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Macrophage Antiviral Activity: Extrinsic Versus Intrinsic Activity

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Peritoneal exudate cells from strains of mice both resistant and susceptible to challenge with mouse hepatitis virus strain JHM were examined for extrinsic and intrinsic antiviral activity. Thioglycolate-elicited and resident peritoneal cells from uninfected mice were able to suppress viral growth in a permissive cell. The active cell in both populations is an adherent, radiation-resistant, Thy-1.2 antigen- and Ia antigen-negative cell. The suppression of virus replication was not related to nonspecific cellular cytotoxicity directed against the permissive host cell, and no interferon was detected. The expression of extrinsic antiviral activity was not related to the ability of the host to resist mouse hepatitis virus infection by virtue of either age or genetic background. The expression of intrinsic antiviral activity, on the other hand, correlated with the ability of the host to resist virus challenge, indicating a characteristic distinction between these two in vitro mechanisms of macrophage-mediated antiviral activity with regard to host resistance to viral infection. Further, the ability of a macrophage to support viral replication itself was independent of the ability of the macrophage to suppress virus growth in another cell.

Macrophages are important in determining the outcome of viral infections, especially those due to herpesvirus and mouse hepatitis virus (MHV) (1, 4, 11, 12). Two terms, extrinsic and intrinsic, have been used to denote different in vitro macrophage-mediated antiviral activities (12). Extrinsic macrophage-mediated antiviral activity is the ability of macrophages or their products to suppress virus growth in another susceptible cell type (12). Thus, it is an action by, rather than a property of, the macrophage. Intrinsic macrophage-mediated antiviral activity, on the other hand, is used to describe the in vitro restriction of virus replication in macrophages from mice resistant to viral infection (12). These two in vitro properties of macrophages have been proposed as relevant mechanisms for limiting virus dissemination and thereby affording protection to the host.

Extrinsic macrophage-mediated antiviral activity has been examined in the context of herpesvirus infection (12). Macrophages from a variety of sources can exhibit anti-herpesvirus extrinsic activity in vitro (8, 10, 11, 16). Mice that exhibit an age-dependent resistance to herpesvirus have macrophages that exhibit a parallel acquisition of extrinsic antiviral activity in vitro (17). In addition, macrophages that do not express extrinsic activity when prepared from uninfected mice will exhibit activity if they are prepared from mice shortly after the animal is infected with herpesvirus (13). In this case, however, the extrinsic activity is not specific for herpesvirus and is probably interferon mediated (8, 13).

The age-dependent acquisition of intrinsic macrophage-mediated antiviral activity has been described for both MHV and herpesviruses (1, 2, 4, 5, 12, 24). In addition, the genetics of host resistance have been determined for three strains of MHV (1, 7, 19). For two of these interactions, there is a parallel between the susceptibility, semisusceptibility, or resistance of the host and the ability of the virus to replicate in macrophages from these hosts (2, 25).

We have been interested in the basis of resistance to the neurotropic JHM strain of MHV (JHMV). JHMV is a member of the coronavirus group of enveloped, positive-stranded RNA viruses (6). It produces an acute demyelinating encephalomyelitis and chronic demyelination in mice and rats (3, 14, 23, 26). Macrophages are known to play an important role in the prevention of systemic MHV infections after parenteral infection (1). We have recently shown that macrophages play a role in the prevention of JHMV-induced central nervous system infection (20). In this study, we examined the intrinsic and

extrinsic antiviral activities of macrophages obtained from mice susceptible and resistant to JHMV challenge.

MATERIALS AND METHODS

Mice. SJL, C57BL/6 (B6), BALB/c, and A.SW strains of mice were purchased from Jackson Laboratories, Bar Harbor, Maine. C57BL/10Sn (B10) and B10.S mice were bred in the Immunogenetics Mouse Colony, University of Southern California.

Effector cell preparation. Peritoneal exudate (PE) cells were elicited by the intraperitoneal injection of 3% thioglycolate broth 3 days before peritoneal lavage with Hanks balanced salt solution containing 10 IU of heparin per ml. The PE cells were removed and washed, and the viability was determined as previously described (20). In some experiments, PE cells were subjected to 2,000 rads with a ^{60}Co source emitting at 1,000 rads/min.

Assay for extrinsic antiviral activity. To test for extrinsic antiviral activity, cells from the DBT line, a continuous mouse astrocyte cell line (22), were grown in 24-well (16-mm) plates with Dulbecco modified minimal essential medium containing 5% newborn calf serum (Biocell, Carson, Calif.). Each well, containing approximately 5×10^5 DBT cells, was infected with 10 to 15 PFU of the DL-plaque-sized variant of JHMV for 1 h at 37°C. The derivation of the DL variant from suckling mouse brain passage 8 of JHMV has been previously described (6).

After removal of the inoculum, 0.5 ml of RPMI 1640 containing 2% newborn calf serum and 10 mM HEPES was added to each well. The effector PE cells were added at final concentrations of 1×10^4 to 5×10^6 cells per well at 2 to 4 h after infection. Control wells received medium only. The cultures were incubated for 18 h, and the supernatants from four wells were pooled and frozen at −70°C before virus titration. Released infectious virus was measured by plaque assay on monolayers of DBT cells as previously described (22). The results are expressed as the mean titer of four wells. Percentage of released virus was calculated as: (Experimental/Control) × 100. The control virus titers varied from 4×10^4 to 1×10^5 PFU/ml.

Antiserum depletion. T cells were depleted by treating 2×10^7 cells per ml with congenic anti-Thy-1.2 serum [(PL/J × B6-*Thy-1*^{a})F_1 anti-B6] and selected, unadsorbed rabbit complement. Ia-bearing cells were removed by treatment with A.TL anti A.TH serum and complement (15). The cells were reconstituted to 10^7 viable cells per ml. Both antisera have been extensively characterized for their ability to deplete the relevant cell type.

Nonspecific cytotoxicity. Nonspecific cytotoxicity was determined by adding 5.0 μCi of [^{3}H]thymidine (ICN Pharmaceuticals, Irvine, Calif.) to 1×10^6 L929 or DBT cells in 75-cm^2 flasks. After 24 h at 37°C, the targets were trypsinized, washed, and counted. Target cells were added to 24-well plates at 4×10^4 cells per well in 0.5 ml of medium. Dilutions of the test PE cells at the quantities used in the extrinsic antiviral assay in 0.5 ml of medium were also added. The total release was determined by adding 0.5 ml of 1.0% sodium dodecyl sulfate in place of PE cells. After 48 h, 0.2 ml of the supernatant was removed and counted as previously described (6). The specific release was calculated by the following formula: % release = [(Target + PE cells) − (Target only)]/[(Target + sodium dodecyl sulfate) − (Target only)] × 100.

Intrinsic antiviral activity. PE cells were placed in 35-mm plates at 4×10^6 viable cells per plate. After 2 to 3 h, the cultures were washed vigorously three times with serum-free Dulbecco modified minimal essential medium. The remaining adherent cells were infected with 0.2 ml of JHMV (multiplicity of infection approximately 1.0) for 1 h at 37°C. At various times postinfection, the cells were scraped into Dulbecco modified minimal essential medium containing 2% fetal calf serum and then disrupted by one cycle of freezing and thawing. The virus content was determined by plaque assay on DBT cells as described above.

RESULTS

Extrinsic antiviral activity. SJL mice at 12 weeks of age are resistant to intracranial challenge with JHMV, whereas 6-week-old SJL mice are not. PE cells from antiviral antibody-negative 12-week-old SJL donors confer resistance on syngenic, young, susceptible animals (20). To determine whether PE cells exhibited extrinsic macrophage-mediated antiviral activity, an assay similar to that described for herpesvirus (8, 10, 13, 16) was developed. The addition of either thioglycolate-elicited or resident peritoneal cells from 12-week-old SJL mice to cultures of infected DBT cells effectively suppressed virus replication at effector-to-target ratios of 0.5:1 or greater (Table 1). Cell-associated virus

TABLE 1. Suppression of JHMV growth in DBT cells by resident and thioglycolate-elicited PE cells from SJL mice

Cell type	Effector-to-target ratio	Virus titer[b] (PFU/ml)	% Released virus
Elicited PE[a]			
	10:1	4.0×10^1	0
	5:1	7.8×10^2	0
	2:1	2.7×10^3	4
	1:1	2.2×10^4	32
	0.5:1	4.6×10^4	68
	0.2:1	6.8×10^4	100
Resident PE			
	10:1	6.4×10^1	0
	5:1	9.9×10^1	0
	2:1	6.1×10^3	9
	1:1	2.8×10^4	41
	0.5:1	5.0×10^4	73
	0.2:1	6.9×10^4	100
No cells added		6.8×10^4	100

[a] Harvested 72 h after intraperitoneal injection of 0.5 ml of thioglycolate.

[b] Determined by plaque assay on DBT cells.

in these cultures was decreased in a manner similar to the suppression of the release of virus into the culture media (data not shown). Both of these PE cell populations are equally competent at virus suppression, demonstrating that macrophage activation is not required for expression of the antiviral response. The suppression of virus replication was not dependent upon T cells or Ia-bearing cells, since treatment of the PE cell population with congenic anti-Thy-1.2 serum or anti-Ia serum plus complement did not decrease extrinsic antiviral activity (Table 2). This demonstrates that neither T cells nor Ia-bearing cells are required for the suppression of virus replication. The inactivity of heat-killed cells (Table 2) and cells killed by alternate cycles of freezing and thawing (data not shown) indicates that viability is required for protection. The suppression of virus replication was also refractory to irradiation of the PE cell population at 2,000 rads (Table 2). These results are consistent with the interpretation that a classical macrophage mediates this response.

Supernatants from macrophages cultured alone, macrophages cultured with both infected and uninfected cells, and infected cells alone were tested for type I and type II interferon (21). In our assay system with vesicular stomatitis virus as the challenge, 1,200 U of mouse reference interferon (National Institute of Allergy and Infectious Diseases) had a titer of 1,000 U. No antiviral activity was detected in any of these supernatants. This was not a surprising finding, since we have previously shown that JHMV does not induce interferon in cells capable of synthesizing interferon (21). In addition, no interferon has been detected in the brains of either susceptible or resistant SJL mice during JHMV infection, even though infectious virus was present (Stohlman, unpublished data).

Age-dependent activity. Age-dependent changes in susceptibility have been described for a number of host-virus relationships including those of herpesvirus (4, 5, 18) and MHV (20, 24). They have been correlated with changes in the ability of the host macrophages to restrict virus replication (4, 5, 17, 18). PE cells from susceptible 4- and 6-week-old and resistant 12-week-old SJL mice were tested for extrinsic antiviral activity to determine if it paralleled the age-related change in the resistance of SJL mice to JHMV (20). There was essentially no difference among the three age groups tested (Table 3), indicating that changes in resistance to JHMV do not parallel changes in the ability of the macrophage to suppress virus growth in a susceptible cell. If anything, the macrophages from 4-week-old animals are slightly more ac-

TABLE 2. Suppression of JHMV replication in DBT cells by thioglycolate-elicited PE cells, PE cells depleted of T cells, PE cells depleted of Ia-bearing cells, and heat-killed PE cells

Treatment	Effector-to-target ratio	Virus titer[c] (PFU/ml)	% Released virus
Expt 1			
No cells added		6.4×10^4	100
Untreated	10:1	3.7×10^1	0
	5:1	4.3×10^2	0
	1:1	1.7×10^4	26
Anti-Thy-C[a] 1.2 +	10:1	7.6×10^1	0
	5:1	5.8×10^2	3
	1:1	1.9×10^4	30
Anti-Ia + C	10:1	8.4×10^1	0
	5:1	1.3×10^2	2
	1:1	2.1×10^4	33
NMS[b] + C	10:1	7.4×10^1	0
	5:1	1.3×10^2	2
	1:1	2.0×10^4	31
Expt 2			
No cells added		6.6×10^4	100
Untreated	10:1	1.6×10^2	0
	5:1	5.1×10^2	0
	1:1	2.0×10^4	30
Irradiation[d]	10:1	2.1×10^2	0
	5:1	4.9×10^2	0
	1:1	2.1×10^4	30
Heat killed	10:1	6.3×10^4	94
	5:1	6.7×10^4	100
	1:1	6.5×10^4	98

[a] C, complement.
[b] NMS, normal mouse serum.
[c] Determined by plaque assay on DBT cells.
[d] 2,000 rads.

TABLE 3. Comparison of the antiviral activity of PE cells from 4-, 6-, and 12-week-old SJL mice

Age (weeks)	Effector-to-target ratio	Virus titer (PFU/ml)	% Released virus
No cells added		7.2×10^4	100
12 (resistant)	10:1	1.1×10^2	0
	5:1	7.2×10^2	0
	2:1	1.4×10^3	2
	1:1	2.2×10^4	31
	0.5:1	4.6×10^4	64
6 (susceptible)	10:1	9.6×10^1	0
	5:1	3.0×10^2	0
	2:1	5.7×10^2	0
	1:1	2.0×10^4	28
	0.5:1	5.0×10^4	70
4 (susceptible)	10:1	2.3×10^2	0
	5:1	7.0×10^2	0
	2:1	2.2×10^3	3
	1:1	1.4×10^4	27

tive. To insure that the cells from the young mice were similar to those described for 12-week-old mice, PE cells from 4- and 6-week-old mice were tested after irradiation and depletion of the Ia- and Thy-1.2-bearing cells. No loss of antiviral activity was found after these treatments (data not shown).

To insure that the apparent antiviral activity was not due to nonspecific cytotoxic activity resulting in the destruction of the host cells, both DBT and L929 cells, which are known to be susceptible to nonspecific cellular cytotoxicity (9), were tested as targets. Although PE cells from both 6-week-old and 12-week-old SJL mice did exhibit nonspecific cytotoxicity against L929 cells, no killing of DBT cells was detectable at any effector-to-target ratio (Table 4).

Extrinsic antiviral activity by susceptible strains. It appeared from the experiments described above that the ability of PE cells to express extrinsic antiviral activity did not correlate with the age-related resistance to JHMV. It then became of interest to examine the relationship between extrinsic antiviral activity and the genetic basis of resistance to JHMV. We have previously shown that of the 13 strains of mice tested, only SJL mice were resistant to JHMV challenge (22). To determine whether the susceptible genotype would correlate with the inability to suppress virus growth, PE cells from B10.S, BALB/c, and B6 mice were tested. PE cells from susceptible mice were as active in suppressing JHMV as PE cells from resistant SJL mice (Table 5). This shows that the PE cells from susceptible animals are competent at suppressing viral growth and suggests that if extrinsic antiviral activity plays a role in determining resistance to viral infection in vivo, it is not the only mechanism involved.

Intrinsic antiviral activity. We have previously shown that PE cells from both resistant SJL and susceptible B10.S mice exhibited intrinsic antiviral activity; that is, JHMV would not replicate in adherent PE cells from either strain (20). To increase the sensitivity of the intrinsic antiviral assay, we tested different methods of detection and found that scraping and a single cycle of freezing liberated the greatest quantity of infectious virus. Using this approach, we were still unable to detect viral replication in thioglycolate-elicited adherent PE cells from either 6-week-old or 12-week-old SJL mice; however, we were able to detect modest virus replication in adherent PE cells from B10.S mice (Table 6). In addition to B10.S, we tested adherent PE cells from other susceptible strains. The thioglycolate-elicited PE cells from all three strains of susceptible mice supported JHMV replication (Table 6).

TABLE 4. Nonspecific cytotoxicity of PE cells from 6- and 12-week-old SJL mice tested against DBT and L929 cell lines

Age (weeks)	Effector-to-target ratio	% Specific release with cell line:	
		L929	DBT
12	5:1	34	0
	2:1	25	0
	1:1	24	0
	0.5:1	18	0
6	5:1	32	0
	2:1	26	0
	1:1	25	0
	0.5:1	20	0

TABLE 5. Extrinsic antiviral activity of thioglycolate-elicited PE cells from different strains of mice

Mouse strain	Effector-to-target ratio	Virus titer (PFU/ml)	% Released virus
SJL	10:1	3.8×10^2	0
	5:1	9.3×10^2	0
	2:1	6.1×10^3	9
	1:1	2.7×10^4	40
	0[a]	6.8×10^4	100
B6	10:1	6.4×10^2	0
	5:1	1.4×10^3	2
	2:1	1.7×10^3	2
	1:1	1.3×10^4	19
	0[a]	6.6×10^4	100
BALB/c	10:1	4.3×10^2	0
	5:1	5.8×10^2	0
	2:1	7.9×10^3	11
	1:1	2.9×10^4	40
	0[a]	7.2×10^4	100
B10.S	10:1	5.7×10^3	0
	5:1	7.3×10^3	11
	2:1	1.8×10^4	27
	1:1	2.6×10^4	39
	0[a]	6.6×10^4	100

[a] No cells were added.

DISCUSSION

Cells of the macrophage series can play a major role in the ability of the host to defend against viral infection. In vitro, these cells exhibit both intrinsic and extrinsic antiviral effects which are believed to play a role in the defense of the host (11, 12). Extrinsic antiviral activity is the ability of macrophages to suppress virus replication in another cell which supports virus replication. The mechanism of this suppression when due to macrophages from a naive animal is not clear; however, extrinsic activity from herpesvirus-infected mice has been attributed to the action of interferon (8, 12). Intrinsic resis-

TABLE 6. Growth of JHMV in the adherent PE cells from different strains of mice

H postinfection	Virus titer[b] in strain:				
	SJL[a]	BALB/c	B6	B10	B10.S
6	≤10	1.3×10^1	1×10^1	1×10^1	1×10^1
12	≤10	6.3×10^1	9.3×10^1	7.3×10^1	1.0×10^1
24	≤10	2.0×10^3	3.3×10^3	7.0×10^2	0.7×10^1
48	≤10	1.7×10^4	5.7×10^4	9.3×10^3	2.0×10^2
72	≤10	6.0×10^4	5.9×10^4	8.7×10^4	4.3×10^2
96	≤10			9.7×10^4	7.3×10^2

[a] PE cells were prepared from 6-week-old SJL mice.
[b] PFU/5×10^5 adherent PE cells.

tance, on the other hand, is defined as the inability of a macrophage population to support virus replication in vitro. It is related to the ability to either phagocytize and degrade virus, thereby rendering it noninfectious, or to adsorb virus at the cell surface and restrict replication within the cellular cytoplasm (18).

Extrinsic macrophage-mediated antiviral activity directed against JHMV-infected cells was expressed by all PE cells tested, irrespective of whether the PE cells were from resistant or susceptible hosts. The active cells are radiation-resistant, Thy 1.2- and Ia-negative cells. They increase in number but not in frequency after thioglycolate injection, since normal resident PE cells are as active on a per-cell basis as elicited cells. This assay with a coronavirus-infected cell as the target is similar to those used to demonstrate extrinsic antiviral activity against herpesviruses. In contrast to the systems described for herpesvirus, extrinsic anti-JHMV activity is expressed without regard to the genetically determined susceptibility or resistance of the host.

The adoptive transfer of PE cells from resistant to younger susceptible mice partially protects from both herpesvirus and coronavirus infection (4, 20). In addition, the in vivo pattern of resistance to herpesvirus correlates with the expression of extrinsic antiviral activity (17). Therefore, to further examine resistance to JHMV mediated by PE cells, we examined age-related changes in the expression of extrinsic anti-JHMV activity. Thioglycolate-elicited PE cells from young, susceptible SJL mice expressed as much or more extrinsic antiviral activity as cells from older resistant mice. Thus, the expression of extrinsic macrophage-mediated anti-JHMV activity is expressed by uninfected mice regardless of whether the animal is resistant by virtue of genetic background or by virtue of age. This would indicate that if extrinsic antiviral activity is expressed in animals infected with JHMV, additional mechanisms are required for the expression of host resistance.

Adherent PE cells express intrinsic anti-herpes-virus or anti-MHV activity if they are prepared from mice resistant to fatal infection. In both instances, PE cells from younger susceptible mice support virus replication. We have previously shown that there is no such age-dependent correlation of intrinsic antiviral activity against JHMV-induced encephalomyelitis (20). In this report, we have extended our observations to other inbred strains of mice susceptible to JHMV-induced acute encephalomyelitis (19). Only adherent cells from the resistant SJL strain expressed intrinsic antiviral activity. Thus, intrinsic antiviral activity may be a dominant mechanism for determining in vivo resistance to JHMV.

The data presented in this report show that the mechanisms of macrophage-mediated antiviral activity directed against the murine coronavirus JHMV is different from the activity expressed against herpesviruses. Anti-JHMV extrinsic activity is virtually identical whether or not the animal is resistant or susceptible to acute disease. In addition, only resistant animals express intrinsic antiviral activity; however, there is no age-dependent change in intrinsic antiviral activity to JHMV as is expressed against herpesvirus (18). These data indicate that there is a clear difference among the mechanisms of host resistance to infection by enveloped viruses. In addition, there is no relationship between extrinsic antimacrophage activity and the ability to survive acute JHMV infection. Extrinsic antiviral activity in herpesvirus-infected hosts, on the other hand, apparently correlates with the suppression of virus dissemination from a primary target organ to secondary organs (11). In addition, our data suggest that macrophages that are able to support virus replication are nonetheless able to suppress replication in another cell, which is consistent with the concept that extrinsic antiviral activity is the result of some action by macrophages, rather than an inherent property of macrophages.

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