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In Vivo and In Vitro Models of Demyelinating Disease: Efficiency of Virus Spread and Formation of Infectious Centers among Glial Cells Is Genetically Determined by the Murine Host

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Resistance or susceptibility of various mouse strains to central nervous system disease caused by different strains of coronavirus is well known. Data from the present study draw attention to an additional, genetically determined mechanism controlling CV infections. The resistance to A59 and JHM virus (JHMV) associated with SJL mice was maintained in explanted glial cultures which, by contrast, fully supported a productive infection by the serorelated mouse hepatitis virus type 3. A comparative analysis of the infectious process in glial cell explants from SJL and CD.1 mice helped to define the stage at which restriction is manifested. Cultures of oligodendrocytes and astrocytes from these strains of mice were challenged with JHMV or mouse hepatitis virus type 3, and cell-virus interactions were monitored, including adsorption, uptake of inoculum, transcription, and cell-to-cell dissemination. The sequence of early events from adsorption to genome activation occurred with about equal efficiency with both viruses and genetically different cells, indicating that SJL resistance is not due to any deficiency in specific receptors or penetration of the inoculum or general expression of viral functions. However, intercellular spread of the infection was restricted in SJL glial cells owing to an as yet undefined component. Since cells from (SJL × CD.1)F_1 mice were fully susceptible to JHMV, resistance to virus spread must be due to a deficiency in some factor, perhaps a proteolytic activity necessary for dissemination.

Coronavirus (CV) infections in rodents are subject to multifactorial regulation. Among the parameters shown to be involved are the virus serotype, species and genetic constitution of the host, age and developmental stage of the central nervous system at the time of challenge, and route of inoculation (18, 19, 25–27, 31). Thus, SJL mice which are relatively resistant to CVs A59 and JHM virus (JHMV) are highly susceptible to mouse hepatitis virus type 3 (MHV_3). Resistance in this strain may, in part, become manifested in an age-related manner but is also inherent in cells explanted from neonates and young animals, among them macrophages, astrocytes, and neurons (8, 14, 15). One explanation offered to account for resistance of SJL mice to CV is the paucity or absence of viral receptors on the target cells (4). However, detailed analyses by crossbreeding of genetically defined JHMV-susceptible B10 and -resistant SJL mice (31) imply that lack of receptors per se cannot account for the low susceptibility.

The availability of routinely prepared explant cultures of glial cells from mouse brain (35) prompted us to do an in vitro analysis of the CV infectious process in anticipation that an explanation may be forthcoming for the resistance associated with SJL mice.

(Preliminary information from these studies was presented at the 3rd International Coronavirus Symposium at Asilomar in 1986 [36].)

MATERIALS AND METHODS

Continuous line of cells. L-2 mouse fibroblasts (22) were cultured as previously described (35), except that in addition to Eagle minimal essential medium (Flow Laboratories, Inc., McLean, Va.), the complete medium (CM) contained 8% Nu-serum (Collaborative Research, Inc., Waltham, Mass.) plus 2% fetal bovine serum (Bocknek Laboratories).

Viruses. CV strains A59, JHM, and MHV_3 were assayed as PFU per milliliter as previously outlined (20). The viruses were propagated by inoculating subconfluent cultures of L-2 cells with a multiplicity of infection (MOI) of approximately 0.1 PFU per cell. After adsorption for 60 min at 22°C, the unadsorbed inoculum was removed by washing. Fresh CM was added, and the cultures were incubated at 37°C in a humidified atmosphere with 5% CO_2. When approximately 90 to 100% of the cells in the monolayer had been recruited into syncytia, the released extracellular virus was collected by initial centrifugation at 1,000 × g for 30 min to remove larger cell debris and then concentrated by spinning in an SW28 rotor of a Beckman centrifuge at 22,000 rpm for 60 min. The pelleted virus obtained was resuspended in a small volume of CM and again concentrated by centrifugation at 22,000 rpm for 60 min through a cushion of 20% sucrose. The pelleted virus was resuspended in CM, divided into aliquots, and stored at −70°C. Isotopically labeled virus was obtained by carrying out the infection for 12 h at 37°C in the presence of 5 μCi of [^{3}H]uridine (23.4 Ci/mmol; NEN-Du Pont) per ml.

Mice and primary glial cultures. Primary glial cultures from neonatal mice were prepared as previously outlined (35) with minor modifications. Mixed cultures consisting of oligodendrocytes and astrocytes were obtained from dissociated cerebral hemispheres of 1- to 2-day-old mice by propagating in 35-mm culture dishes (Nunc, Roskilde, Denmark) cells derived on an average from 1.8 brains in basal modified Eagle medium plus 10% fetal calf serum (BME10) (GIBCO Laboratories, Grand Island, N.Y.).

Cultures enriched with respect to oligodendrocytes or

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astrocytes were prepared from the primary explants of mixed cells as previously described (35). Cell types in primary cultures were characterized as before (35) by indirect immunofluorescence to myelin basic protein in oligodendrocytes and glial fibrillary acidic protein in astrocytes. Staining for nonspecific esterase determined the frequency of macrophage-type cells. In our mixed cultures, oligodendrocytes and astrocytes predominated, with 0 to 3% cells staining with nonspecific esterase. Cultures enriched specifically for oligodendrocytes were >90% myelin basic protein positive, while astrocyte-enriched cultures were >95% glial fibrillary acidic protein positive. Breeding and crossbreeding were done in-house from stock CD.1 and SJL mice purchased from Jackson Laboratory, Bar Harbor, Maine.

Monitoring virus adsorption and sequestration. Glial cultures kept at 4°C for 10 min were inoculated with [^{3}H]uridine-labeled CV at an MOI of 1 PFU per cell. After adsorption for 60 min on a continuous rocking platform, the unattached virus was removed by three consecutive washes with 1 ml of cold (4°C) phosphate-buffered saline, pH 7.4. Cell-virus complexes were released by scraping off the cells into 1 ml of cold phosphate-buffered saline. Trichloroacetic acid-precipitable counts per minute (cpm) of cell-associated labeled virus were determined with a liquid scintillation counter.

Internalization of inoculum virus after warming to 37°C was quantitated at designated intervals by determination of cell-associated cpm following release of any remnant extracellular virus with 0.5 mg of proteinase K per ml (12). Efficiency of CV association with host cells was also determined by infectious centers (ICT) assay, in which the inoculum was adsorbed for 60 min at 22°C and allowed to penetrate during incubation for 90 min at 37°C, prior to removal of the extracellular inoculum by proteinase K treatment. Samples were taken immediately after penetration (time zero) and after further incubation at 37°C for 24 and 48 h. After release, the glial cells were separated into monodisperse suspensions, which could be serially diluted, mixed with 10^6 L-2 cells, and plated onto 35-mm dishes. Upon attachment, the mixture of L-2 and glial cells was overlaid with 0.5% methylcellulose in CM and incubated at 37°C for 24 h to allow development of plaques. The plaques were enumerated on fixed and stained monolayers (20).

Determination of virus antigens. Expression of virus antigens in glial cells was monitored by immunoperoxidase staining. For this purpose, cultures propagated on 12-mm-diameter glass cover slips were infected and sampled as described in Results. Fixation with acetone for 2 min at 22°C permeated the cells while preserving antigenic reactivity. After attachment of monoclonal anti-JHMV nucleocapsid antibody (kindly supplied by M. Buchmeier, Scripps Research Clinic), anti-mouse immunoglobulin G Vectastain ABC kit (Vector Laboratories Inc.) was used for specific tagging.

Dot-blot analysis of CV RNA. Mixed glial cultures were inoculated and then incubated at 37°C and sampled at 24, 48, and 72 h postinoculation to ascertain expression of virus-specified RNA. Cell-associated RNA was extracted (9), and specificity was determined by dot blotting (6) with cDNA probes (26) against standard quantities of RNA per sample applied to the nitrocellulose.

Enhancement of infectivity by proteases. Glial cultures undergoing infection were exposed to various proteases in a serum-free BME medium. Briefly, explanted cortical cells from CD.1 and SJL mice were inoculated with JHMV or MHV_3 at various MOIs and then incubated for 4 h at 37°C in BME10. The BME10 was removed, and cultures were washed in BME without serum and then treated with 0.1 μg of trypsin, chymotrypsin, or thermolysin (Sigma Chemical Co., St. Louis, Mo.) per ml in serum-free medium for 10 h at 37°C. At the end of incubation, the cultures were monitored for PFU in the supernatant and frequency of cell-cell fusion.

TABLE 1. Replication of CV in oligodendrocytes and astrocytes from resistant and susceptible mice

Mouse strain from which cultures originated	MOI	Titer (10^2 PFU/ml)[a]			
		Oligodendrocytes		Astrocytes	
		JHMV	MHV_3	JHMV	MHV_3
SJL	1	11	3,400	5	750
	10	49	2,100	ND[b]	ND
CD.1	1	180	180	1,950	3,000
	10	390	575	ND	ND

[a] Values shown are averages from duplicate cultures sampled 24 h after inoculation.
[b] ND, Not done.

RESULTS

Virus replication in glial cells from susceptible, resistant, and hybrid mouse strains. Intracerebral inoculation of the viscerotropic MHV_3 strain into either CD.1 or SJL/J mice causes a rapid, fatal encephalomyelitis (unpublished data). By contrast, SJL/J mice are highly resistant to intracerebral infection with the neurotropic JHMV (14, 31), whereas CD.1 mice develop a central nervous system disease (unpublished data). To test whether the appropriate match between host and virus strain associated with central nervous system disease susceptibility could be reproduced in vitro, cultures of mixed glial cells or cultures enriched for oligodendrocytes or astrocytes were challenged with MHV_3, JHMV, or A59 virus and monitored at intervals for the amount of PFU released into the supernatant. MHV_3 was replicated to high titers irrespective of the glial cell type or strain of mouse (Table 1). Data on the time course of virus production (Table 2) reveal that MHV_3 was produced efficiently, so that maximum titers were evident within 48 h in SJL cells and 72 h in CD.1 cells. No further increase in PFU occurred thereafter, undoubtedly because of extensive lysis of the massive syncytia which had formed. By contrast, production of JHMV was restricted in the SJL mixed glial and enriched oligodendrocytic or astrocytic cultures (Tables 1 and 2). The SJL restriction was evident as early as 12 h after inoculation (Table 2). The highest JHMV titers, recorded within 24 h, did not increase further during the subsequent 48 h and were lower by more than 2 orders of magnitude than with MHV_3. Inoculation with 10-fold-greater MOIs appeared to have only a small influence on JHMV titers in SJL cells (Table 1). There was a restriction over replication of A59 in SJL but not in CD.1 cells, corresponding to that observed with JHMV (Table 2). However, with A59 the cytopathic effect was different from that evident with JHMV or MHV_3, because no formation of syncytia was detected (data not shown), confirming earlier observations by others on permissive A59 infection of C57BL/6 primary glial cultures (16). Our results on infection with A59 also demonstrated that cell-cell fusion and syncytium formation are not a prerequisite for the efficient replication of some CVs.

Since there was no appreciable difference in virus yields from mixed glial as compared with oligodendrocytic or

TABLE 2. Comparison of CV replication in glial cultures[a] from purebred and hybrid mice

Mouse strain from which cultures originated	Time after inoculation (h)	Titer (10^2 PFU/ml)[b]		
		JHMV[c]	MHV_3[c]	A59[c]
SJL	12	0.17 ± 0.29 (3)	590 ± 114 (3)	6 ± 2 (6)
	24	23 ± 12 (14)	435 ± 292 (14)	13 ± 0.5 (6)
	48	48 ± 20 (14)	886 ± 62 (10)	2 ± 0.6 (3)
	72	22 ± 9 (12)	475 ± 119 (6)	ND[d]
CD.1	12	350 ± 216 (5)	77 ± 12 (4)	ND
	24	3,260 ± 2,110 (13)	778 ± 30 (11)	21,300 ± 19,600 (3)
	48	9,440 ± 500 (9)	3,340 ± 2,300 (7)	95,800 ± 2,700 (3)
(CD.1 × SJL)F_1	24	961 ± 34 (5)	1,120 ± 730 (5)	ND
	48	6,260 ± 4,320 (5)	2,400 ± 2,000 (5)	ND
	72	943 ± 480 (5)[e]	213 ± 52 (5)[e]	ND

[a] Primarily oligodendrocytes and astrocytes (35).
[b] The values are means with standard deviations. The number of cultures tested is shown in parentheses.
[c] MOI, 1 PFU per cell.
[d] ND, Not determined.
[e] Decrease in titers was attributed to rapid cell killing.

astrocytic cultures, in all subsequent experiments we used mixed cultures.

To test whether restriction over JHMV replication in SJL cells was a genetically dominant or recessive trait, we compared virus produced in glial cells from susceptible, resistant, and F_1 hybrid mice. The F_1 hybrids were bred from matings of CD.1 females with SJL males. A comparison of JHMV and MHV_3 yields (PFU per milliliter) in CD.1, SJL, and (CD.1 × SJL)F_1 cultures (Table 2) clearly revealed that glial cells from hybrid and CD.1 mice were equally permissive for JHMV, thus demonstrating that resistance is recessive. The decline in virus titers evident between 48 and 72 h in Table 2 was attributed to rapid cell lysis, which could be observed by daily microscopic examination.

Early events during CV-glial cell interactions. To determine whether the SJL restriction occurs during an early stage of cell-virus interaction, we examined the efficiency of JHMV and MHV_3 adsorption and uptake by glial cells from resistant and susceptible mice.

Cultures were inoculated at 4°C for 60 min with [^{3}H]uridine-labeled virus, and cell-associated cpm were determined. The results (Table 3) revealed that about equal quantities of inoculum were adsorbed to glial cells from SJL and CD.1 mice. These data indicated that SJL restriction over JHMV is not likely to be due to an absence or paucity of viral receptors.

The next stage of virus-glial cell interaction examined was sequestration of the inoculum. The rate of uptake, expressed as the percentage of cpm cleared from the surface, was about equal in SJL and CD.1 glial cultures (Table 4). Between 68 and 100% of the stably adsorbed inoculum was internalized within 3 h (Table 4). Reduction in cell-associated cpm during the interval from the end of adsorption at 4°C and incubation at 37°C for 1 h was most probably due to desorption of the inoculum, indicating that only a small fraction of the input virus remained firmly attached. By comparison, after adsorption of these agents to the highly efficient L-2 host cells, about 30% of the inoculum initiated stable attachments (data not shown).

Cell-to-cell spread of virus. Previous studies with SJL peritoneal macrophages infected with JHMV (15) and continuous LM-k cells infected with A59 (21) have indicated a defect in the spread of these CVs from initial foci, thereby restricting infection. Since neither adsorption nor internalization appear to be the steps limiting JHMV replication in SJL glial cultures, we examined the possibility that virus dissemination was affected. For this purpose, cells from inoculated SJL and CD.1 glial cultures were analyzed for the frequency of ICT formation. After infection and incubation for 0, 24, and 48 h at 37°C, adherent cells were released and assayed as described in Materials and Methods. Immediately after adsorption, CD.1 cells infected with either JHMV or MHV_3 became ICT about five times as frequently as SJL cells (Table 5). The incidence of ICT in CD.1 cultures infected with JHMV and MHV_3 was amplified about 20-fold during 48 h. Incidence of ICT after infection of SJL cells with MHV_3 increased about 50 times in 24 h and 80 times in 48 h. By contrast, there was a marginal increase in the frequency of ICT in SJL cultures inoculated with JHMV during the 48-h duration of the experiment (Table 5), indicating that SJL restriction may involve cell-to-cell spread of virus.

The above data were corroborated by determining at 12 and 24 h postinfection the time-related differences in the

TABLE 3. Adsorption of inoculum CV labeled with [^{3}H]uridine

Mouse strains from which cultures originated	JHMV[a]		MHV_3[a]	
	Total cpm added	% of initial cpm adsorbed[b]	Total cpm added	% of initial cpm adsorbed[b]
SJL	2,300–8,500	22 ± 2 (6)	2,200–19,000	11 ± 1 (5)
CD.1	2,200–7,200	23 ± 2 (6)	2,100–18,000	10 ± 1 (5)

[a] All cultures were inoculated with an MOI of 1 PFU per cell.
[b] Averages and standard deviations. Number of samples tested is in parentheses.

TABLE 4. Sequestration of [^{3}H]uridine-labeled inoculum CV in glial cells

Mouse strain from which cultures originated	Time (h) after inoculation	JHMV		MHV_3	
		Total cell-associated virus (cpm)[a]	% of adsorbed cpm not removed by proteinase K	Total cell-associated virus (cpm)[a]	% of adsorbed cpm not removed by proteinase K
SJL	0	512	0	2,143	0
	1[b]	121	62	256	66
	3[b]	85	69	246	100
CD.1	0	490	0	1,855	0
	1[b]	104	68	245	65
	3[b]	74	78	246	93

[a] Values are averages from duplicate cultures which are representative of data obtained in additional experiments.
[b] After adsorption at 4°C, cultures were washed and incubated at 37°C with BME10 for 1 or 3 h.

frequency of cells positive for virus antigen. Results (Table 6) revealed a rise in the incidence of syncytia and CV antigen-positive cells in CD.1 cultures inoculated with MHV_3 or JHMV and in SJL cultures inoculated with MHV_3, but not in SJL cultures inoculated with JHMV. These observations, which are entirely consistent with the ICT data, also suggest that SJL restriction over JHMV is due to inability of this virus to spread between cells.

Expression of CV RNA in glial cells from susceptible and resistant mice. Because of the above evidence that JHMV is restricted in SJL cells at a step subsequent to internalization, we investigated whether viral genome expression, in terms of RNA synthesis, might be affected. For this purpose, cells were sampled at intervals postinoculation and the extracted RNA was tested for viral specificity with cDNA probes in dot blots. The specific hybrids formed with the ^{32}P-labeled probes were monitored in autoradiograms of the type illustrated in Fig. 1A. Data from densitometric scans of each dot blot in Fig. 1A, when expressed in terms of area (square millimeters) (Fig. 1B), provided a semiquantitative assessment of CV RNA. These results demonstrated that rates of RNA synthesis were approximately equal at 12, 24, and 48 h after infection of CD.1 cells with JHMV or MHV_3 and SJL cells with MHV_3. By comparison, production of JHMV RNA in SJL cultures was delayed, as evidenced by displacement of the points to the right in Fig. 1B. However, JHMV RNA accumulated gradually in SJL cells so that by 72 to 120 h postinoculation the amount present was about the same as in the other three infections. While surprising, these data imply that the initial target cells continued to synthesize JHMV RNA efficiently.

From these results, it is concluded that synthesis of JHMV RNA in SJL glial cells is unlikely to be the rate-limiting step in the formation of infectious progeny.

Attempts to enhance infectiousness of JHMV in SJL cells. Previous work has demonstrated that infectiousness of bovine (32) and murine (37) CVs can be increased by treatment with proteases, coincident with greater syncytium formation. More recently, investigators have shown that proteolytic cleavage of the E2 glycoprotein of A59 CV activates the Cl 17-1 cell-to-cell fusing property of this agent (11, 33). To check the possibility that restriction on JHMV spread in SJL glial cells resulted from some deficiency in an exoprotease needed after synthesis of RNA, we treated glial cultures with trypsin, chymotrypsin, or thermolysin according to published protocols (23). A minor (about 25%), albeit reproducible, increase in PFU released into the supernatant was observed with trypsin and chymotrypsin, while thermolysin had no effect on the virus titer (data not shown). Microscopic examination of living and fixed cultures failed to reveal any increase in syncytia. Additional experiments to show activation of viral dissemination after protease treatment of SJL glial cultures were also attempted. Experiments including cocultivation of SJL ICT with uninfected CD.1 or L-2 cells and treatment of infected SJL cells with combinations of proteases all failed to enhanced virus spread or release of PFU. Therefore, any putative SJL deficiency in protease activation of JHMV infectiousness remains to be demonstrated.

TABLE 5. ICT assay of glial cells

Mouse strain from which cultures originated	Time (h) after infection	ICT/1.2 × 10^6 cells[a]	
		JHMV[b]	MHV_3[b]
SJL	0	153 ± 62 (6)	124 ± 59 (6)
	24	825 ± 572 (9)	5,932 ± 2,262 (5)
	48	538 ± 395 (9)	>10,000 (4)
CD.1	0	871 ± 317 (5)	394 ± 81 (5)
	24	>10,000 (2)	1,417 ± 439 (3)
	48	>10,000 (2)	>10,000 (5)

[a] Values are averages and standard deviations. Number of samples tested is in parentheses.
[b] All cultures were inoculated with an MOI of 1 PFU per cell.

TABLE 6. Frequency of glial cells containing CV antigen

Mouse strain from which cultures originated[a]	Virus strain[b]	Time (h) postinoculation	% of antigen-positive cells	Frequency of syncytia/10^3 cells[c]
CD.1	MHV_3	12	1.4 ± 0.5	1 (2)
		24	15.7 ± 3.1	20 (3)
	JHMV	12	2.2 ± 0.4	2 (3)
		24	9.0 ± 2.3	13 (4)
SJL	MHV_3	12	2.6 ± 0.8	1 (4)
		24	13.3 ± 2.2	33 (3)
	JHMV	12	1.8 ± 0.7	2 (2)
		24	0.7 ± 0.3	0 (0)

[a] Cell density was approximately 3 × 10^5 cells per well.
[b] All cultures were inoculated with an MOI of 1 PFU per cell.
[c] Average number of nuclei per syncytium is in parentheses.

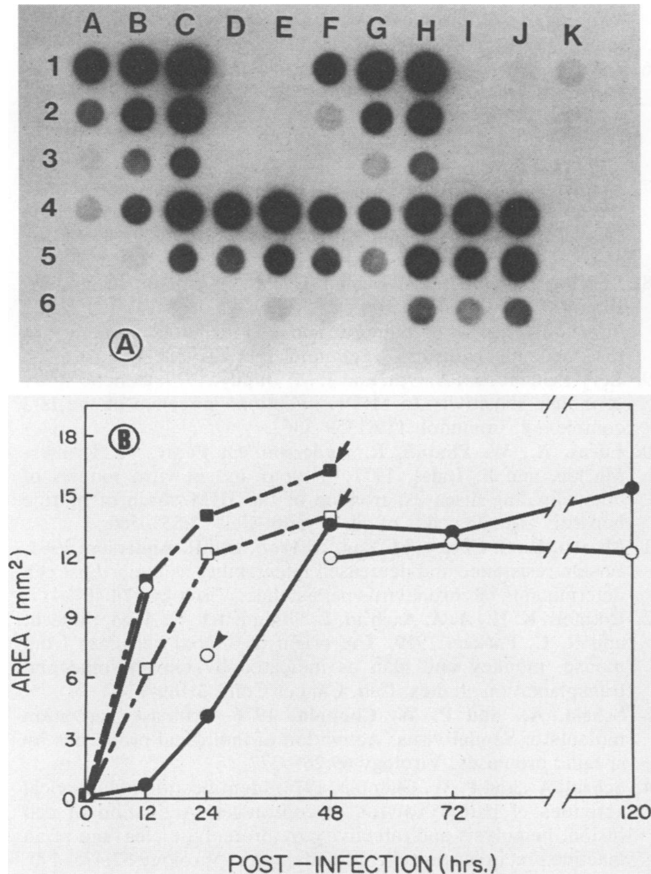

FIG. 1. (A) Dot-blot analysis of CV RNA present at various times after infection of mixed glial cultures. Lanes: A to C, CD.1 JHMV RNA 12, 24, and 48 h, respectively, postinfection at 1:1 (1), 1:10 (2), and 1:100 (3) dilution; F to H, CD.1 MHV_3 RNA 12, 24, and 48 h, respectively, postinfection at 1:1 (1), 1:10 (2), and 1:100 (3) dilution; A to E, SJL JHMV RNA 12, 24, 48, 72, and 120 h, respectively, postinfection at 1:1 (4), 1:10 (5), and 1:100 (6) dilution; F to J, SJL MHV_3 RNA 12, 24, 48, 72, and 120 h, respectively, postinfection at 1:1 (4), 1:10 (5), and 1:100 (6) dilution; I to K, RNA from uninfected CD.1, SJL, and L cells, respectively, at 1:1 (1), 1: 10 (2), and 1:100 (3) dilution. (B) Densitometric scans comparing dot-blot analyses of CV-specific RNA in the CD.1 and SJL glial cell cultures infected with JHMV or MHV_3 shown in panel A. The concentrations of [^{32}P]cDNA bound are expressed as the area in square millimeters occupied by individual dots. Arrows, Sampling terminated owing to extensive cytopathic effect. Arrowhead, Abnormally low value. Symbols: ○, SJL MHV_3 infection; ●, SJL JHMV infection; □, CD.1 MHV_3 infection; ■, CD.1 JHMV infection.

DISCUSSION

The low efficiency of JHMV in vitro replication in glial cells from SJL mice, documented in this study, should be related to resistance of adult SJL mice to this agent (14, 31; unpublished data). On the basis of crossbreeding studies, resistance to JHMV in SJL mice has been postulated to be controlled by a single gene (14) or by two factors (31). The ability to challenge glial cells in vitro with CVs enabled us to demonstrate unequivocally that strain-specific susceptibility of CD.1 mice and resistance of SJL mice is a genetic trait already inherent in neonatal cells. Since peritoneal macrophages (14, 15, 30), neurons (14), and perhaps all potential host cells in the body conform to the same constitutive control over virus replication, the age-related onset of SJL resistance must involve an independent, perhaps immune, mechanism, implying that two independent factors are controlling JHMV expression in vivo. The level of resistance may be greater with cells of nonglial origin, as evidenced by the lack of virus production previously reported with JHMV in SJL primary macrophages and neurons (14, 15, 30). In view of the very low contamination, if any, by brain-derived macrophage cells in both CD.1 and SJl cultures, one can discount the influence of macrophages on suppression of JHMV replication, as observed when high concentrations of peritoneal macrophages were deliberately added to continuous cultures of glial cells (30).

Our genetic evidence showed that (i) closely related CV serotypes can either multiply efficiently, as found with MHV_3, or are greatly restricted, as is the case with JHMV and A59 and that (ii) based on data from hybrid F_1 mice, susceptibility is a dominant trait, in line with results from previous in vitro studies (8, 15). From these findings one can assume that some cellular factor interacting with a viral component which is necessary for production of infectious particles is missing from SJL cells. In our systematic dissection of a sequence of steps in cell-virus interaction, analysis of adsorption showed that CV receptors for JHMV and MHV_3 are about equally abundant in CD.1 and SJL glial cells. These findings are supported by an ICT assay which showed that the frequency at which cells become infected initially is not determined by resistance or susceptibility of the mouse strain. Our data are therefore inconsistent with recent findings by others (4) who concluded, on the basis of a solid-phase assay in which virions were bound to isolated plasma membranes of hepatocytes and enterocytes fixed onto nitrocellulose sheets, that SJL restriction to CV A59 is due to a lack of receptors on target cells. Our studies monitoring the uptake and sequestration of the inoculum indicated that this stage of the infection also proceeds with equal efficiency regardless of the virus type or strain of mouse from which glial cells are derived. Nor is the expression of viral RNA, assessed by dot blots and immunomicroscopic detection of antigens, appreciably affected in the initial SJL cell targets of JHMV. Results from ICT assays and frequency of cells expressing CV antigen led us to conclude that low yields of JHMV in SJL cells are due to the inability of the progeny released from initially infected targets to spread the infection. This could be related to an absence of JHMV-induced syncytia from SJL cultures and their presence in CD.1 cultures. It should, however, be remembered that spread of virus and syncytiogenesis are not necessarily connected, as demonstrated with A59 virus.

If JHMV is restricted in SJL cells because the infection cannot spread from the primary targets, our attempts to facilitate virus spread by treatment with proteases were inconclusive, although a similar approach revealed interrelationships between posttranslational modification of peplomer glycoproteins of the orthomyxoviruses (13, 17), paramyxoviruses (23, 24), and CVs (11, 32, 33, 37) and activation of infectiousness, virulence, and cell fusion. In the CVs, the enzymatic target is the E2 glycoprotein, involved in cell-cell fusion, attachment, and infectiousness (5, 7, 10, 34). The limited PFU enhancement obtained with trypsin and chymotrypsin in our studies provides inadequate data to support any claim that a protease is, indeed, the missing SJL factor required for activation of JHMV.

In conclusion, the present study demonstrated the existence of a genetically regulated factor controlling the intercellular spread of CV. This type of regulation over the

infectious process should be added to the list of other mechanisms examined by us previously, including influence of cellular immunity (28, 29), cell-specified tropisms (2), and a developmental time clock of glial cell differentiation in the central nervous system (1–3, 35) which may affect the disease process.

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