLE I
LD₅₀ of MHV-3 for BALB/c Mice

Group*	Dose MHV-3‡ PFU	Mortality %
1	1	71
2	10	67
3	100	84
4	1,000	100

* Each group contained seven mice.

‡ MHV-3 given in 100 μ l of PBS by intraperitoneal injection.

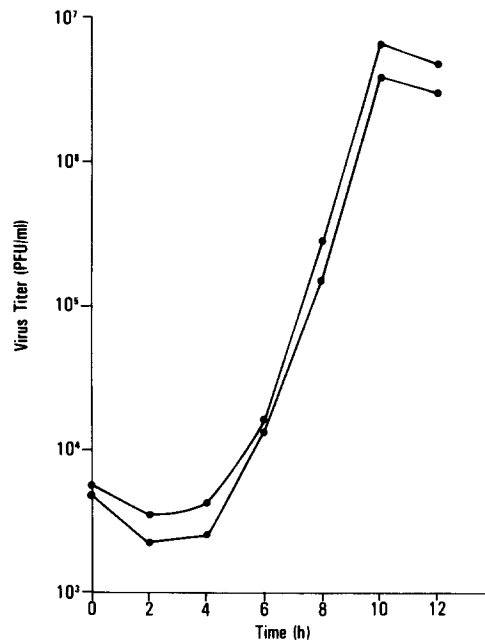


FIG. 1. One-step growth curve of MHV-3 in 2×10^7 17 CL1 cells. Cells were infected in suspension with MHV-3 at an MOI of 0.1 (29). After virus adsorption for 30 min at 37°C, the cells were collected by low-speed centrifugation and resuspended in media containing 2% FCS, and replicate cultures were seeded at 1×10^6 cells per 12 \times 35-mm culture dish. At appropriate intervals, cultures were frozen, and the cells were harvested by freeze-thawing, scraped with a rubber policeman, and disrupted by sonication. The homogenates were then clarified by centrifugation, and virus titer was determined by plaque assay at serial 10-fold dilutions. Each data point represents the mean of duplicate cultures, and each line represents individual experiments.

Packaging, Cambridge, Mass.) in 1 ml DMEM fortified with 2% fetal calf serum and were infected at an MOI of 0.1 with MHV-3. Virus was adsorbed to the cells for 60 min at 37°C. Unadsorbed virus was removed by washing with DMEM, the media were replaced, and the cells were further incubated at 37°C. Cultures were frozen at appropriate intervals, treated as described above, and assayed for virus by plaque assay at serial 10-fold dilutions (Fig. 2). In contrast to the growth of MHV-3 in a totally permissive cell line (17 CL1), in BALB/c monocytes, a prolonged period of 16 h was observed postinfection, during which time no virus replication was evident. However, subsequent to the 16-h lag period, replication was progressive, with peak yields 24 h after infection. In contrast, no virus replication was detected in A/J or C3H/St monocytes within 48 h postinfection. These observations parallel the observations of acute fulminant hepatitis in BALB/c mice and resistance of A/J mice to overt hepatitis or death.

Basal Cellular PCA. The total cellular content of PCA immediately after isolation of cells and before any form of in vitro culture was determined for A/J, BALB/c, and C3H/St mice and was remarkably consistent at 56–81 mU/10⁶ PBM. This activity rises spontaneously to a maximum stable concentration of 515 mU/10⁶ PBM within 6 h of culture in serum-free medium in the absence of added stimulus (Table II). When the control cultured cells were fractionated by adherence into lymphocytes and

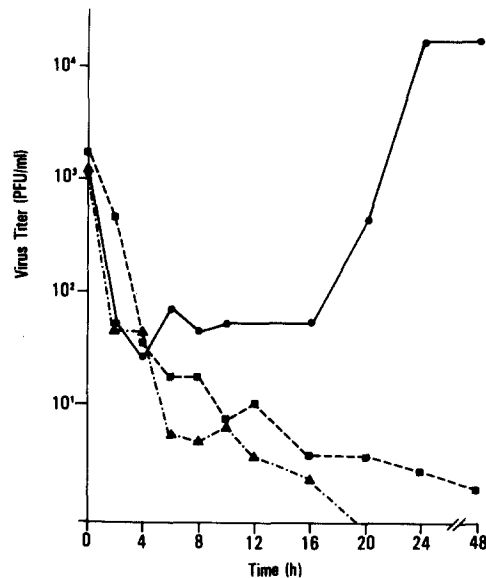


FIG. 2. One-step growth curve for MHV-3 in BALB/c (●), C3H/St (■), and A/J (▲) monocytes. Monocytes were isolated from PBM by adherence to plastic. 3×10^6 monocytes were added to each well of 24-well cluster plates and infected with MHV-3 at an MOI of 10^{-1} . The infection was allowed to continue for various time intervals and then the virus was recovered by freeze-thawing and scraping of the plates with a rubber policeman. The virus was clarified by centrifugation and then assayed in a plaque assay. Each data point represents the mean of duplicate cultures.

TABLE II
Basal Levels of PCA in PBM

Strain	Total content PCA/ 10^6 PBM*	
	Time zero‡	6 h in culture§
	<i>mU</i>	
A/J	56 ± 14	485 ± 110
BALB/c	74 ± 12	515 ± 75
C3H/St	81 ± 20	500 ± 100

* Total content PCA is determined by measuring an aliquot of freeze-thawed and disrupted cells.

‡ Time zero is defined as time immediately after isolation of cells.

§ Cells are cultured with no stimulus in RPMI 1640 for 6 h in 5% CO₂ at 37°C.

monocytes, 74% of the basal PCA was accounted for in the latter cells (esterase positive) in all strains tested, which agrees with results reported previously (16).

MHV-3 Induction of PCA. PBM were isolated from each of the mice strains, and 1×10^6 PBM were incubated with 10^6 PFU of MHV-3 for 18 h at 37°C in 1 ml of RPMI 1640 supplemented with 2% heat-inactivated FCS at 37°C. The cells were washed three times with RPMI 1640, disrupted, and assayed for total PCA. As a positive control, 1×10^6 PBM from each strain was stimulated with *Escherichia coli* 0111:B4 LPS at 10 µg/ml, as previously described (16). Cells from all three strains responded to LPS with a six- to eightfold increase in PCA (Table III), in accord with previous observations (16). The PCA response of BALB/c PBM to 10^6 PFU MHV-3

was greater than previously observed for any stimulus, reaching levels of $>70,000$ mU/ 10^6 PBM (Table III). In contrast to the profound PCA response of BALB/c PBM, no response to MHV-3 was observed in PBM from A/J mice, even though a normal PCA response was observed to LPS stimulation. A moderate PCA response was observed for PBM from C3H/St mice stimulated with MHV-3, but the response was only 70% of the response observed for LPS stimulation (Table III). Thus, the PCA response of PBM to MHV-3 stimulation exhibited profound strain differences. The production of the PCA by PBM correlates with the susceptibility of the strain to hepatic disease. Furthermore, the PCA response of BALB/c mice was of a magnitude 15–20 times greater than that observed for the response to LPS or antigen-antibody complexes (14–16). No increase in PCA above basal levels was observed when 1×10^6 PBM from BALB/c, A/J, or C3H/St mice were cultured with an aliquot from mock-infected DBT, L2, or 17 CL1 cells (data not shown).

Dose-response titrations were examined for MHV-3-induced PCA in which 1×10^6 PBM from BALB/c mice were incubated for 6 h with MHV-3. When 1 PFU of MHV-3 was used, a fourfold increase in PCA as compared with unstimulated control cultures was observed (Fig. 3). A dose-dependent increase of PCA achieved a 168-fold maximum increase of PCA with 10^8 PFU of MHV-3 (Fig. 3).

Comparable experiments were conducted with PBM from C3H/St and A/J mice. When 1×10^6 PBM from C3H/St mice were incubated for 6 h with increasing amounts of MHV-3, no PCA response was observed until concentrations of 100 PFU of MHV-3 were added; and the maximum PCA response was observed at 10^5 PFU of MHV-3, with a 7.2-fold maximum increase in PCA observed over control cultures. In contrast, no PCA response was observed for PBM from A/J mice over a dose titration of $1-10^6$ PFU of MHV-3.

To determine the kinetics of MHV-3-induced amplification of PCA, 1×10^6 PBM from BALB/c, C3H/St, and A/J mice were each incubated with 10^6 PFU of MHV-3 for various time intervals and assayed for total cellular PCA. A 10-fold PCA response was observed for BALB/c PBM that were cultured for 1 h with MHV-3 (Fig. 4). There was a progressive increase of PCA with time of exposure, which reached a maximum of 84,500 mU at 12 h. This level of PCA was maintained for 48 h, which was the maximum interval of observation. In contrast, the PCA response of C3H/St mice did not rise until 2 h after exposure to MHV-3 and was maximum at 8 h (Fig. 4) at 2,800 mU, which was a 6.8-fold increase over control. Finally, there was no increase in PCA from A/J mice above a background level of 385 mU at any time interval of cultivation (Fig. 4).

MHV-3 Stimulation of Cell Populations. When isolated lymphocytes or monocytes

TABLE III
MHV-3 Induction of PCA in PBM in Inbred Strains of Mice

Strains	Control*	PCA (mU/ 10^6 PBM) LPS‡	MHV-3§
A/J	540 \pm 120	4,250 \pm 200	710 \pm 150
BALB/c	605 \pm 140	3,850 \pm 100	74,560 \pm 1,250
C3H/St	550 \pm 200	4,100 \pm 150	2,850 \pm 105

* Control levels taken from cells cultured for 6 h after isolation.

‡ 10 μ g LPS added to PBM in experiment.

§ 10^6 PFU in a volume of 100 μ l added to 10^6 PBM.

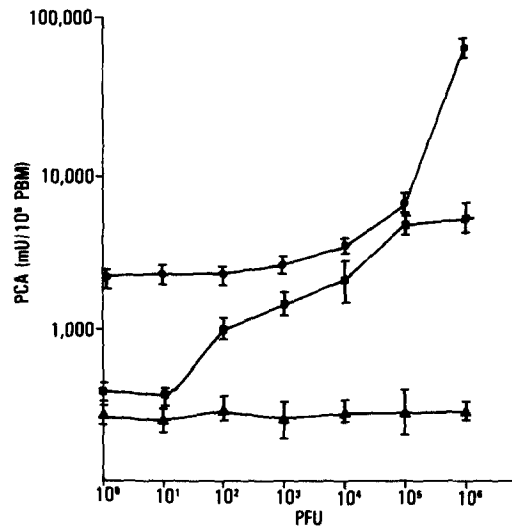


FIG. 3. Dose-response curve for MHV-3-induced PCA in PBM from BALB/c, C3H/St, and A/J mice. 1×10^6 PBM from Balb/c (●), C3H/St (■), and A/J (▲) mice were infected with serial 10-fold dilutions of virus for 18 h, and then PBM was assayed for total content of PCA in a one-stage clotting assay.

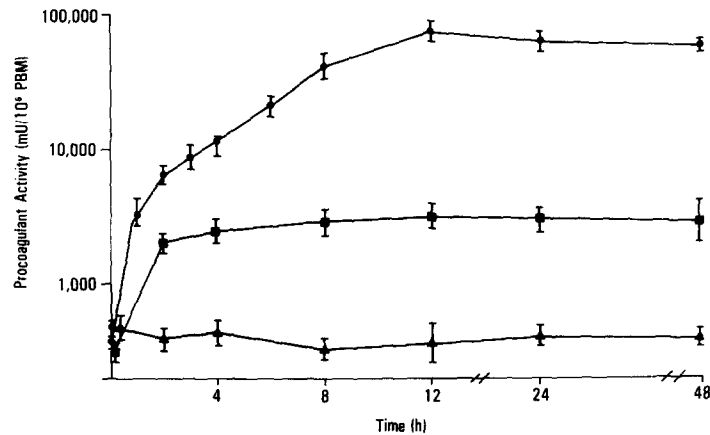


FIG. 4. Time-course for the induction of murine PCA by MHV-3. 1×10^6 PBM from BALB/c (●), C3H/St (■), and A/J (▲) mice were cultured with 1×10^6 PFU of MHV-3 and then freeze-thawed and were sonicated and assayed in a one-stage clotting assay for total content of PCA.

from either C3H/St or BALB/c mice were directly stimulated with MHV-3, no increase of cellular PCA was observed (Table IV). However, when PBM were first stimulated by MHV-3 for 18 h and then subsequently fractionated into lymphocytes and monocytes by adherence to plastic for 48 h, the increase in PCA observed for the whole PBM was localized to the monocyte population. This is similar to the increase demonstrated for LPS stimulation (14). The PCA response of PBM from BALB/c mice was 168-fold, increasing from 505 to 81,400 mU. 98% of the increase in PCA could be accounted for in the monocyte fraction, which demonstrated an increase in total cellular PCA from 3,200 (control) to 722,000 mU/ 10^6 monocytes (Table V).

TABLE IV
Failure of Induction of PCA by Direct MHV-3 Stimulation of Cells Isolated from PBM from BALB/c and C3H/St Mice

MHV-3 stimulated cells*	BALB/c		C3H/St	
	mU	Stimulated/basal	mU	Stimulated/basal
PBM	84,300	156	3,500	9.2
Lymphocytes‡	64	1.3	56	0.9
Monocytes§	3,450	1.1	2,950	0.8

* 10^6 PFU of MHV-3 added in all cultures.

‡ Lymphocytes separated by adherence to plastic and directly stimulated by MHV-3. Lymphocytes were <2% esterase positive.

§ Monocytes separated on the basis of adherence to plastic and directly stimulated by MHV-3. Monocytes were >96% esterase positive.

TABLE V
Cellular Source of MHV-3-induced PCA in Subpopulations from PBM in BALB/c and C3H/St Mice

Cell population*	PCA/ 10^6 cells			
	BALB/c		C3H/St	
	mU	Stimulated‡/basal	mU	Stimulated‡/basal
PBM	81,400	168	3,150	7.1
Lymphocytes§	48	0.9	51	1.3
Monocytes	722,000	172	28,500	8.1

* Cells were held in culture for 18 h and then assayed for total content of PCA.

‡ 10^6 PFU of MHV-3 added in all cultures.

§ Lymphocytes separated on the basis of adherence to plastic after MHV-3 stimulation of PBM. Lymphocytes represent 86% of the total cell population and are <2% esterase positive.

|| Monocytes separated on the basis of adherence to plastic after MHV-3 stimulation of PBM. Monocytes represent 12-14% of PBM and are >96% esterase positive.

Similar analyses were conducted with C3H/St cells. Although the total PCA was considerably less than that observed for PBM from BALB/c mice (Table IV), the cellular distribution was similar. Even with cellular subfractionation there was no increase in PCA in monocytes from A/J mice (data not shown). It is apparent that the monocyte is the cellular source of increased PCA; however, direct stimulation of monocytes did not result in increased PCA. A further requirement appeared essential for the monocyte PCA response.

Lymphocyte Requirement for Generation of Monocyte PCA. To further explore the cellular requirements for the induction of the monocyte PCA response, monocytes were prepared by 48-h incubation and adherence to plastic. Lymphocytes were vigorously washed away and >99% of the remaining cells in the 16-mm Diam plastic wells demonstrated active uptake of neutral red. Upon detachment and preparation of cytologic smears, virtually all cells were positive for nonspecific esterase. Such monocytes, though admittedly now with a more macrophagelike cytology and behavior,

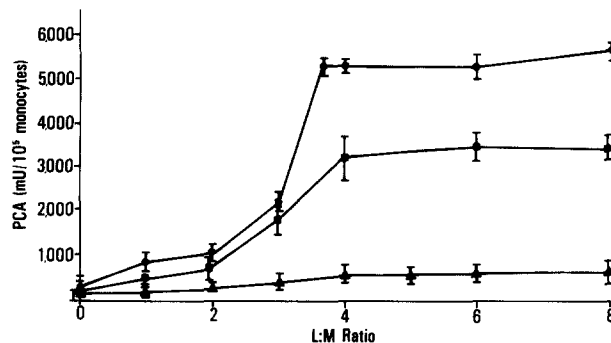


FIG. 5. Lymphocyte requirement for the induction of PCA by MHV-3. 1×10^5 monocytes from BALB/c (●), C3H/St (■), and A/J (▲) mice were stimulated with 1×10^5 PFU of MHV-3, either alone or with increasing numbers of lymphocytes for 16–18 h. Monocytes were then isolated from the lymphocytes and assayed for total content of PCA.

were prepared from C3H/St, A/J, and BALB/c mice. They were stimulated either directly by MHV-3 alone attached in the 16-mm plastic wells or in the presence of variable numbers of lymphocytes, i.e., with lymphocyte:monocyte ratios from 0:1 to 8:1. Monocytes were then isolated from lymphocytes by vigorous washing and assayed for total cellular PCA. Monocytes (1×10^5) exposed to 10^6 PFU of MHV-3 in the absence of lymphocytes exhibited no PCA response, nor was the response at lymphocyte:monocyte ratios of less than 2:1 particularly notable (Fig. 5). However, at lymphocyte:monocyte ratios of 2.5:1 or greater, a significant PCA response was observed and it reached maximum levels as the ratio approached 4:1. No further increase in PCA was noted as the ratio was increased to 8:1. These data indicate that monocytes are the cellular source of the MHV-3-induced PCA, but the presence of lymphocytes is necessary for induction of the PCA response in monocytes.

Discussion

This study further characterizes the generation of monocyte procoagulant activity in response to stimuli, a response mediated by the coagulation pathways. In previous reports, we have demonstrated that monocytes from a wide variety of mouse strains, including the A/J, BALB/c, and C3H/St used in this study, produce an equivalent PCA response to such agents as LPS (14, 16) and antigen-antibody complexes (15). Others (13, 17–20) have observed this response in a variety of species, including human and rabbit, using lectins, LPS, and antigen-antibody complexes.

In the present study, a genetically restricted pathogenic agent, MHV-3, stimulates the PCA response of cells only from strains of mice susceptible to the pathologic effects of the virus. This, to our knowledge, is the first example of a normal genetic restriction of the PCA response and is characterized by maximum output of PCA by BALB/c mice that are fully susceptible to MHV-3, with a fatal outcome within 6–7 d, a moderate PCA response of C3H/St mice who develop enzymatic and histologic lesions of hepatitis (G. A. Levy, J. L. Leibowitz, and T. S. Edgington, manuscript in preparation), and no PCA response of mice fully resistant to MHV-3, as exemplified by the A/J strain. We have shown that these strains are all functionally capable of

producing a PCA response to LPS² and also to antigen:antibody complexes (unpublished data) and that the magnitude of the PCA response is equivalent.² Therefore, the lack of response by the A/J strain to MHV-3 is specific for the stimulus. The response of cells from C3H/St mice is comparable to that observed for other unrestricted stimuli, such as LPS² or antigen:antibody complexes (15) and before this study would have been considered a maximum response. Of great interest is the unprecedented response of cells from BALB/c mice. It represents ~30 times the response observed for any other previously examined stimulus, including those reported by other investigators (17-20). Whether or not the increased magnitude of the response represents increased synthesis of procoagulant molecules, a new and as yet uncharacterized initiator of the coagulation pathways or conformation realignment of either tissue factor or the monocyte prothrombinase (39) and amplification of activity by a cofactor such as factor Va (40) remains to be investigated.

MHV-3-induction of the PCA response is also more rapid than was previously observed, with a significant increase of PCA within 1-1.5 h *in vitro* and with maximum activity at 12-18 h. This contrasts to the 2-3-h lag period before monocyte PCA increases in response to LPS-triggered lymphocytes. Virus growth in a fully susceptible cloned cell line (17 CL1) was not detectable until 6 h after infection, and in monocytes from BALB/c mice infectious virus was not produced until 18 h postinfection. Thus, the induction of the PCA response in PBM antedates production of infectious virus and cannot be ascribed to it *per se*. Furthermore, little or no viral replication occurred in monocytes from C3H/St mice, and yet a robust generation of monocyte PCA followed. Thus, these initial observations suggest that the induction of the PCA response by PBM does not depend on virus replication.

The induction of this activity temporally precedes induction by other stimuli, such as LPS or antigen-antibody complexes, as reported previously. At 2 h, although only 6% of total virally induced PCA is seen in the susceptible BALB/c strain, the quantity of PCA exceeds a maximum response by other agents, such as LPS. We have recently described (16) the kinetic characteristics and metabolic requirements for the induction of lymphoreticular PCA. We demonstrated, using LPS as a model stimulus, that lymphocyte triggering by this stimulus was a brief event, requiring only 5-10 min for detectable effect and 30 min for maximum triggering. Lymphocyte contact and induction of monocytes required 15 min for complete effect, but monocytes then required an additional 4 h for production of maximum PCA. In the viral induction of PCA, it appears there are differences in the kinetics of induction of PCA, with an initial response in just 1.5 h, although maximum PCA induction requires 12-16 h. This is not simply a matter of recruiting more monocytes or macrophages because we have demonstrated (14) in cytologic assays that virtually all monocytes/macrophages are recruited in the previously described responses. Thus, it is possible that different cellular mechanisms or products are operative for the induction of PCA by different stimuli.

We have extended these observations by demonstrating that lymphocyte collaboration is necessary for the induction of monocyte PCA. When highly purified monocytes from C3H/St or BALB/c strains of mice are directly stimulated by the

² Levy, G. A., and T. S. Edgington. The major histocompatibility requirement for cellular collaboration in the murine lymphoid procoagulant response stimulated by bacterial lipopolysaccharide. Manuscript submitted for publication.

virus, no induction of PCA follows. These results parallel the PCA response to other stimuli (14-16). In studies involving human PBM (41, 42), we have observed that both helper and suppressor lymphocytes exist that appear central to the induction and regulation of the PCA response to LPS. Indeed, the induction of monocyte PCA by helper T cells can be markedly attenuated by T γ cells. This finding may be significant in explaining the response to MHV-3. It is possible that the failure of response by PBM from A/J mice could reflect an excess of suppressor cells rather than a lack of lymphocyte recognition and generation of triggered T helper cells. Furthermore, the different responses observed in C3H/St and BALB/c mice could reflect similar disproportions in the suppressor and helper cell participants. Another possible explanation of the differences in PCA induction by MHV-3 could reflect differences in the capacity of monocytes to receive signals from MHV-3-triggered lymphocytes or otherwise accept lymphocyte collaboration for this specific stimulus. The lack of a monocyte subpopulation (which is inducible) by MHV-3 within A/J mice might explain the failure of induction of PCA in this strain. Application of the cytologic PCA assay previously described (16), in which we can visualize individual cells and the presence or absence of PCA, promises to provide additional information.

A number of investigators (26, 27) have demonstrated that the susceptibility of adult mice of different strains to MHV, using mortality as the index, parallels the cytopathic effects that the virus has in mouse peritoneal macrophages *in vitro*. The cellular basis for the resistance of A strain macrophages is not known; however, it has been observed (26) that resistance can be overcome by increasing the infecting dose, suggesting that resistant strains do not entirely lack receptors for MHV-3. It has also been demonstrated (43-45) that young or immunologically depressed A strain mice develop disease similar to that occurring in susceptible strains, in contrast to the fully resistant adult A mouse. Further observations suggest that the state of susceptibility is under the influence of T cells (46-48), humoral factors (28), and splenic adherent cells (27). It has also been demonstrated (46, 47) that nude mice and mice treated with cyclophosphamide (49) develop full susceptibility to MHV-3 infection. Thus, it appears that a complex series of interactions between lymphocytes, monocytes, and humoral factors may be required to explain the balance between susceptibility or resistance to this virus.

The present study reports that PBM from susceptible strains of mice respond to MHV-3 with a rapid and robust PCA response. In contrast, there is a failure of induction of PCA in the fully resistant A/J strain. Although most mechanistic details remain to be resolved, this system provides a biochemical marker for susceptibility to MHV-3-induced disease and perhaps may play a role in the pathogenesis of disease induced by this virus.

Summary

The *in vitro* induction of procoagulant activity (PCA) in murine peripheral blood mononuclear cells (PBM) by murine hepatitis virus type 3 (MHV-3) correlates with the disease susceptibility in three strains of mice. PBM from BALB/c mice, a strain in which MHV-3 infection results in fatal acute fulminant hepatitis, responds to the virus with a robust PCA response, whereas PBM from C3H/St mice, a strain which develops mild acute hepatitis followed by chronic hepatitis, only exhibit a modest PCA response. In contrast, PBM from A/J mice, a strain fully resistant to MHV-3,

generate no increase in PCA above control levels. The induction phase of MHV-3 PCA is rapid, with an increase within 1–1.5 h, with maximum activity at 18 h, and it precedes MHV-3 replication in either 17 CL1 cells, a fully permissive cell line, or in monocytes from these strains of mice. The PCA response of BALB/c PBM exceeds the response to any other known stimulus. No induction occurs upon direct stimulation of monocytes by MHV-3, but in the presence of lymphocyte collaboration, the PCA response is observed first at a lymphocyte:monocyte ratio of 2:1 and reaches a maximum as the lymphocyte:monocyte ratio approaches 4:1.

This response appears to provide a functional marker for susceptibility to MHV-3 infection in inbred strains of mice and could be important in the pathogenesis of MHV-3-induced disease.

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