Macrophages and Resistance to JHM Virus CNS Infection

Stephen A. Stohlman and Jeffrey A. Frelinger

University of Southern California School of Medicine

2025 Zonal Avenue, Los Angeles, CA 90033

INTRODUCTION

The macrophage is an important cell in determining the outcome of viral infections, especially those due to herpes and mouse hepatitis viruses (Morgensen, 1979). Mouse hepatitis virus (MHV), is a member of the coronavirus group which are positive stranded RNA viruses (Lai and Stohlman, 1978) and produce a wide spectrum of diseases in their natural hosts (McIntosch, 1973). The principle organs attacked by MHV during infection are the liver and the central nervous system (CNS). Bang and co-workers have demonstrated a genetic basis for the resistance of mice to fatal hepatitis caused by MHV and that in vitro macrophages from resistant animals exhibit resistance parallel to that determined in vivo (Bang, 1978). They have shown that MHV can only replicate in vitro in macrophages from susceptible animals. This type of resistance appears to be analogous to the intrinsic resistance described for herpes viruses (Johnson, 1964). Intrinsic resistance to viral replication limits virus dissemination thereby affording protection to the target organ and the host. Recently, we have demonstrated the ability of passively transferred macrophages to confer resistance to the JHM strain of MHV (MHV-JHM) induced acute CNS disease (Stohlman et al, 1980). However, macrophages from both susceptible and resistant animals exhibited intrinsic resistance to MHV-JHM replication.

We have examined the ability of macrophages to exhibit extrinsic anti-viral activity, the ability of macrophages to suppress MHV replication in another cell, correlated this with intrinsic anti-viral activity, and examined the macrophages from resistant and susceptible mice for a number of macrophage markers.

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ABSTRACT

Thioglycollate elicited peritoneal exudate cells from resistant SJL mice, younger susceptible SJL mice, and susceptible ASW, BALB/c, and C57/BL6 mice all exhibit extrinsic antiviral activity. The active cell was characterized as a Thy 1.2 negative, Ia negative, radiation resistant adherent cell. The antiviral activity was not due to nonspecific cellular cytotoxicity directed against the susceptible cell nor interferon. Adherent PE cells from resistant and susceptible SJL mice were similar with respect to the number of phagocytes, nonspecific esterase containing, Fc, and C_3b receptor bearing cells. Finally, extrinsic antiviral activity was not dependent upon intrinsic antiviral activity.

MATERIALS AND METHODS

Mice: SJL, C_{57}BL/6, and BALB/c were all purchased from Jackson Laboratories, Barr Harbor, Maine. ASW mice were obtained from the Immunogenetics Mouse Colony, USC.

Effector Cell Preparation: Peritoneal exudate (PE) cells were elicited by the intraperitoneal injection of thioglycollate broth 3 days prior to peritoneal lavage with Hanks balanced salt solution containing 10 IU of heparin/ml. The PE cells were removed, washed and the viability determined as previously described (Stohlman et al, 1980). In some experiments, PE cells were subjected to 2000 R with a 60_{Co} source emitting at 1000 R/min. Nonadherent cells were prepared as previously described (Stohlman et al, 1980).

Assay for Extrinsic Anti-Viral Activity: To test for extrinsic anti-viral activity, DBT cells, a continuous mouse astrocyte cell line (Stohlman and Weiner, 1978) were grown in 24 well (16 mm) plates using Dulbecco's modified MEM containing 5% Newborn calf serum (Biocell, Carson, California). Monolayers contained approximately 5 x 10^5 cells. All wells were infected with 10-15 pfu of the DL plaque size variant of MHV-JHM for 1 hr at 37°C.

Following removal of the inoculum, 0.5 ml of RPMI-1640 containing 2% NBC serum and 10 mM Hepes was added to each well. At 2-4 hr after inoculation, the effector PE cells at final concentration of from 10^4 to 5 x 10^6 cells per well were added in quadruplicate in a 0.5 ml volume. Control wells received medium only. Cultures were incubated for 18 hr and the supernatants from the 4 wells were pooled before the samples were frozen at -70°C. Released progeny virus was determined by plaque assay on monolayers of DBT cells as previously described (Stohlman and Weiner, 1978). The average virus yield in the 4 wells was determined in either triplicate or quadruplicate assays.

Antisera Depletion: T cells were depleted by treating 2 x 10^7 cells/ml with congenic anti-Thy 1.2 serum PL/J x B6-Thy1^a) F_1 anti-B6 and rabbit complement (C). Ia bearing cells were removed as described by treatment with A.TL anti A.TH serum and C (Niederhuber, et al, 1975). Cells were reconstituted to 10^7 viable cells/ml.

Nonspecific Cytotoxicity: Nonspecific cytotoxicity was determined by adding 5.0 uCi of ^{3}H-thymidine (ICN Pharmaceuticals) to 1 x 10^6 L929 or DBT in 75 cm^2 flasks. After 24 hr at 37°C the targets were trypsinized, washed, and counted. Target cells were added to 24 well plates at 4 x 10^4 cells per well in 0.5 ml. Dilutions of the test PE cells at the quantities used in the extrinsic anti-viral assay in 0.5 ml were also added in quadruplicate. Total release was determined by adding 0.5 ml of 1.0% SDS in place of PE cells. After 48 hrs, 0.2 ml of the supernatant was removed and counted as previously described (Stohlman and Lai, 1979).

ADCC and ADP: Antibody dependent cellular cytolysis (ADCC) and antibody dependent phagocytosis (ADP) were determined simultaneously using the method of Walker (1977). TNP-coated SRBC were labeled with ^{51}Cr (NEN) and incubated with PE cells at an effector to target ratio of 5:1 for 2 hrs at 37°C with constant rocking.

Intrinsic Antiviral Activity: PE cells were placed in 35 mm plates at 4 x 10^6 viable cells/plate. After 2-3 hrs the cultures were washed vigorously 3x with serum free DMEM. The remaining adherent cells were infected with 0.2 ml of MHV-JHM for 1 hr at 37°C. At various times post infection the cells were scraped into DMEM 2% fetal calf serum and then disrupted by two cycles of freezing and thawing. The virus content was determined by plaque assay on DBT cells (Stohlman and Weiner, 1978).

RESULTS

Extrinsic anti-viral activity: SJL mice exhibit an age-dependent resistance to intracranial (i.c.) challenge with MHV-JHM. We have previously shown that PE cells from non-immune SJL donors confer resistance to syngenic susceptible animals (Stohlman et al, 1980). To determine if extrinsic macrophage-mediated anti-viral activity was a protective mechanism operating under these circumstances, an assay for extrinsic antiviral activity was established similar to those developed for herpes virus (Morahan et al, 1980). Table 1 shows that the addition of thioglycollate elicited PE cells from 12 week old SJL mice to cultures of infected cells effectively suppresses virus replication at effector to target ratios in excess of 1:1.

TABLE 1

Extrinsic anti-viral activity of PE cells, PE cells depleted of Ia bearing cells, and heat killed PE cells

TREATMENT	EFFECTOR TO TARGET RATIO	PERCENT RELEASED VIRUS
None		
	10:1	0
	5:1	0
	1:1	26
	None	100
Anti-Theta + C[2]	10:1	0
	5:1	0
	1:1	28
Anti-Ia + C[2]	10:1	0
	5:1	2
	1:1	33
NMS[1] + C[2]	10:1	0
	5:1	2
	1:1	31
Irradiation (1000 R)	10:1	0
	5:1	0
	1:1	30
Heat Killed	10:1	94
	5:1	100
	1:1	98

[1] NMS = normal mouse serum

[2] C = complement

Suppression of the release of virus into the supernatant was not dependent upon T cells since treatment of the PE cell population with congenic anti-Thy 1.2 serum had no effect. Protection from lethal i.c. challenge is mediated by a PE cell population lacking Ia antigen (Stohlman et al, 1980). Therefore, PE cells depleted of Ia bearing cells were tested for anti-viral activity. Table 1 also shows that removal of the Ia bearing cells did not significantly reduce the anti-viral activity of the remaining cell population. Furthermore, the activity was also not decreased by irradiation of the effector PE cell population. Heating at 56°C for 30 min. completely abolished protection. In addition, the nonadherent PE cell population exhibited markedly reduced protection. Partial protection in the nonadherent population is probably

due to contamination with a cell population that is able to adhere in an additional two hr inoculation (approximately 10%). This residual population contains nonspecific esterase, Fc and C_3b receptor positive cells (data not shown). Experiments were run in parallel comparing thioglycollate elicited and resident peritoneal cells to determine if thioglycollate treatment enhanced the protective capacity. Table 2 shows that resident cells were as effective in suppressing virus replication as thioglycollate elicited PE cells, which is in agreement with our previous finding that splenic macrophages are also effective in confirming protection (Stohlman et al, 1980).

TABLE 2

Comparison of extrinsic anti-viral activity of Resident and Thioglycollate elicited PE cells

	EFFECTOR TO TARGET RATIO	PERCENT RELEASED VIRUS
Elicited PE cells		
	10:1	0
	5:1	4
	2:1	32
	1:1	26
Resident PE cells		
	10:1	0
	5:1	9
	2:1	41
	1:1	28

To insure that the apparent anti-viral activity was not due to nonspecific cytotoxic activity resulting in the destruction of the host cells, DBT and L929 cells were tested as targets. SJL PE cells exhibited nonspecific cytotoxicity against L929 cells. No killing of DBT cells was detectable at any effector to target ratio (data not shown).

Age-Dependent Activity: PE cells from 4, 6, and 12 week old SJL were tested for extrinsic anti-viral activity to determine if the basis of the age-related change in resistance to MHV-JHM could be correlated with an inability of PE cells from mice younger than 12 weeks of age to suppress viral growth. Table 3 shows that there was essentially no difference in anti-viral activity between the three age groups tested. To insure that the cells responsible for the anti-viral activity of the PE cells from 4 and 6 week old animals was similar to that described above, these PE cells were also tested following irradiation. In addition,

PE cells from 6 week old animals were tested following Ia and Th 1.2 depletion. No loss of anti-viral activity was found following these treatments (data not shown). PE cells from 6 week old SJL were also tested for nonspecific cyotoxicity against DBT and L929 cells. Similar to the results with PE cells from 12 week old SJL, there was no nonspecific cytotoxic activity against the DBT cells. The level of activity against L929 cells was comparable to that found for PE cells from 12 week old SJL, a maximum of approximately 30% of the total counts released.

TABLE 3

Comparison of the anti-viral activity of PE cells from 4, 6 and 12 week old SJL mice

AGE	EFFECTOR TO TARGET RATIO	PERCENT RELEASED VIRUS
12 weeks	10:1	0
	5:1	0
	2:1	2
	1:1	31
	0.5:1	64
6 weeks	10:1	0
	5:1	0
	2:1	0
	1:1	28
	0.5:1	70
4 weeks	10:1	0
	5:1	0
	2:1	3
	1:1	27

Histochemical Analysis: Adherent PE cell populations from 6 and 12 week old SJL were examined for the number of nonspecific esterase positive, phagocytic, Fc and C_3b receptor bearing cells. The number of adherent cells in any particular PE cell preparation varied from 50-80%, however, in any given experiment the number of adherent cells from 6 and 12 week old cells varied no more than $\pm$ 5%, and the variation was not consistent with age.

Table 4 shows that the adherent PE cells from 6 week old and 12 week old mice are slightly different with respect to the number of cells able to phagocytize 1.0 u latex beads, and in the number of cells with C_3b receptors. The difference is probably not large enough to be correlated with the dramatic change in the ability of these cell populations to prevent acute CNS disease. The two populations were similar with respect to the number of cells positive for nonspecific esterase and Fc receptor.

TABLE 4

Macrophage marker associated with the adherent PE cell population from 6 and 12 week old SJL mice

MARKER	PERCENTAGE ADHERENT CELLS POSITIVE 6 week	12 week
Phagocytosis[1]	91.9 ± 0.1	87.4 ± 0.6
Nonspecific Esterase	88.3 ± 3.1	91.6 ± 1.7
Fc Receptor	91.6 ± 1.06	92.5 ± 0.1
C_3b Receptor	68.4 ± 0.9	72.1 ± 1.40

[1] Determined by uptake of latex beads

ADCC and ADP: Antibody dependent cellular cytolysis (ADCC) and antibody dependent phagocytosis (ADP) of TNP-modified sheep red blood cells were examined using 6 and 12 week old SJL PE cells as effectors. Table 6 shows three of these experiments. There is no difference in the ability of the PE cells from the 6 or 12 week old SJL to lyse TNP-coated SRBC or to phagocytize antibody coated SRBC. Increased ADCC activity with increased dilution of antibody is a regular finding in ADCC systems (Lovchik and Hong, 1977).

Intrinsic Viral Resistance: MHV stains replicate in PE cells obtained from susceptible animals (Bang, 1980; Virelizier and Allison, 1976). However, MHV-JHM would not replicate in adherent PE cells from either resistant 12 week old SJL, susceptible 6 week old SJL or from susceptible B10.S mice (Stohlman et al, 1980). Adherent PE cells from 6 week old SJL and BALB/c were tested for their ability to support MHV-JHM replication. Table 6 shows that the adherent PE cells from BALB/c mice would support MHV-JHM while those from SJL mice would not support virus replication.

Extrinsic Activity in Susceptible Strains: Thioglycollate elicited PE cells from ASW, BALB/c, and C_{57}BL/6 mice were examined for extrinsic antiviral activity in parallel experiments with PE cells from SJL. Table 7 shows that PE cells from these three strains of mice which are all susceptible to i.c. challenge with MHV-JHM (Stohlman and Frelinger, 1978) are as efficient as PE cells from SJL mice in suppressing viral growth in DBT cells.

TABLE 5

Antibody dependent cellular cytolysis and phagocytosis of PE cells from 6 week old and 12 week old SJL mice

EXPERIMENT NUMBER	AGE WEEKS	ANTIBODY DILUTION (RECIPROCAL)	PERCENT OF TOTAL ADCC	ADP
1	6	100	6.0	30.0
		1000	6.7	35.4
	12	100	5.3	41.6
		1000	6.3	36.0
2	6	100	7.7	36.0
		1000	10.7	24.2
	12	100	7.7	32.4
		1000	11.4	24.0
3	6	100	17.0	29.2
		1000	24.7	16.9
	12	100	18.6	27.1
		1000	22.6	17.1

TABLE 6

Replication of MHV-JHM in adherent PE cells from SJL and BALB/c mice

MOUSE STRAIN	HOURS POST INFECTION	TITER
BALB/c	6	0^{1}
	12	3×10^{2}
	24	1.1×10^{3}
	48	2.9×10^{4}
	72	3.9×10^{3}
SJL	6	0^{1}
	12	0^{1}
	24	0^{1}
	48	0^{1}
	72	0^{1}

[1]) No virus deleted

TABLE 7

Extrinsic anti-viral activity of thioglycollate elicited PE cells from different strains of mice.

MOUSE STRAIN	EFFECTOR TO TARGET RATIO	PERCENT RELEASED VIRUS
SJL	10:1	0
	5:1	0
	2:1	9
	1:1	40
ASW	10:1	0
	5:1	0
	2:1	6
	1:1	42
C_{57}BL/6	10:1	0
	5:1	2
	2:1	2
	1:1	19
BALB/c	10:1	0
	5:1	0
	2:1	11
	1:1	40

DISCUSSION

Cells of the macrophage series play a major role in the hosts ability to defend against viral infection. Macrophages exhibit both "intrinsic" and "extrinsic" anti-viral effects as part of the hosts immune defense. Intrinsic resistance is the inability to support virus replication (Morahan and Morse, 1979). It is related to the ability either to phagocytize viruses, thereby rendering them noninfectious, or to adsorb virus at the cell surface and restrict a complete replication cycle within the milieu of the cellular cytoplasm. Extrinsic antiviral activity, on the other hand, is the ability to suppress virus replication in another cell susceptible. The mechanism of this suppression, which can be demonstrated with in vitro systems in the absence of other cells of the immune system, is not clear (Morahan et al, 1980). It is expressed by both circulating monocytes and PE cells that have properties attributable to macrophages (Morahan and Morse, 1979).

Intrinsic antiviral activity of macrophages has been implicated in resistance to both herpes virus and MHV infections (Morahan and Morse, 1979; Bang, 1978; Virelizier and Allison, 1976). In the case of herpes simplex virus, the intrinsic resistance

is correlated with an age-dependent acquisition of host resistance (Johnson, 1964; Stevens and Cook, 1971). Intrinsic resistance to MHV has been related to the genetic basis of resistance to acute viral hepatitis (Bang, 1978). JHM, the neurotropic strain of MHV, causes an acute encephalomyelitis with both acute and chronic demyelination in mice (Weiner, 1973; Herndon et al, 1975). CNS disease in SJL mice can be prevented by the passive transfer of PE cells (Stohlman et al, 1980). However, in vitro, macrophages from mice that are both susceptible and resistant to i.c. challenge with MHV-JHM exhibit extrinsic anti-viral activity which clearly sets these results apart from the results reported for both HSV and other strains of MHV.

In this communication we report the first evidence of extrinsic anti-viral activity for a virus other than a member of the herpes virus group. PE cells from SJL mice resistant to i.c. challenge with MHV-JHM exhibit extrinsic anti-viral activity. The cell responsible was characterized as a macrophage based on refractiveness to irradiation, negative selection for adherence, and the absence of Thy 1.2 antigen. In addition to these properties, all in common with the cell type capable of confirming resistance to susceptible young SJL (Stohlman et al, 1980), the active cell type lacked suface Ia antigens similar to the cells active in the in vivo model. The anti-viral activity was dependent on the effector to target ratio and was not related to either interferon or the lysis of the infected cells by nonspecific cytotoxicity.

We examined the extrinsic anti-viral activity of PE cells from 12 week old SJL mice to help understand its possible role in macrophage mediated age-dependent resistance to i.c. challenge. Intrinsic anti-viral activity had previously been ruled out since macrophages from both susceptible and resistant SJL mice were refractory to infection with MHV-JHM (Stohlman et al, 1980). Evidence presented indicates that PE cells from 4, 6 and 12 week old SJL all have equal ability to suppress viral growth in a second cell type.

In addition to trying to correlate extrinsic antiviral activity with the age-dependent change in resistant of SJL mice we have also examined the PE cell populations from these two age groups for other markers associated with macrophages. No differences significantly large to account for the dramatic change in resistance were noted in the percent adherent cells, the number of adherent cells that were phagocytic, contained nonspecific esterase, Fc or C_3^b receptor, or in the ADCC or ADP activity of PE cells from these two age groups.

Since we have previously shown that SJL is the only strain of mice capable of surviving a lethal i.c. challenge with MHV-

JHM (Stohlman and Frelinger, 1978), we examined other strains of susceptible mice for their ability to express macrophage mediated extrinsic anti-viral activity against MHV-JHM. We found that PE cells from BALB/c, ASW, and C_{57}BL/6 mice, which are susceptible to MHV-JHM, suppressed virus replication to the same extent as PE cells from the resistant SJL strain. This observation is in contrast to the apparent differential expression of intrinsic anti-viral activity. SJL adherent cells show complete intrinsic anti-viral activity, while the adherent cells from BALB/c are permissive. This indicates that macrophages can exhibit extrinsic anti-viral activity quite apart from intrinsic anti-viral activity and that these two functions may not be directly correlated with the ability to survive an acute CNS viral infection or an *in vivo* model of protection based on the passive transfer of macrophages.

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