Mediators of Protection Against Lethal Systemic Vesicular Stomatitis Virus Infection in Hamsters: Defective Interfering Particles, Polyinosinate-Polyctydylate, and Interferon

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Homologous defective interfering (DI) particles protected adult Syrian hamsters against lethal systemic infection with vesicular stomatitis virus (VSV) serotype Indiana. The DI particles had to be biologically active, but did not have to be administered at the same inoculation site as the infectious virus. Serum and tissue levels of VSV postinoculation were significantly lower in DI-protected animals than in unprotected controls, suggesting that true autointerference was occurring. However, some aspects of protection also must be mediated through nonspecific mechanisms, since susceptible hamsters could be protected against VSV Indiana by coinjection with heterologous DI particles prepared from VSV serotype New Jersey or by simultaneous administration of polyinosinic acid-polycytidylic acid. By measuring serum levels of putative hamster interferon (type 1), we found that animals coinjected with VSV and DI particles or polyinosinic acid-polycytidylic acid produced significant levels of interferon. Since similarly high serum levels of interferon were measured in recipients of VSV alone (animals that eventually died from infection), there appeared to be no correlation between protection against lethal disease and induced levels of serum interferon. Instead, serum interferon levels correlated positively with amounts of VSV PFU found in serum and tissues of infected animals, the lowest levels being found in serum of animals protected with homologous DI particles. The data are consistent with the hypothesis that autointerference by DI particles as well as various host defense mechanisms (possibly including induction of interferon) participates in protecting hamsters against lethal VSV infection.

A physiological role for defective interfering (DI) particles in vivo viral infections has been postulated (14) and has been studied with several viruses by using mice or rats as hosts. Studies with standard virus and DI particles have been conducted with vesicular stomatitis virus (VSV) (6, 8), influenza virus (24), reovirus (26), lymphocytic choriomeningitis virus (22, 27), Semliki Forest virus (7), and rabies virus (4). These studies had two common factors. First, viral administration was either intracerebral (i.c.) or intranasal, resulting in infections of the central nervous system. None of the studies involved the effects of DI particles on systemic viral infections. Second, interferon was considered not to play an important role in the observed prolonged survival or decreased viral titers in infected tissues after concomitant inoculation of wild-type virus and DI particles, since no interferon-like activity could be demonstrated.

One model system for studying the prophylactic effects of DI particles is the lethal infection of mice after i.c. injection of VSV; however, using this system, different investigators have reported contradictory results. Holland and co-workers (8, 16) have shown that simultaneous i.c. injection of VSV and large numbers of highly purified homologous DI particles can provide complete protection or significant prolongation of life. This protection was shown to require biologically active DI particles, to be specific for homologous virus, and to be unrelated to the action of interferon. In contrast, Crick and Brown (6), using a similar experimental system, found that biological activity is not a prerequisite and that protection by DI particles is not specific; i.e., they found that chemically inactivated DI particles can protect against both homologous and heterologous VSV challenge. They concluded that DI particles enlist a host mechanism, probably immunological, and that most of the observed protection is not due to true ho-
mologous interference. Both groups of investigators, however, agree that induction of and concomitant antiviral activity due to interferon are not important components of DI particle protection in vivo.

We have developed a model for studying the prophylactic effects of DI particles on a lethal systemic disease; this model is not confined to the restricted environment of the central nervous system and allows for potential interaction of host defense systems. Our model uses adult Syrian hamsters and VSV serotype Indiana (VSV\textsubscript{IND}). We have reported (9) that VSV causes a lethal systemic infection in certain inbred strains of Syrian hamsters after intraepitoneal (i.p.) inoculation of the virus. Death of infected hamsters occurs within 72 h, and histological studies (10) indicate that VSV exhibits a tropism for cells of lymphoreticular origin. More importantly, histological examination of brain tissue at the time of death shows little or no involvement of the central nervous system.

The present study demonstrated that hamsters highly susceptible to i.p. infection by VSV could be protected by biologically active homologous DI particles, by heterologous DI particles, and by treatment with the synthetic polyribonucleotide polyinosinic acid-polycytidylic acid [poly(I)-poly(C)].

MATERIALS AND METHODS

**Animals.** Syrian hamsters of the inbred LSH and MHA strains were used when they were 3 months old or older. LSH hamsters were bred in facilities at the University of Texas Health Science Center at Dallas under the direction of William Duncan; MHA hamsters were obtained from Lakeview Hamster Colony Inc., Charles River Breeding Laboratories.

**Viruses.** VSV\textsubscript{IND} has been described previously (5). Two homologous VSV\textsubscript{IND} DI particles were used: DI-2 (VSI ts\textsuperscript{+} ATCC DI 0.54), a DI from the 5' end of the VSV genome (17) and DI-LT (VSI HR ATCC DI 0.67), a long DI from the 3' end of the genome of a heat-resistant VSV\textsubscript{IND} variant (23). DI stocks were partially purified by a single banding in 5 to 30% sucrose gradients. VSV serotype New Jersey (VSV\textsubscript{NJ}), was kindly provided by Amiya Banerjee. A single-size class of DI particles (DI-NJ) was generated after one high-multiplicity passage of VSV\textsubscript{NJ} in R(B77) cells. DI-NJ also was partially purified by banding in a 5 to 30% sucrose gradient. The number of DI particles in the stock preparations was calculated by using the size of the DI particles relative to that of a standard virion, the percentage of protein in and the weight of a standard virion, and the amount of protein in the DI preparations as determined by the method of Lowry et al. (19). The number of physical particles in all DI preparations was approximately 10\textsuperscript{10}/ml; however, this method only gives an upper limit to the actual number of biologically active DI particles, the number of which is at least 10- to 100-fold lower. Since these stocks were gradient purified one time only, they contained contaminating standard virions; the degree of contamination was determined by plaque assay on monolayers of L cells and was approximately 1 PFU of VSV per 10\textsuperscript{10} physical particles.

**In vitro interference assays.** The ability of DI-NJ to interfere with the production of homologous VSV\textsubscript{NJ} and heterologous VSV\textsubscript{IND} was demonstrated by in vitro interference assays. Briefly, approximately 2 × 10\textsuperscript{6} PFU of standard virus and 10\textsuperscript{12} DI particles were adsorbed to monolayers of R(B77) cells (approximately 2 × 10\textsuperscript{6} cells) in 100-mm petri dishes for 45 min at 37°C; Dulbecco modified minimal essential medium containing 5% fetal calf serum was then added. After incubation for 16 h, the medium was removed and clarified of cellular debris by centrifugation at 2,000 rpm for 15 min, and the supernatants containing viral particles were stored at −70°C. The degree of interference was determined by the relative numbers of PFU in supernatants from control and infected cultures, which were enumerated by plaque assay on monolayers of L cells by using an agar overlay.

**Injection of virus and DI particles.** In all experiments hamsters were infected by i.p. injection of 10\textsuperscript{4} PFU of VSV\textsubscript{IND}, a number significantly greater than that required to produce lethal disease (the 50% lethal dose is less than 10 PFU) (9). For coinfection with standard virus and DI particles, 10-fold serial dilutions of DI particles were mixed with standard virus to generate ratios of DI particles to VSV particles of from 10\textsuperscript{5}:1 to 10\textsuperscript{2}:1; 0.2 ml of the virus mixture was injected i.p. with a 25-gauge needle. The operational ratios are upper limits for the number of DI particles in each inoculum (see above). Intravenous injections of DI particles were performed via the saphenous leg veins immediately before i.p. injection of standard virus. All dilutions of viral particles for injection were made in balanced salt solution.

**Injection of poly(I)-poly(C).** Poly(I)-poly(C) (P-L Biochemicals, Inc.), a known inducer of interferon, was diluted in phosphate-buffered saline to a concentration of 1 mg/ml. Poly(I)-poly(C) (100 µg/100 g of body weight) was injected i.p. at either 24 h before or immediately before injection of virus.

**Interferon assays.** Antiviral activity in sera obtained from infected hamsters was measured by plaque reduction of VSV\textsubscript{NJ} or VSV\textsubscript{IND} on HaK cells (hamster kidney fibroblasts) that were obtained from Paul Gleesen. Briefly, hamsters were bled by cardiac puncture at various times after injection of virus; serum was prepared from whole blood and stored at −70°C. Before assay, the serum was diluted 1:4 in Dulbecco modified minimal essential medium, centrifuged at 100,000 × g for 90 min, and then subjected to UV irradiation for 15 min. Aliquots (1 ml) of twofold serial dilutions of irradiated serum in Dulbecco modified minimal essential medium containing 5% neonatal calf serum were added to replicate monolayers of HaK cells in 35-mm petri dishes. After 20 h of incubation at 37°C, the test samples were aspirated, the cells were washed once with phosphate-buffered saline, and 50 to 100 PFU of the test virus was added in a volume of 0.1 ml. After 1 h at 37°C to allow for virus adsorption, 2.5 ml of 0.9% noble agar in Dulbecco modified minimal essential medium plus 5% neonatal calf serum was added. Plaques were counted 2 days later. Interferon titers were expressed as the reciprocal of the dilution giving a 50% reduction in viral plaques.
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DI PROTECTION IN SYSTEMIC VSV INFECTIONS

TABLE 1. Effect of coinjecting standard VSV and homologous DI particles i.p. on lethal infections of hamsters

<table>
<thead>
<tr>
<th>Ratio of DI particles to VSV</th>
<th>No. deada/total no. injected with the following particleb (% survival)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DI-2</td>
</tr>
<tr>
<td>10^4:1</td>
<td>1/10  (90.0)</td>
</tr>
<tr>
<td>10^6:1</td>
<td>3/14  (78.6)</td>
</tr>
<tr>
<td>10^8:1</td>
<td>9/14  (35.7)</td>
</tr>
<tr>
<td>10^10:1</td>
<td>9/14  (35.7)</td>
</tr>
<tr>
<td>10^12:1</td>
<td>12/14 (43.3)</td>
</tr>
<tr>
<td>0/12</td>
<td>1/9   (88.9)</td>
</tr>
<tr>
<td>0f</td>
<td>8/10  (20.0)</td>
</tr>
</tbody>
</table>

a A total of 10^4 PFU of VSV was injected i.p. with graded doses of DI particles.
b On day 14 postinjection.
c MHA and LSH hamsters were injected with DI-LT and VSV; only LSH hamsters received DI-2 and VSV.
d NT, Not tested.
e Only DI-2 particles were injected; no wild-type virus was added.
f Only 10^4 PFU of VSV (no DI particles) was injected.

Statistical analyses. The significance of the data (P values) was determined by using the standard normal distribution for differences between sample proportions.

RESULTS

Effects of homologous DI particles on lethal VSV infection. We first determined whether homologous DI particles, which are capable of in vitro interference (17, 23), could provide protection from lethal systemic infections caused by i.p.-injected VSV. Table 1 shows the effects of coinjecting either DI-2 or DI-LT with wild-type VSV. Significant protection (>75% survival) was achieved at DI-2/VSV ratios of 10^4:1 and 10^6:1, whereas at ratios of 10^10:1 and lower, the majority of the recipient hamsters died. Similarly, when another homologous DI particle, DI-LT, was coinjected with VSV, 100% of hamsters that received doses of 10^5:1 and 10^6:1 survived, and at the lowest dose of 10^8:1, 50% of the recipient hamsters survived. Thus, both homologous DI particles could protect LSH hamsters from death. In this and subsequent experiments, more than 65% of the animals that died did so on days 2 or 3 postinfection. No animal died more than 12 days after injection of virus. The fact that DI-LT appeared to be more effective than DI-2 at lower ratios of DI particles to VSV may imply that the stock preparation of DI-LT contained more biologically active DI particles. Alternatively, if protection was not due to true interference, then DI-LT may be more efficient than DI-2 at eliciting one or more protective host defense mechanisms.

Serum and tissue homogenates from LSH hamsters that had previously received VSV and DI particles at various times were tested for PFU by plaque assay. PFU were detected in 8 of 26 hamsters tested up to 48-h postinfection (see Fig. 3). In serum from only one animal were PFU detected at more than 32 h after infection. The distribution of PFU in 10-fold serial dilutions of individual samples suggested that DI particles were present and were interfering with the assay. This implies that DI particles are replicated in the hamster and can interfere with standard virus production at sites distant from the site of virus and DI particle inoculation.

Effects of UV-inactivated DI particles on lethal VSV infection. Whether inactive DI particles can protect against i.c. infection of mice with VSV is not certain since the results of Jones and Holland (16) and of Crick and Brown (6) are contradictory. To test whether inactive DI particles can protect in the VSV-hamster system, comparable amounts of DI-2 or UV-inactivated DI-2 were mixed with VSV and injected i.p. into separate groups of hamsters. A third group received VSV mixed with 3 x 10^9 PFU equivalents of UV-inactivated VSV. The survival data for animals in these and control groups are shown in Table 2. The majority of hamsters that received VSV only, VSV plus UV-inactivated DI-2, or VSV plus UV-inactivated VSV died. In comparison, hamsters that received untreated DI-2 plus VSV showed a significantly higher percentage of survivors. These results agree with the data of Jones and Holland (16) in that biologically active DI particles were necessary for prophylaxis and suggest that specific interference occurred. It has been shown by others (1; Horodyski and Holland, unpublished data) that VI inactivation of DI particles destroys their ability to interfere in vitro.

TABLE 2. Effect of UV-inactivated DI particles on lethal VSV infection in LSH hamsters

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>No. deadb/total no. injected</th>
<th>% Survival</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^10 UV-inactivated DI-2+ VSV</td>
<td>10/12</td>
<td>16.7</td>
<td>&gt;0.11</td>
</tr>
<tr>
<td>3 x 10^9 UV-inactivated VSV + VSV</td>
<td>9/10</td>
<td>10.0</td>
<td>&gt;0.11</td>
</tr>
<tr>
<td>10^10 DI-2 + 10^4 PFU of VSV</td>
<td>3/8</td>
<td>62.5</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>10^10 UV-inactivated VSVc</td>
<td>0/4</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>10^9 PFU of VSV</td>
<td>8/8</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

a All injections were i.p.
b On day 14 postinjection.
c Compared with survival of those receiving only VSV.
d DI-2 and wild-type VSV were kept on ice and exposed to UV light for 40 min at a distance of 8 cm.
TABLE 3. In vitro interference of homologous and heterologous DI particles with standard VSV.

<table>
<thead>
<tr>
<th>Infection</th>
<th>Titer (log_{10})</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSV_{IND}</td>
<td>9.4 x 10^{9}</td>
<td></td>
</tr>
<tr>
<td>VSV_{NJ}</td>
<td>1.4 x 10^{9}</td>
<td></td>
</tr>
<tr>
<td>VSV_{IND} + DI-2 (IND)</td>
<td>9.6 x 10^{5}</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>VSV_{IND} + DI-NJ</td>
<td>7.8 x 10^{9}</td>
<td>16.1</td>
</tr>
<tr>
<td>VSV_{NJ} + DI-NJ</td>
<td>4.8 x 10^{7}</td>
<td>96.6</td>
</tr>
</tbody>
</table>

a R(B77) cells were infected with standard virus or with standard virus and DI particles as described in the text.

b Values are averages of two experiments.

Survivors from all groups were rechallenged i.p. with 10^{4} PFU of VSV at 10 days after the initial injection; all survived. This implies that hamsters that had survived VSV infections, including those originally receiving only UV-inactivated VSV, were immunized against subsequent VSV infection. This was confirmed by VSV neutralization assays with sera taken 2 to 4 weeks postinfection from surviving hamsters, picked at random, which were not rechallenged.

Effects of administering DI particles and standard VSV via different routes. Since specific interference can occur in vitro only if DI particles coinfect a cell with standard virus, it was of interest to determine whether protection could be achieved when DI particles were injected by a route different from that by which virus was administered. LSH hamsters were injected i.p. with VSV immediately after receiving 10^{10} DI-2 particles intravenously. Of 14 hamsters tested, all but 1 survived. Thus, DI particles rendered hamsters resistant to VSV even if the particles were not administered at the same site as the infectious virus. This implies (i) that VSV expresses a cellular or tissue tropism in hamsters such that DI particles and standard virus are transported to or migrate to a common tissue site where interference can occur or (ii) that a systemic protective mechanism other than true interference is being evoked.

Effects of heterologous DI particles on lethal VSV infection. To choose between these possibilities, we took advantage of the fact that homologous but not heterologous DI particles can interfere with VSV replication in cultured cells. A single-size class of DI particles was generated from VSV_{NJ}. DI-NJ is uncharacterized except for the fact that in vitro it shows only minimal interference with VSV_{IND} replication (Table 3). Coinfection of hamsters with VSV_{IND} and DI-NJ revealed that 79\% of hamsters receiving heterologous DI-NJ survived the infection (Table 4). We conclude, therefore, that the prophylactic effect of DI particles in systemic VSV infections in hamsters may occur through a mechanism that is not true interference.

Effect of poly(I)-poly(C) on lethal VSV infection. Although protection by heterologous DI particles did not exclude the possibility that true interference was achieved by homologous DI particles in vivo, it strongly implicated another mechanism, possibly interferon. DI particles are known to be more efficient at inducing interferon than is wild-type VSV, which is highly cytopathic. The possible role of interferon in attenuation of lethal VSV disease was tested by i.p. coinjections of LSH hamsters with VSV and poly(I)-poly(C), an agent known to induce interferon (Table 5). More than 80\% of hamsters that received poly(I)-poly(C) either 24 h before or at the same time as VSV survived the infection, irrespective of whether a small or large dose of virus was coadministered. In addition, 100 \mu g of poly(I)-poly(C) per 100 g of body weight appeared to be sufficient to elicit maximal protection, since a three-times-greater amount of poly(I)-poly(C) resulted in the same percentage of hamsters surviving 10^{4} PFU of VSV. These results are consistent with the hypothesis that interferon induced by homologous and heterologous DI particles protects susceptible hamsters from lethal VSV infections. The following studies were designed to determine the extent to which Syrian hamsters can and do produce interferon in response to VSV, DI particles, poly(I)-poly(C), or a combination of these.

Production of interferon after injection of poly(I)-poly(C). The ability of hamsters as a species to produce interferon in response to poly(I)-poly(C) has been questioned by at least two investigators (18, 21), although others (15, 25) have shown measurable interferon in serum, decreased viral titers in tissues, or both after i.p. injection of poly(I)-poly(C). We therefore tested the ability of 100 \mu g of poly(I)-poly(C) administered i.p. to elicit interferon production in LSH hamsters.

TABLE 4. Effect of heterologous DI-NJ particles on VSV_{IND} infection\(^{a}\) of LSH hamsters.

<table>
<thead>
<tr>
<th>VSV(^{b})</th>
<th>DI particle</th>
<th>Ratio of DI particles to PFU</th>
<th>No. dead/total no. injected</th>
<th>% Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>DI-NJ</td>
<td>10^{6}:1</td>
<td>3/14</td>
<td>78.6(^{d})</td>
</tr>
<tr>
<td></td>
<td>DI-NJ</td>
<td>0/8</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DI-2 (IND)</td>
<td>10^{6}:1</td>
<td>0/8</td>
<td>100.0</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>6/8</td>
<td>25.0</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) All injections were i.p.
\(^{b}\) +, 10^{6} PFU of VSV was injected; --, no VSV was injected.
\(^{c}\) On day 14 postinjection.
\(^{d}\) \(P < 0.01\) compared with controls receiving only VSV.
TABLE 5. Effect of poly(I)-poly(C) on lethal VSV infection of LSH hamsters

<table>
<thead>
<tr>
<th>PFU of VSVa</th>
<th>Time poly(I)-poly(C) administeredb (h before virus)</th>
<th>Amt of poly(I)-poly(C) (μg)c</th>
<th>No. deadd/total no. injected</th>
<th>% Survival</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>24</td>
<td>100</td>
<td>1/9</td>
<td>88.9</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>150</td>
<td>0</td>
<td>100</td>
<td>0/10</td>
<td>100.0</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>10⁴</td>
<td>0</td>
<td>100</td>
<td>2/12</td>
<td>83.3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>10⁴</td>
<td>0</td>
<td>300</td>
<td>1/6</td>
<td>83.3</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>10⁴</td>
<td>0</td>
<td></td>
<td>6/8</td>
<td>25.0</td>
<td></td>
</tr>
</tbody>
</table>

a All injections were i.p.
b Injected i.p. 24 h before virus or immediately preceding virus.
c Per 100 g of body weight. Weights varied between 95 and 135 g.
d On day 14 postinjection.
e Compared with percent survival of untreated controls.

hamsters. Serum was obtained at various times after injection of poly(I)-poly(C) and was assayed for antiviral activity by the plaque reduction method with VSVIND or VSVNJ as the test virus. The 100-μg amount of poly(I)-poly(C) was sufficient to induce measurable interferon; the antiviral activity remained relatively constant from 4 to 48 h postinjection (Fig. 1). Sera from eight uninjected LSH hamsters had no detectable interferon activity (titer, <2).

Levels of serum interferon after injection of VSV alone, with DI particles, or with poly(I)-poly(C). To determine whether interferon participates in DI particle-mediated protection against lethal VSV infection, we looked at serum interferon levels in LSH hamsters at different times after injection of 10⁴ PFU of VSV, 10⁴ PFU of VSV plus 10¹⁰ DI-2 particles, or 10⁴ PFU of VSV plus poly(I)-poly(C). In all three instances substantial amounts of interferon were generated in hamsters (Fig. 2). It is evident that levels of serum interferon do not correlate with protection from lethal VSV infection, since injection of VSV alone resulted in levels of interferon equivalent to those found in animals that received VSV plus poly(I)-poly(C) and greater than those found in animals that received VSV plus DI particles. We thus conclude that either serum interferon levels are an insufficient index of tissue interferon that successfully protects hamsters from VSV, or interferon production is not a critical factor in protection.

The numbers of PFU of VSV present in sera that were tested for interferon activity were determined by plaque assay before high-speed centrifugation and UV treatment of the sera. Viral PFUs as a function of interferon titers for individual animals from which serum was obtained at 24 to 48 h postinfection are plotted in Fig. 3. The data indicate that there is some correlation between serum interferon levels and PFU of VSV. It also can be seen that maximum serum titers of 10⁷ to 10⁸ PFU/ml were attained in animals that received VSV alone or VSV plus poly(I)-poly(C). These results indicate that the mechanism of protection in hamsters treated with poly(I)-poly(C) is not inhibition of virus replication. It also should be noted that of the 16 hamsters that received VSV plus DI particles, only 5 had measurable PFU whereas all had measurable levels of interferon.

Since maximum interferon titers of 6,000 to 7,000 U/ml of serum were obtained from hamsters injected with VSV, this virus appears to be an efficient inducer of interferon in Syrian hamsters, even in hamsters susceptible to the acute, lethal disease.

Characterization of VSV-induced interferon. Identical titers of interferon were found in all cases when both VSVIND and VSVNJ were used as test viruses. This indicated that anti-VSVIND antibodies, if present in sera from infected ham-
FIG. 2. Serum interferon levels after injection of VSV alone or coinjection of VSV with DI particles or poly(I)-poly(C). Hamsters received the indicated amounts of VSV and DI particles or 100 μg of poly(I)-poly(C) per 100 g of body weight. o, no detectable interferon (titer, <2). The open symbol represents the mean titer of sera from 8 uninfected animals; all other symbols are as described in the legend to Fig. 1.

FIG. 3. Correlation between serum interferon levels and VSV PFU in hamsters injected with VSV, VSV plus DI particles, or VSV plus poly(I)-poly(C). Titers of VSV and interferon were determined as described in the text. Each point represents data from one animal, and only those values obtained from sera taken at 24 to 48 h postinfection were used. When no VSV PFU were detected, one-half of the lowest detectable value (50 PFU) was used; this value is represented by the dotted line. The placement of solid lines was determined by linear regression; correlation coefficients were 0.71, 0.85, and 0.84 for animals that received VSV, VSV plus DI particles, and VSV plus poly(I)-poly(C), respectively.
TABLE 6. Summary of effects of various prophylactic treatments on protection, interferon production, and viremia in VSV-infected hamsters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protectiona</th>
<th>Serum interferonb</th>
<th>Serum VSV PFUc</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Homologous DI particles</td>
<td>+</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>UV-inactivated DI</td>
<td>-</td>
<td>NT*</td>
<td>NT</td>
</tr>
<tr>
<td>Heterologous DI particles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(I)-poly(C)</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

a Injection at same time as that of 10⁴ PFU of VSV.

b +, Significant protection compared with controls; - , no significant protection.

c +, Maximum interferon titers of approximately 100 U/ml; ++, maximum interferon titers ≥1,000 U/ml.

d +++, Maximum titers ≥10⁴ PFU/ml; +/−, usually not detected or maximum titers <10⁴ PFU/ml.

e NT, Not tested.

To treat the species specificity of interferon induced in hamsters after VSV infection.

The antiviral activity present was assumed to be type I interferon since it met the following criteria: (i) specific for the species of origin; (ii) nonspecific for viruses; (iii) active after centrifugation at 100,000 × g; (iv) active after UV irradiation; (v) sensitive to trypsin; and (vi) resistant to pH 2 (data not shown).

DISCUSSION

To our knowledge, these experiments are the first to examine the protective effects of DI particles on a systemic viral infection. In Syrian hamsters DI particles obviously were very effective in preventing the fatal disease that normally follows i.p. injection of VSV. The observed protection, however, probably was not due solely to true autointerference since heterologous as well as homologous DI particles and poly(I)-poly(C) protected against death. Interferon probably plays a role in DI-induced protection of hamsters; however, since high levels of serum interferon were achieved in hamsters that received only VSV (and that ultimately died from infection), interferon production alone, as measured by serum levels, is insufficient to account for the protective effect.

It is likely that the different protective treatments elicit different host defense mechanisms. Table 6 summarizes the effects of the prophylactic treatments of VSV-infected hamsters with respect to protection, interferon, and viremia. Although there was an obvious correlation between viremia and interferon levels, there was no correlation between protection and serum interferon concentrations. The lack of detectable PFU in serum and tissues of animals protected by homologous DI particles suggests that in vivo autointerference may have been occurring. It has been suggested (16), however, that autointerference may not be the only effect of DI particles. This is supported by our findings (i) that intravenous injections of DI particles protected against lethal i.p. injections of VSV and (ii) that heterologous DI-NJ protected against lethal VSVIND infections.

In similar studies with mice inoculated i.c., other investigators (6, 8) detected little or no interferon in the central nervous system after coinfection with virus and DI particles, possibly due to poor interferon production in the central nervous system. Perhaps the best mechanism to provide for early protection after i.c. infection of mice with VSV is true interference, and, to be effective, this requires large numbers of DI particles to insure coinfection of all cells. This idea is supported by the fact that Doyle and Holland (8) had to use extremely large ratios of DI particles to VSV (more than 1,000-fold higher than we used to protect hamsters) to observe protection in the murine model. Gresser et al. (11) have shown, however, that potent interferon preparations administered after the onset of VSV replication in brains of infected mice can provide some protection from death.

Irrespective of the participation of interferon in the outcome (i.e., death or survival) of VSV infection of hamsters, it probably does limit the final amount of infectious VSV produced. It has been demonstrated (20) that interferon-treated cells produce VSV progeny that are less infectious than those produced in normal cells. The concordant rise and fall of VSV PFU in sera and tissues (10) and of serum interferon levels (Fig. 2) support this idea. The data we obtained after VSV infection of hamsters closely resembles that of Bradish and Titmuss (2), who followed the same parameters in mice injected i.p. with Semliki Forest virus; however, their finding that prophylactic administration of fungal double-stranded RNA influenced the extent of viral replication but did not alter virulence is in contrast to our findings.

Another possible effect of injection of poly(I)-poly(C) is that it alters the primary site of VSV replication in hamsters. After i.p. injection of the virus, the first round of VSV replication probably occurs in peritoneal macrophages with subsequent systemic spread of the virus. Since poly(I)-poly(C) induces macrophages to produce interferon (13), and since interferon-treated macrophages do not support VSV replication as well as do untreated macrophages (12), the bulk of VSV replication may be shifted to other tissues or cell types in poly(I)-poly(C)-treated hamsters. Protection also could be achieved...
through other effects of poly(I)-poly(C), e.g., activation of NK cells or antibody enhancement.

The facile detection of interferon in serum of hamsters treated with poly(I)-poly(C) or injected with VSV alone deserves mention. Hamsters are generally considered to be inefficient producers of interferon, especially after treatment with poly(I)-poly(C). The results presented in this paper prove that hamsters do make substantial amounts of interferon.

Our results show that a number of factors can be involved in early protection of hamsters against acute systemic VSV infection.

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LITERATURE CITED


