Peritoneal Barrier to the Spread of Semliki Forest Virus in Mice (42831)

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Abstract. **The LD_{50} for encephalitis caused by Semliki forest virus in 6- to 8-week-old mice is 1 plaque-forming unit (PFU) in C3H/Ten strain of mice when injected intracerebrally, iv, or in the footpad; however, the LD_{50} by the ip route is 4×10^3 PFU. In the ICR strain of mice at the same age, the LD_{50} for the intracerebral route is 1 PFU, 10^3 PFU for the iv and footpad routes, and 4×10^3 PFU for the ip route. A number of *in vivo* and *in vitro* experiments were done to explain the relative resistance to Semliki forest virus injection by the ip route. The results suggest that the viruses are adsorbed to and enter adherent cells of the peritoneal cavity but do not replicate and release progeny virus. After inoculation with the virus, viral antigens could only be observed in methanol-treated cells as a halo by immunofluorescence at or just below the plasma membrane of only a small fraction (<0.5%) of peritoneal adherent cells. Naturally occurring interferon-α/β (<1 unit/ml) was found to probably play a marginal role, if any, in the resistance.**
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Semliki forest virus (SFV) is a positive-stranded RNA virus in the family *Togaviridae* (1), many members of which can cause a variety of pathologic conditions including rash, fever, arthritis, and encephalitis in many animal species (2). Natural infection by togaviruses is transmitted by the bite of persistently infected mosquitoes and is systemically spread by the hematogenous route; however, animals can be artificially infected with these viruses, including SFV, by various routes of injection and, depending on which route is used, the relative susceptibility will vary widely (3–9). In the mouse, the central nervous system is the target organ most directly affected, which results in a lethal encephalitis (10, 11). In general, the intracerebral route of injection requires the lowest dose (4, 12), whereas the ip route even in young weanling mice, frequently requires doses several orders of magnitude higher (3–5, 9).

In our previous studies (13–17), we found that the LD_{50} for SFV is 4×10^3 plaque-forming units (PFU) by the ip route in the Swiss ICR or C3H strains of mice in contrast to an LD_{50} of 1 by the intracerebral route. However, Kraaijeveld *et al.* (18), using the BALB/c strain of mice, reported that the LD_{50} for the ip or intracerebral routes was only 1–2 PFUs of SFV. In that model, virus given in excess above the LD_{50} appeared to spread quickly out of the peritoneal cavity with about 20% of an inoculum of 175 PFU present in serum at 45 min and about 70% at 180 min, a time interval preceding any virus multiplication. Furthermore, what remaining virus could be recovered from the peritoneal cavity suggested that the virus was not adsorbed to, or inactivated by peritoneal cells or soluble factors. This may account for the sensitivity of BALB/c mice to SFV when given by the ip route.

In this report, we confirm our previous findings, and those of others, that the level of PFU required to produce an LD_{50} is much higher (>1000-fold) by the ip route than by other routes in C3H and ICR mice. We found, as did Kraaijeveld *et al.* (18), that virus does not replicate in peritoneal cells. However, in contrast to their work, where virus did not appear to adsorb to peritoneal cells and offered no barrier to virus entry into the blood, we found that elements in the peritoneal cavity prevent the dissemination of the virus into the blood. To explain this, we found that naturally occurring interferon (IFN)-α/β plays only a marginal role, if any, in the resistance but a subpopulation of resident peritoneal cells *in vivo* and adherent peritoneal cells *in vitro* rapidly adsorbs added SFV, although the virus does not replicate in these cells.

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Materials and Methods

Animals. C3H/Ten mice were obtained from the breeding colony of the University of Tennessee Medical Center (Knoxville, TN) and ICR mice were obtained

from Harlan Sprague-Dawley (Indianapolis, IN). The mice were used at 6–8 weeks of age.

Virus. The SFV strain was originally obtained from Casals and passaged three times through neonatal mouse brains. After plaque purification, the virus was propagated in primary chick embryo fibroblasts (CEF) for three passages and then stored at −70°C. Viral titers were obtained by infecting CEF monolayers with serial dilutions of virus stock and overlaying the cells with modified Eagle's medium-agar (one part modified Eagle's medium, one part 1.8% Noble agar) containing 100 units of penicillin and 100 μg of streptomycin/ml and 2% fetal bovine serum (FBS; Sterile Systems, Logan, UT). After 2 days, a 1/11,000 dilution of neutral red was added and the numbers of plaques were counted. The stock titer was 2.7×10^9 PFU/ml. For *in vivo* assay, a virus stock was used, which had been passaged twice in newborn mouse brains. The stock titer was 1.2×10^9 PFU/ml. All dilutions were made in brain-heart infusion broth.

Cells. Mouse L929 fibroblasts were cultured routinely in McCoy's modified 5A medium (GIBCO, Grand Island, NY) supplemented with 10% FBS, 50 units of penicillin and 50 μg of streptomycin/ml, Hepes, and $NaHCO_3$ added to pH 7.4.

CEF were prepared from 10- to 11-day-old embryonated eggs after decapitation and evisceration. They were cultured in Medium 199 supplemented with 5% FBS, 50 units of penicillin and 50 μg of streptomycin, Hepes, and $NaHCO_3$ to pH 7.4.

Peritoneal cells were harvested from fluid recovered after injection into mice of 5 ml of cold RPMI 1640 supplemented with 10% FBS, penicillin-streptomycin, Hepes, and $NaHCO_3$ to pH 7.4. The fluid was centrifuged at 800*g* for 10 min and the cell pellet suspended in RPMI 1640 medium. Viability was determined by trypan blue dye exclusion and the cells were usually seeded into 6- or 24-well microtiter plates at a concentration of 1 mouse equivalent (approximately 2×10^6 cells) per 2 cm^2 growth area. For immunofluorescence experiments, the wells contained round, glass coverslips, which could be removed to determine cell counts on a hemocytometer.

Peritoneal Fluid. Peritoneal fluid was examined for antiviral substances. It was obtained from normal C3H/Ten mice by injecting 5 ml of cold Hanks' balanced salt solution ip, massaging the abdomen, and withdrawing the fluid. Cells were removed by centrifugation at 800*g* for 10 min and fluid was used undiluted or diluted with McCoy's medium.

Effect of Interferon-α/β. L929 cells were pretreated with normal peritoneal fluid, uv-treated fluid from SFV-infected mice, or interferon-α/β (100 IRU/ml) for 24 hr before SFV infection. As controls, the fluid or IFN were irradiated with uv light (virus controls showed that exposure to uv irradiation for 3 min inactivated 99.4% of the SFV) or incubated for 1 hr with anti-IFN-α/β (Lee Biomolecular Research Lab) diluted 1/20 in growth medium. After 3 days of incubation at 37°C, the number of plaques were determined.

To test the effect of anti-IFN-α/β *in vivo*, ICR mice were treated ip with 1.25×10^3 standard neutralizing units (SNU) or 8.6×10^3 SNU of rabbit anti-IFN-α/β serum or an equivalent amount of normal serum, then infected 24 hr later with 0.8 LD_{50} of SFV. The animals were observed twice each day for 21 days.

Immunofluorescence. The bottoms of 24-well microtiter plates (Falcon, Becton-Dickinson Labware, Oxnard, CA) were etched with acetone and then sterile, round glass microscope coverslips were placed in each well. After washing with phosphate-buffered saline (PBS), 1 ml of approximately 2×10^6 peritoneal cells (1 mouse equivalent) from normal or SFV-infected mice suspended in RPMI 1640 medium was added to each well. After incubation at 37°C for 3.5 and 18 hr, the nonadherent cells were washed off with RPMI 1640 medium. The number of cells was determined by inverting a representative coverslip onto a hemocytometer, counting the cells within 1 mm^2, and multiplying by 201 (2.01 cm^2, the area of the microtiter well bottom).

For *in vitro* infections of the adherent cells, 1 ml of SFV diluted in RPMI 1640 to the appropriate multiplicity of infection (moi) was added, the cells were incubated for 1 hr at 37°C and then washed with RPMI 1640 medium, and finally incubated an additional 30 min. The cells were air dried and either fixed for 10 min in absolute methanol or not fixed. Duplicate wells (containing normal or infected cells) were treated with rabbit preimmune serum γ-globulin or anti-SFV γ-globulin for 1 hr at 37°C, washed three times with PBS, and then treated with fluorescein isothiocyanate-conjugated goat anti-rabbit γ-globulin (Zymed, San Francisco, CA) diluted 1/20. All sera were preabsorbed with adherent peritoneal cells. After 1 hr at 37°C, the cells were washed three times in PBS, air dried, mounted on a glass slide with a 1:1 mixture of glycerin and PBS, and examined with a Nikon uv microscope.

Indomethacin Treatment. Indomethacin solution was made by dissolving 13 mg in 4 ml of 5% $NaHCO_3$ heated to 50°C. Distilled water was added to a final volume of 16 ml and 0.2 ml (0.13 mg) was injected (5 mg/kg) ip on Days 1, 2, and 3. One hour after the injection on Day 2, 1 LD_{50} of SFV was given ip. Controls included diluent or PBS. All mice were observed twice per day for 21 days.

Results

Based on our previous findings that 1 PFU of SFV given intracerebrally and 4×10^3 PFU given ip were the LD_{50} for either 6- to 8-week-old C3H/Ten mice, an inbred strain of mice, or 6- to 8-week-old Swiss ICR, an outbred strain, we first chose to compare the LD_{50} in these two strains of mice when the virus was given

by other routes of inoculation. We believed that uniform high dose levels would be required for all peripheral challenges with our strain of SFV in contrast with the ip challenge of SFV used in the BALB/c strain (18) where 1–2 PFU produced an LD_{50}. The results presented in Table I show that the LD_{50} in C3H mice given SFV by the intracerebral, iv, or footpad routes is 1–2 PFU/mouse but by the ip route it is 4×10^3 PFU. In the ICR strain, the LD_{50} for the intracerebral route and the ip route is the same as for the C3H strain. However, in contrast to the C3H strain, the LD_{50} by the iv route in ICR mice is 10^3 PFU. It is apparent that the relative resistance of mice to what may be described as moderately virulent (11) SFV given by different peripheral routes does vary among strains of mice; however, one common finding for C3H and ICR strains is that much higher doses are required when the mice are challenged by the ip route. It is the C3H strain on which we focused our studies to investigate why the mice were relatively resistant to inoculation by the ip route. It is those results that are presented below.

Antiviral Factors in the Peritoneal Cavity. It has been reported that SFV injected ip can disseminate rapidly into the vascular system (18) presumably via the subdiaphragmatic lymphatics (6). On the basis of the LD_{50} data in Table I, it was assumed that if 1 PFU entered the circulation in an C3H mouse, the mouse would succumb to encephalitis; however, the high LD_{50} required for ip inoculation suggested that the virus was rapidly and directly inactivated or otherwise inhibited in its replication in the peritoneal cavity. To test for the presence of antiviral factors, cell-free peritoneal washings from normal mice were used to treat SFV *in vitro* for 30 min before infection of L929 cells in culture to detect any substance that would directly inactivate virus or to pretreat L929 cells for 1.5 hr before infection with SFV to detect substances that might indirectly interfere with virus production. The number of plaques on L929 cell monolayers after each treatment are given in Table II and indicate that in two experiments, pretreatment of the monolayers with peritoneal wash resulted in a reduction of 25 and 27%, respectively, in the number of plaques. Additionally, pretreatment of the inoculum caused a 23 and 27% reduction which could be a direct effect on the virus or, more likely, a transfer of peritoneal factors with the inoculum to the monolayers. The reduction by either pretreatment was considered to be caused by naturally occurring low levels of IFN-α/β in the peritoneal wash. Apparent inactivation of the inhibiting factor was attained by heating the peritoneal fluid at 100°C for 30 min, which is consistent with the factor being heat labile (e.g., IFN-α/β) under these conditions.

Table I. PFU/LD_{50} and Average Day of Death for Mice Infected with SFV by Various Routes of Injection

Route	C3H/Ten		ICR/Harlan	
	PFU/LD_{50}	Average day of death[a]	PFU/LD_{50}	Average day of death[a]
Fpad	2	10.3	10^3	9.5
iv	1	10.3	10^3	8.0
ic	1	6.6	1	6.7
ip	4×10^3	8.9	4×10^3	9.0

[a] At doses resulting in 1 LD_{50}.

Interferon Induction. Although antiviral factors appeared to be present in normal peritoneal washings, it did not appear to be at a level sufficient to account for the putative peritoneal barrier to dissemination of SFV. On the assumption that the antiviral factor was IFN-α/β and would increase above the naturally occurring low level with virus infection, mice were injected ip with 1 LD_{50} of SFV (4×10^3 PFU) at 24 hr before peritoneal fluid harvest, which was then used to pretreat L929 monolayers 24 hr before infection. As a control, 100 IRU/ml of commercial IFN-α/β was also added to L929 cells for 24 hr before infection with SFV. Anti-IFN-α/β was added to some cultures containing the known amount of IFN-α/β and to those with peritoneal

Table II. Number of SFV Plaques on L929 Monolayers Pretreated with Normal Peritoneal Wash Before Infection or Infected with Virus Inoculum Pretreated with Normal Peritoneal Wash

Pretreatment with peritoneal wash of:	Experiment 1 (PFU/ml ± SD)	% change[a]	Experiment 2 (PFU/ml ± SD)	% change
L929 monolayers for 1.5 hr, then washed with medium before infection	143 ± 11.8	−25	291 ± 17.5	−27
SFV inoculum for 30 min before addition to monolayers	148 ± 28.7	−23	290 ± 20.0	−27
Controls				
SFV inoculum pretreated with heated (100°C, 30 min) peritoneal wash before addition to monolayers	211 ± 6.2	+10	ND[b]	
No pretreatment of cells or inocula before infection with SFV	192 ± 16.8		400 ± 11.4	

[a] Number of plaques on monolayers after treatment of cells or virus inoculum with normal peritoneal wash/number of plaques on untreated control × 100.
[b] ND, not done.

fluid to determine whether the antiviral effect would be inhibited. Ultraviolet light from a germicidal lamp was used to inactivate any residual SFV in the peritoneal washes.

The results presented in Table III show that pretreatment of cells with peritoneal wash from normal mice caused a 48% reduction in the number of plaques, compared with that of the control of SFV only. This reduction was completely abrogated by specific anti-IFN-α/β serum indicating that IFN-α/β was responsible for the reduction. Peritoneal wash from mice infected with SFV for 24 hr and then irradiated caused a 78% reduction in the number of plaques, which was also completely abrogated by anti-IFN-α/β serum.

Since the reduction in the number of plaques in monolayers pretreated with peritoneal wash from SFV-infected mice was equivalent to that observed with 100 IRU of IFN-α/β added to other monolayers (i.e., 78% reduction vs 82%), it was of interest to determine whether IFN was the protective factor. Anti-IFN-α/β was given to mice 24 hr before challenge with 0.8 LD_{50} of SFV. This dose was chosen because a loss of protection would be observed as a significant increase in the number of deaths. Two doses of anti-IFN-α/β were used: 2.5×10^3 SNU and 8.6×10^3 SNU/mouse. The results in Table IV show that there was a marginal increase, if any, in mortality ($p_t < .2$) with mice receiving rabbit anti-IFN-α/β compared with that of control mice receiving normal rabbit serum. It appears that the amount of IFN-α/β found in normal mice (approximately 1 IRU) or induced in mice infected for 24 hr (<100 IRU) is not sufficient to inhibit all of the SFV given ip and that another barrier to virus dissemination from the peritoneal cavity must be operative.

Indomethacin Treatment. Prostaglandins produced by macrophages have been implicated as immunoregulatory (19, 20) and their possible contributing role in the peritoneal barrier was tested. Mice were treated with indomethacin, which is known to inhibit arachidonate metabolism not only in the cyclooxygenase pathway leading to prostaglandin synthesis (21) but also in the lipoxygenase pathway (22). If metabolic products of these pathways (e.g., prostaglandins, leukotrienes) were inhibiting the virus directly or indirectly, the treatment with indomethacin should enhance virus infectivity. Thus, after treatment, mice were infected with a suboptimal dose (0.8 LD_{50}) of SFV ip. The results in Table V indicate that indomethacin had no significant effect on survival ($p_t < .5$) in contrast to a more virulent infection that might have been anticipated.

Peritoneal Adherent Cells. We next investigated a possible direct antiviral role of peritoneal adherent cells. Mice were infected with SFV ip at varying doses and peritoneal adherent cells were harvested 30 min later. It should be noted that about 36% of virus inocula given ip at doses equal to or less than 1 LD_{50} (4×10^3 PFU) was detected free in the peritoneal fluid at 45 min and 5–6% at 180 min; however, no virus was

Table IV. Mortality and Average Day of Death of Mice Given Anti-IFN-α/β and SFV[a] ip

Treatment	Number dead/total	% deaths	Average day of death	p_t[b]
1.25×10^3 SNU of anti-IFN-α/β serum	5/10	50	9.9	<.2
8.6×10^3 SNU of anti-IFN-α/β serum	6/10	60	8.7	<.2
Normal rabbit serum	3/10	30	9.0	

[a] 0.8 $LD_{50} = 3.2 \times 10^3$ PFU.
[b] Student's *t* test.

Table III. Percentage of plaque reduction of SFV on L929 Monolayers Pretreated for 24 hr with Peritoneal Wash from Normal Mice or Peritoneal Wash from 24-hr-Infected Mice

Pretreatment of monolayers with peritoneal wash from:	Number of plaques	% plaque reduction[a]
Normal mice; added 16 hr before SFV	78 ± 9	48
Normal mice given anti-IFN-α/β	152 ± 6	0
Mice infected with SFV for 24 hr; wash was uv irradiated before use	32 ± 4	78
Mice infected with SFV for 24 hr; wash was uv-irradiated and added with anti-IFN-α/β to each culture	146 ± 7	0
Controls		
IFN-α/β, 100 IRU added to each culture 16 hr before SFV	27 ± 5	82
IFN-α/β (100 IRU) plus anti-IFN-α/β added to each culture 16 hr before SFV	155 ± 8	0
UV-irradiated IFN-α/β added to each culture	27 ± 3	82
UV-irradiated inoculum added to each culture	1 ± 1	99.3
SFV only	149 ± 7	0

[a] Done in triplicate. Experiment repeated with similar results.

detected in the blood at any time. The peritoneal cells were seeded into 24-well plastic microtiter plates with each well containing a round, glass coverslip. After 22 hr of incubation, the cells were washed, treated with methanol, and then with rabbit anti-SFV or normal γ-globulin. Finally fluorescein isothiocyanate-conjugated goat anti-rabbit IgG serum was added. A typical result is shown in Figure 1. Only a low fraction (≤1 in 200) of cells were positive for viral antigens and the antigens appeared to be localized on or just below the plasma membrane. Although the cells had been infected for 22 hr before staining, viral antigens were not present throughout the cytoplasm, suggesting that no significant virus replication was taking place. Viral antigens could not be observed in cells that were not pretreated with methanol.

Table V. Mortality and Average Day of Death of Mice Given Indomethacin and SFV[a] ip

Treatment	Number dead/total	% deaths	Average day of death	p_t
Indomethacin + SFV	4/10	40	10.8	<.5
Indomethacin + virus diluent, no virus	0/10	0		
0.1 *M* $NaHCO_3$[b] + SFV	6/10	60	8.9	

[a] 0.8 $LD_{50} = 3.2 \times 10^3$ PFU.
[b] Indomethacin diluent.

To test further for the susceptibility of peritoneal cells to SFV, the *in vitro* infection of 18-hr cultured adherent peritoneal cells was made at varying moi of 0.002–20. The results were similar to the *in vivo* infection in that only a small fraction of methanol-treated cells (≤1 in 200) contained viral antigen at or just below the plasma membrane. These findings suggest that a small subpopulation of peritoneal adherent cells can take up SFV within 30 min but the virus does not replicate. Presumably, any free virus that was not initially adsorbed to these cells could enter the sudiaphragmatic lymphatics and reach the retrosternal lymph nodes; there is no direct route of entry into the blood except via the lymphatics (6). We suspect that virus entering the lymphatics would be adsorbed by cells similar to the subpopulation in the peritoneal cavity since any virus reaching the blood (1–2 PFU) would be equivalent to a LD_{50}.

It was possible that the replicative cycle was much slower in the infected subpopulation of adherent cells. Consequently, cells were infected *in vitro* at moi of 20 and cultured for 1, 2, 3, or 4 days before immunofluorescent staining. There was no observable increase in antigen-positive cells and, in fact, viral antigens could not be detected in or on the cells at 4 days. After 4

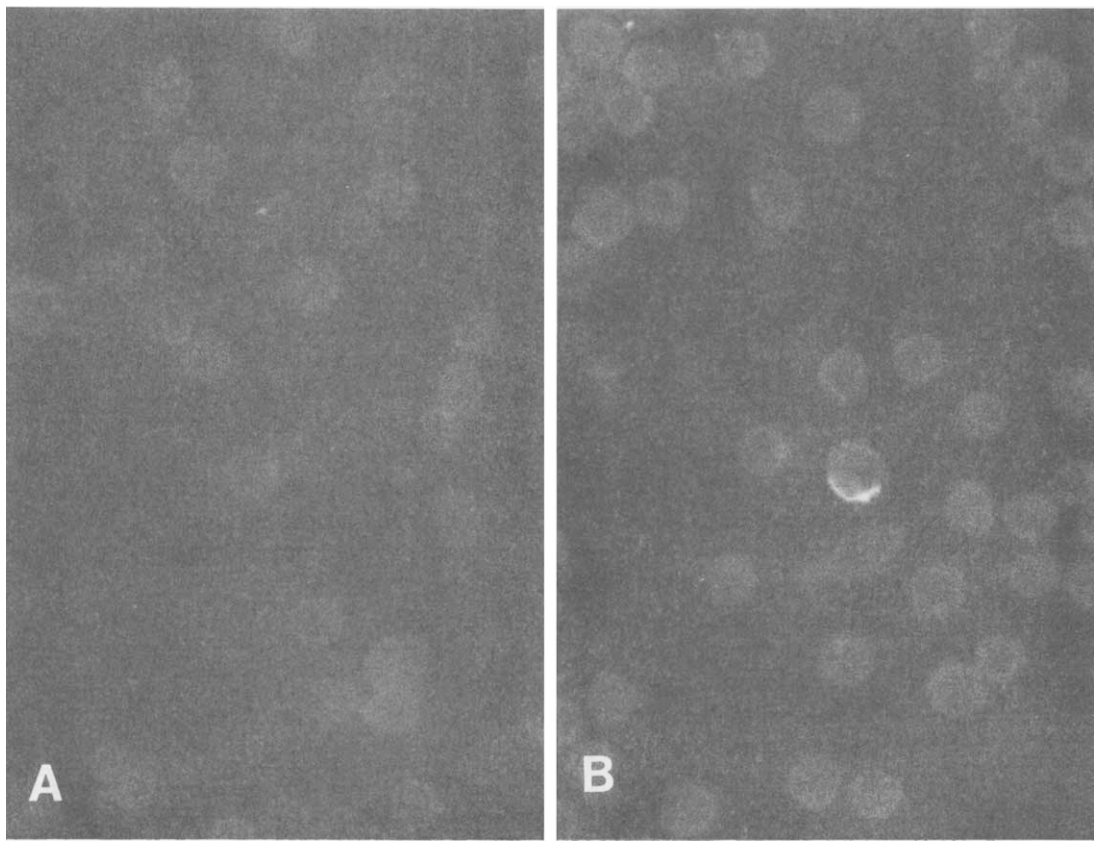

Figure 1. Immunofluorescence of SFV-infected, methanol-fixed peritoneal adherent cells. *A*, Normal rabbit γ-globulin used as first antibody. *B*, Rabbit anti-SFV γ-globulin used as first antibody.

days, infected or mock-infected cells were rounding and becoming detached. Control infections with uv-irradiated killed virus showed a similar pattern of viral antigens in an analogous small subpopulation of adherent cells. To be sure that virus was not destroying cells, the number of adherent cells were determined by removing the coverslip from each well and inverting each onto a hemocytometer. The number of cells per mm^2 was then determined. The results given in Figure 2 show no significant changes in the number of cells through Day 4. It was also noted that the cells were attached to the glass as expected, by fibroblastic-like extensions, and all stained with the vital dye, neutral red.

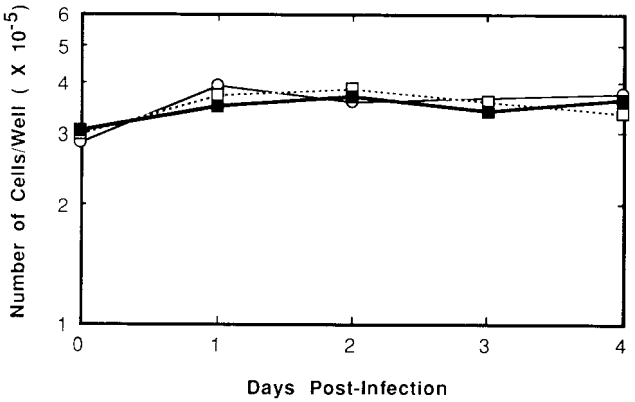

Figure 2. Number of viable adherent peritoneal cells *in vitro* by trypan blue dye exclusion at 0, 1, 2, 3, and 4 days after infection with SFV (moi = 2). Filled squares, SFV infected cells; open squares, mock-infected cells; open circles, normal untreated cells.

Since the number of cells that show immunofluorescence (less than 1 per 200) and the apparent intensity of immunofluorescent label in these cells does not increase with increased moi (Fig. 2), we assumed that only that subpopulation can adsorb SFV. To test this assumption, the fate of the virus in the cells was determined by infecting peritoneal adherent cells *in vitro* with SFV at several moi, 20, 2, 0.2, and 0.002, and culturing them for 0, 1, 3, or 4 days. It is worthy of note that an moi of 0.002 is the estimated equivalent of 4×10^3 PFU (LD_{50})/mouse based on the number of peritoneal adherent cells obtained from a mouse (2×20^6 cells). Samples of supernatant fluids were assayed for live virus on CEF monolayers and the infected cells themselves were assessed for virus by infectious center assays. The results (Fig. 3) indicate that the virus yield in PFU per 100,00 adherent cells decreases from the time of infection through Day 3. On Day 4, a slight increase in titer was observed at moi 2 and 0.2; however, the highest increase with moi of 2 was 1.7 logs less than the level of the inoculum. Likewise, the number of infectious centers (Fig. 4) decreases from the time of infection through Day 3 and then increases on Day 4 in cultures with initial moi of 0.2 and 2. This increase was much less than that expected from the inoculum itself (decrease of 3 logs). On Day 5, the cells were rounding and detaching from the glass surface and

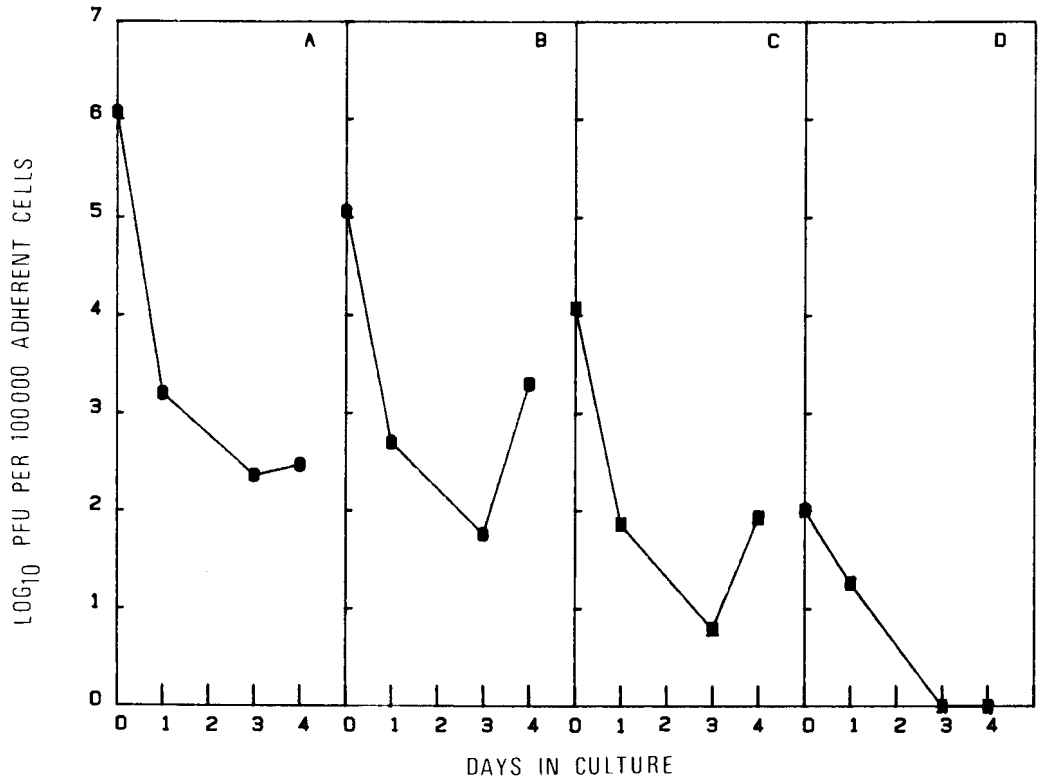

Figure 3. Log_{10} PFU in supernatant fluid per 100,000 adherent peritoneal cells infected with SFV and cultured for 4 days. Panel A, moi of 20; Panel B, moi 2; Panel C, moi of 0.2; Panel D, moi of 0.002.

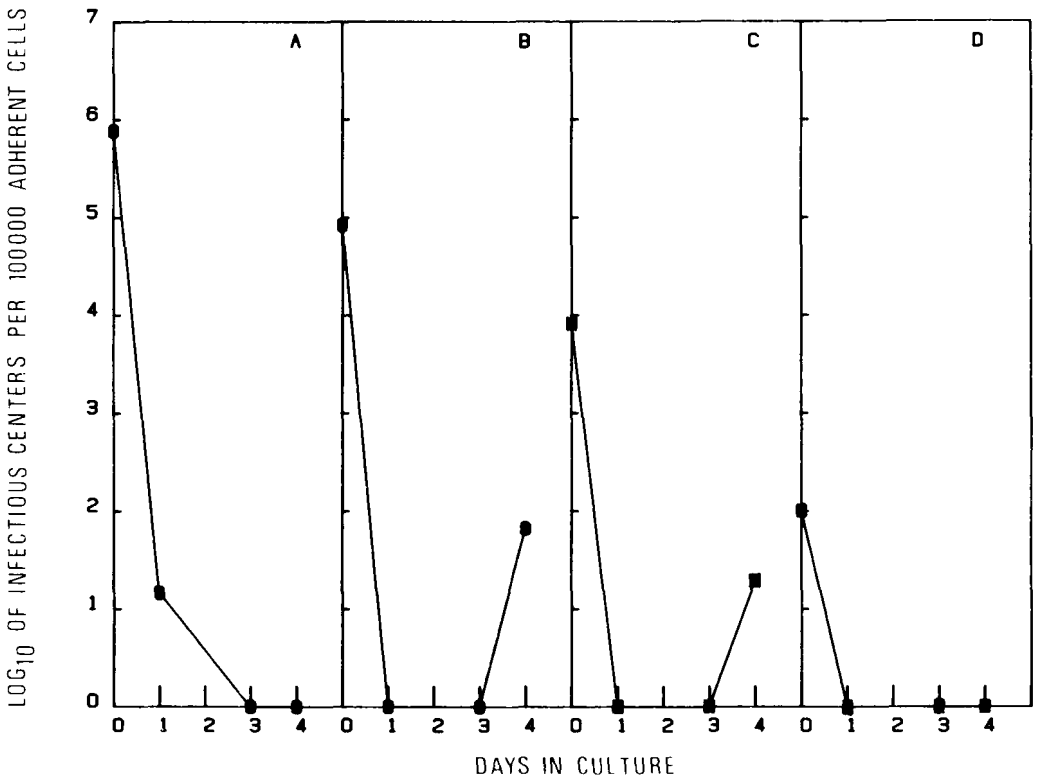

Figure 4. Log_{10} of infectious centers per 100,000 adherent peritoneal cells infected with SFV and cultured for 4 days. Panel A, moi of 20; panel B, moi of 2; Panel C, moi of 0.2; Panel D, moi of 0.002.

could be stained with trypan blue. The total amount of virus per culture was less than on Day 4 but could not be calculated per 10^5 adherent cells. These data suggest that virus is not replicating or disseminating significantly and residual virus from the inoculum is probably being released on Day 4 into the supernatant fluid from dying cells into which the virus had adsorbed.

Discussion

Our strain of SFV produces encephalitis in 6- to 8-week-old mice after injection of appropriate doses by any one of several routes. In the C3H/Ten strain of mice, the iv and footpad routes appear to be as efficient as the intracerebral routes; however, the ip route required a 4000-fold higher dose to cause death. In the ICR strain of mice, the iv and footpad routes required a 1000-fold higher dose for lethality than the intracerebral route but the ip lethal dose, as in C3H/Ten, was 4000-fold higher.

In this study, we focused on the peritoneal cavity to determine what characteristics make this route refractive to infection. It has been reported (18) that virus injected into the peritoneal cavity can disseminate into the vascular system within a short time after administration; however, passage of the virus to the vascular system from the peritoneal cavity occurs via the lymphatic system and there is no direct entry into the blood (6). Since 1 PFU of SFV given iv is potentially lethal in C3H/Ten mice, the refractiveness or resistance of the peritoneal cavity, and the draining lymphatics and lymph nodes, acts at an early stage of infection, otherwise, the virus would disseminate to the vascular system in lethal amounts ($\geq$1 PFU).

The first approach to identify a peritoneal barrier was to determine whether soluble antiviral factors were present normally. Peritoneal wash from normal mice did reduce SFV infectivity of L929 cells to a small degree but not by direct inactivation. Interferon was one of the factors considered to be present because incubation of the L929 cells in peritoneal wash before infection was necessary to show lower numbers of virus plaques. Low levels of IFN-α/β (approximately 1 IRU/ml) appears to be normally present in the peritoneal fluid and this increases ($\leq$100 IRU/ml) after SFV infection. This IFN could be effectively neutralized with anti-IFN-α/β serum *in vitro*. However, administration of high amounts of anti-IFN-α/β serum (1.25×10^3 and 8.6×10^3 SNU) to mice before virus challenge had a relatively small effect on mouse mortality (50–60% compared with 30% in control mice receiving normal serum, $p_t < .2$). An increase in the level of IFN after infection requires a considerable amount of time; for example, Bradish and Allner (3) found maximum titers only after 36 hr of infection and less than 100 units/ml were found in blood during the 36-hr time period. Therefore, it appears unlikely that IFN-α/β plays a major role in limiting virus dissemination from the peritoneal cavity.

In an examination of the infected peritoneal cells themselves *in vivo*, and adherent peritoneal cells *in vitro*, a small fraction (approximately 0.5%) of adherent cells only adsorbed SFV regardless of the dose of virus given. The viral antigens forming a halo-like appearance were located just on the inside of the cells based on the fact that fixation with methanol was necessary to observe them. Since the antigens revealed by immunofluorescence were concentrated at, or near, the plasma membrane and none were found within the cytoplasm, this suggests that these cells take up the virus but do not allow replication or the virus is eclipsed and/or replicating only abortively. As a further indication of this interpretation, uv-inactivated SFV was distributed in essentially the same pattern. In addition, cultures of infected adherent cells did not show an increase in the number of positive cells, nor was there an increase in the intensity of fluorescence in the positive cells.

On the fourth day of culture, a slight increase in virus yields in the supernatant fluids (Fig. 3) and infectious centers (Fig. 4) was noted at moi of 0.2 and 2, suggesting that some small percentage of residual virus from the inocula may have been released from the cells that had adsorbed them, perhaps by a reversible adsorption mechanism prior to the cells dying. Note that no virus increase was found at an moi of 0.002 which is equivalent to 1 LD_{50} *in vivo*. It should be emphasized that the limited small increase in virus (or infectious centers), observed at 4 days with higher moi, was well below the level that might be expected if the virus was replicating. These levels at 4 days do not reach the titers of the inocula. Correlated with the small increase in free virus on Day 4 was the observation that viral antigens could no longer be detected in cells by immunofluorescence either with or without methanol fixation. On Day 5, the cells were rounding and detaching from the glass surface and stained with trypan blue. The total amount of virus per culture was less than on Day 4 but could not be calculated per any number of adherent cells.

Cells that were initially resistant to virus appeared to remain so through 4 days of culture, since the number of immunofluorescent cells did not increase. This is in contrast to a report in which another virus, vesicular stomatitis virus, was shown to replicate in cultured macrophages rather than in freshly harvested ones (23). Consistent with our results, Kraaijveld *et al.* (18) showed that peritoneal cells did not allow SFV multiplication. However, their results in BALB/c mice showed that the cells did not adsorb virulent virus and 1–2 PFU were equivalent to an LD_{50}. In our results, peritoneal cells of both C3H/Ten and ICR mice apparently adsorb the virus, resulting in 4×10^3 PFU being required for an LD_{50} by the ip route. In another study, Johnson (24) reported that large doses of herpes simplex virus were required to produce an LD_{50} for the ip route of infection and estimated that one macrophage in 100,000 might support virus growth.

The data presented herein suggest that there is a small subpopulation of adherent cells in the peritoneal cavity, and probably in the draining lymphatics, which preferentially take up SFV but do not allow replication. Some of the virus, at least, is not degraded, but can be released after several days, presumably to be inactivated by immune factors, such as antibody. The overall consequence of this selective adsorption without replication is to prevent dissemination of the virus into the vascular system. We believe that this is the major factor that accounts for the relatively high resistance to SFV infection by the ip route, as opposed to the iv, ic, and fp routes (approximately 1000- to 4000-fold in the C3H strain). We are attempting to characterize further the subpopulation of adherent cells that adsorbs virus in a way that results in an abortive infection.

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