Protection of Rats against Pseudorabies Virus Infection by γ-Interferon (42954)

VIRGIL E. C. SCHIJNS, BART L. HAAGMANS, HUUB SCHELLEKENS,* AND MARIAN C. HORZINEK
Institute of Virology, Veterinary Faculty, State University Utrecht, Yalelaan 1, 3508 TD Utrecht, The Netherlands and the TNO Primate Centre, Lange Kleiweg 151, 2280 HV Rijswijk, The Netherlands*

Abstract. **Administration of recombinant rat γ-interferon to rats conferred complete protection against an otherwise lethal intraperitoneal pseudorabies virus (PRV) infection. The primary target cell of the virus has been identified as the serosal cell of the peritoneum. Histologic examination showed that after infection of the underlying adventitia, the virus replicates in the myenteric and submucosal plexuses of the gastrointestinal tract; this is followed by centripetal spread to the autonomous and central nervous system. In recombinant rat γ-interferon-treated rats, viral antigen was absent in the primary target cells and was not detected in any other organ. In interferon-treated cultures of peritoneal fibroblasts, which represent another primary target cell population *in vivo*, complete inhibition of PRV replication was observed. The peritoneal macrophage is not susceptible to PRV, as was shown by coculture and immunocytochemical studies. Peritoneal cells from γ-interferon-treated rats showed enhanced major histocompatibility class II antigen expression and extrinsic antiviral activity in PRV-susceptible rat embryo fibroblasts. The results presented in this study indicate that protection by the lymphokine is likely to be based on direct inhibition of viral replication in serosal cells.**
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The lymphokine γ-interferon is produced by activated T lymphocytes and natural killer cells and possesses diverse biologic functions (1). In addition to its antiviral and antiproliferative effects, it is a potent immunoregulator known to induce expression of major histocompatibility complex (MHC) class I and II antigens (2–4), to activate macrophages (5, 6), to affect antibody production (7–9), and to influence natural killer cell function (10, 11) and cell-mediated cytotoxicity (12, 13). Recently, we have demonstrated that administration of recombinant rat γ-interferon (rRIFN-γ) to rats was able to confer complete protection against a lethal pseudorabies virus (PRV) infection (14). In view of the published evidence it was tempting to ascribe the antiviral effect to a stimulation of the host defense system. In our model system, however, the antiviral effect was not abolished in naturally immunocompromised and artificially immunosuppressed rats. Our explanation for the protective effect is that rRIFN-γ had induced an antiviral state in the target cell of PRV (14).

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Pseudorabies or Aujeszky's disease virus is a neurotropic α-herpes virus which causes lethal infections in piglets and leads to latent infections in adult pigs (15, 16). In naturally infected cattle (17) as well as in laboratory rodents and lagomorphs (18, 19), mostly fatal infections of the nervous system occur.

In this study we describe the identification of the primary target cell of PRV after intraperitoneal infection and the protective effect of rRIFN-γ administration.

Materials and Methods

Virus. A field isolate of pseudorabies virus was obtained from the Centraal Diergeneeskundig Instituut, Lelystad, The Netherlands. The batches used in this study were the third and fourth passages in Ratec cells (rat embryo fibroblasts).

Interferon. rRIFN-γ was produced as previously described (20, 21). Preparations were assayed in a cytopathic effect inhibition assay on Ratec cells challenged with vesicular stomatitis virus. Activity is expressed in laboratory units standardized against a rat interferon reference (22). The interferon preparations used in this study had an activity of $0.5–2.0 \times 10^6$ units/ml and a specific activity of 4.0×10^6 units/mg protein. Freeze-dried interferon preparations were diluted in phosphate-buffered saline (PBS), pH 8.0, to a final concentration of 12.5×10^4 units/ml. About 2.5

$\times 10^4$ units in a 0.2-ml volume were given per animal.

Experimental Procedure. Four- to 5-week-old SPF female Wag/Rij rats were obtained from the colony of the REPGO Institutes (Rijswijk, The Netherlands). rRIFN-γ and PRV were both administered intraperitoneally. Rats received two intraperitoneal injections of about 500 units of rRIFN-γ/g body wt in a volume of 0.2 ml on Days -1 and 0. Control rats received PBS injections. On Day 0 rats were infected intraperitoneally with 200 PFU of PRV. rRIFN-γ-treated rats were completely protected against infection and developed no signs of illness. Untreated infected rats developed paralysis, accelerated respiration rates, and neurologic symptoms. They succumbed within 3–4 days postinfection (PI) (14). At Day 1 or 2 PI animals were sacrificed to remove tissues or to isolate peritoneal cells.

Immunohistology. In order to locate PRV antigen in infected rats, a histologic immunoperoxidase (IPOX) and immunofluorescence (IF) study was performed. At 1 or 2 days after infection, rats were anesthetized with ether and perfused with formaldehyde (4% in PBS) via the left ventricle. Brain, spinal cord, heart, lungs, kidneys, intestinal tissue, thymus, and spleen were removed and immersed immediately in the same fixative. Tissues were dehydrated, embedded in paraffin, and sectioned (5 μm). Paraffin sections were examined for the presence of viral antigen using an immunoperoxidase technique. Abdominal tissues of the same groups of rats were frozen at -160°C in semisolid isopentane. Cryostat sections were made (8 μm), fixed in 5% acetic acid in methanol at -20°C for 20 min, washed in PBS (pH 7.2) and distilled water, air dried, and stored at -20°C until examination. Frozen sections were examined for the presence of viral antigen by double IF and/or an IPOX technique. Polyclonal rabbit anti-PRV serum used for the immunoperoxidase technique was obtained from Dr. J. van Dijk (Department of Veterinary Pathology, Utrecht, The Netherlands). Polyclonal rabbit anti-PRV serum used for double immunofluorescence was kindly provided by J. Pol (Centraal Diergeneeskundig Instituut, Lelystad, The Netherlands). Mouse monoclonal antibody directed against rat MHC class II I-a antigens (designated OX-6) was provided by Dr. J. Rozing (Institute of Experimental Gerontology, TNO Rijswijk, The Netherlands). All fluorescein isothiocyanate (FITC)- and peroxidase-conjugated sera were obtained from Nordic (Tilburg, The Netherlands).

Immunoperoxidase. The IPOX technique as reported previously (23) was applied to paraffin and cryostat sections and to fixed cells grown on coverslips. Briefly, sections were deparaffined and rehydrated in Tris-buffered saline (0.05 *M* Tris-hydrochloride buffer solution, pH 7.6) which was also used for all washings. The preparations were incubated with 7.5% H_2O_2 in methanol for 15 min to block endogenous peroxidase activity and preincubated with normal goat serum (1/20, 15 min, 18°C) to reduce nonspecific binding of the secondary antibody. The sections were then treated with rabbit anti-PRV serum (dilution 1/320) in a moist chamber (2 hr, 37°C) and subsequently with goat anti-rabbit peroxidase conjugate (dilution 1/200, 45 min, 18°C). Peroxidase activity was visualized using 0.003% H_2O_2 and 0.5% 3,3′-diaminobenzidine in 0.05 *M* Tris-HCl buffer (pH 8.3). The preparations were counterstained with hematoxylin, dehydrated, and mounted. In each series a PRV-positive and a negative section was included.

Immunofluorescence. Cytospin preparations, cryostat sections, or cells grown on coverslips were fixed in 4% formaldehyde (5 min) and permeabilized with 100% ethanol (5 min). After washing, the preparations were incubated in polyclonal rabbit anti-PRV serum (1/200, 1 hr, 18°C), washed, and incubated in FITC-conjugated goat anti-rabbit serum (1/60) in the dark (1 hr, 18°C). After incubation the preparations were washed, mounted in PBS-buffered glycerol containing 2% diazobicyclo octane, and examined in an epifluorescence microscope.

Peritoneal Cell Culture. Peritoneal cells were obtained by washing the peritoneal cavity with a total volume of 10 ml of PBS (pH 7.2). The cells were pelleted (225*g*, 10 min) and resuspended in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml), which was used in all culture experiments. An average of 3×10^6 cells/rat was obtained consisting of about 60% macrophage-type and 40% lymphoid cells, as determined after May Grunwald-Giemsa staining. Vitality was more than 95%. Freshly isolated peritoneal cells were seeded into 35-mm dishes or 16-mm wells of 24-well plates (Costar, Cambridge, MA) and rinsed 3 hr later to remove nonadherent cells. Adherent cells were identified as macrophages from their morphology and immunoreactivity with the macrophage-specific monoclonal antibody MAC-1. For cytospin preparations about 10^5 freshly isolated peritoneal cells were spun down onto glass slides, air dried, and fixed in acetic acid in methanol (20 min, -20°C). PRV or MHC class II antigens were visualized by double IF and/or the IPOX technique as described above.

Peritoneal Fibroblast Culture. After vigorous washing of the peritoneal cavity, not only peritoneal macrophages and lymphoid cells were released, but also a low percentage of fibroblast-like cells. The fibroblast-like cells may either be derived directly from the connective tissue of the serosa or evolve by *in vitro* differentiation of mesothelial cells. Three hours after plating, nonadherent cells were discarded and fresh medium was added after washing with PBS. The fibroblast-like cells displayed high mitotic activity, in contrast to the nondividing peritoneal macrophages. Within 7–10 days after plating they had reached confluence. Cultures then also contained a relatively low percentage of peritoneal macrophages (less than 2%), as determined by mor-

phology (May-Grünwald-Giemsa staining) and adherence to plastic. Monolayers of these cells were treated with different concentrations of rRIFN-γ for 24 hr before PRV infection (100 PFU); 18–24 hr later they were washed in PBS, fixed in 4% formaldehyde, and permeabilized with 100% ethanol. Presence of viral antigen was demonstrated by double IF and the IPOX technique.

Flow Cytometry. Flow cytometric analysis of PRV antigen and MHC class II I-a expression on freshly isolated peritoneal cells was performed using a RELACS-8 fluorescence-activated cell sorter at the Institute of Radiobiology, Rijswijk, The Netherlands. About 0.5×10^6 peritoneal cells were preincubated in suspension with normal goat serum (1/40, 30 min, 4°C) to block nonspecific binding of the secondary antibody. After washing they were incubated (60 min, 4°C) with 100 μl of either mouse anti-rat I-a serum (designated OX-6), polyclonal rabbit anti-PRV, or monoclonal mouse antiserum directed against the g-I protein of PRV at the appropriate dilutions (PBS, pH 7.2, 0.05% sodium azide, 1% bovine serum albumin). Cells were then washed twice before incubation for 60 min with 100 μl of FITC-conjugated goat anti-mouse (Ig) serum (1/60) or an anti-rabbit IgG conjugate. Cell suspensions were also incubated with antibodies directed against unrelated antigens to determine background staining levels. Approximately 10,000 cells per sample were analyzed.

Determination of Virus Replication in Peritoneal Macrophages; *In Vivo* Susceptibility of Peritoneal Macrophages. Rats were infected with 5000 PFU of PRV. After 1 hr peritoneal cells were isolated and plated on coverslips. Three hours later the nonadherent cells were discarded and culture medium was added. The adherent macrophages were incubated for an additional 18–24 hours, at which time presence of PRV antigen was determined by immunocytochemistry. PRV replication was also examined in a coculture assay; 4×10^5 Ratec cells were added after plating. PRV multiplication was assayed by monitoring plaque formation in Ratec cells.

Susceptibility of Adherent Peritoneal Cells. In each well, freshly isolated macrophages or 5-day-old *in vitro* cultures were incubated with 500 PFU of PRV. One hour after inoculation, the cells were washed thoroughly and 1 ml of medium was added. After incubation for 18 hr, the presence of PRV antigen was determined by IPOX or IF staining. Infected Ratec cells or primary fibroblast cultures served as positive controls. *In vitro* cultured adherent peritoneal cells were also examined for permissiveness by a coculture assay with Ratec cells.

Assay for Extrinsic Antiviral Activity of Peritoneal Macrophages. PRV was adsorbed to Ratec monolayers in 24-well plates (16 mm; Costar) at approximately 100 PFU/250,000 cells. Unadsorbed virus was removed and 1.5×10^6 peritoneal cells/well were added. After incubation at 37°C for 3 hr to allow macrophages to adhere, the plates were washed and each well then received 1 ml of fresh medium. At 18–24 hr PI the monolayers were fixed with 4% aqueous formaldehyde and either stained using the IPOX technique or 0.5% crystal violet to determine the number of plaques.

Results

Viral Spread after Intraperitoneal Infection. In infected rats PRV antigen was present in the cells covering the parietal and visceral peritoneum and in the underlying loose connective tissue of the gut (Fig. 1B). Also, the myenteric (Auerbach's) nerve plexus which is situated in the connective tissue between the layers of the lamina muscularis externa was found to be infected (Fig. 1D–G). Sympathetic ganglion cells and fibers in the submucous nerve plexus of Meissner also contained viral antigen (Fig. 1B, G, and F).

Examination of spinal cord and abdominal tissues revealed PRV antigen in the neurons of abdominal sympathetic ganglia, prevertebral mesenteric ganglia, and spinal cord ganglia (Fig. 2A, C, and D). Viral antigen was equally present in branching axons of the dorsal root (Fig. 2A) and in spinal cord tissue (Fig. 2E and F). Specific dark brown staining was observed in both the nucleus and the cytoplasm of neurons situated in the gray matter and ganglionic cells of the spinal ganglia (Fig. 2D and E). In the spinal cord also, the cytoplasm, probably of glial cells situated in the *fasciculus gracilis* and *cuneatus,* was found to contain viral antigen (Fig. 2F). Antigen was never seen in the brain, heart, lungs, spleen, kidneys, or thymus. In one animal, a few infected cells were detected in the adrenal gland.

***In Situ* Examination of PRV Spread.** The histologic data showed that cells forming the superficial layer of the visceral serosa represent the primary target cells after intraperitoneal infection. These are very thin, flat mesothelial cells forming a single-layered continuous sheet (24). Using the IPOX technique on spread-mounted preparations of mesenterial membranes from infected rats we were able to demonstrate viral dissemination *per continuitatem;* foci of infected cells are seen (Fig. 1H) not unlike the plaques formed in monolayer cell cultures.

Intrinsic Resistance of the Peritoneal Macrophage to PRV Infection. The peritoneal cavity contains predominantly macrophages which are easily isolated by lavage. To examine whether the peritoneal macrophage represents another primary target cell for PRV after intraperitoneal infection, we applied double IF to cytospin preparations obtained from rats at 18 and 42 hr PI. The macrophages which amounted to 60% of the total cell population did not show viral antigen. Flow cytometric examination for expression of PRV antigen revealed only background fluorescence levels (results not shown). Peritoneal cells from rats infected

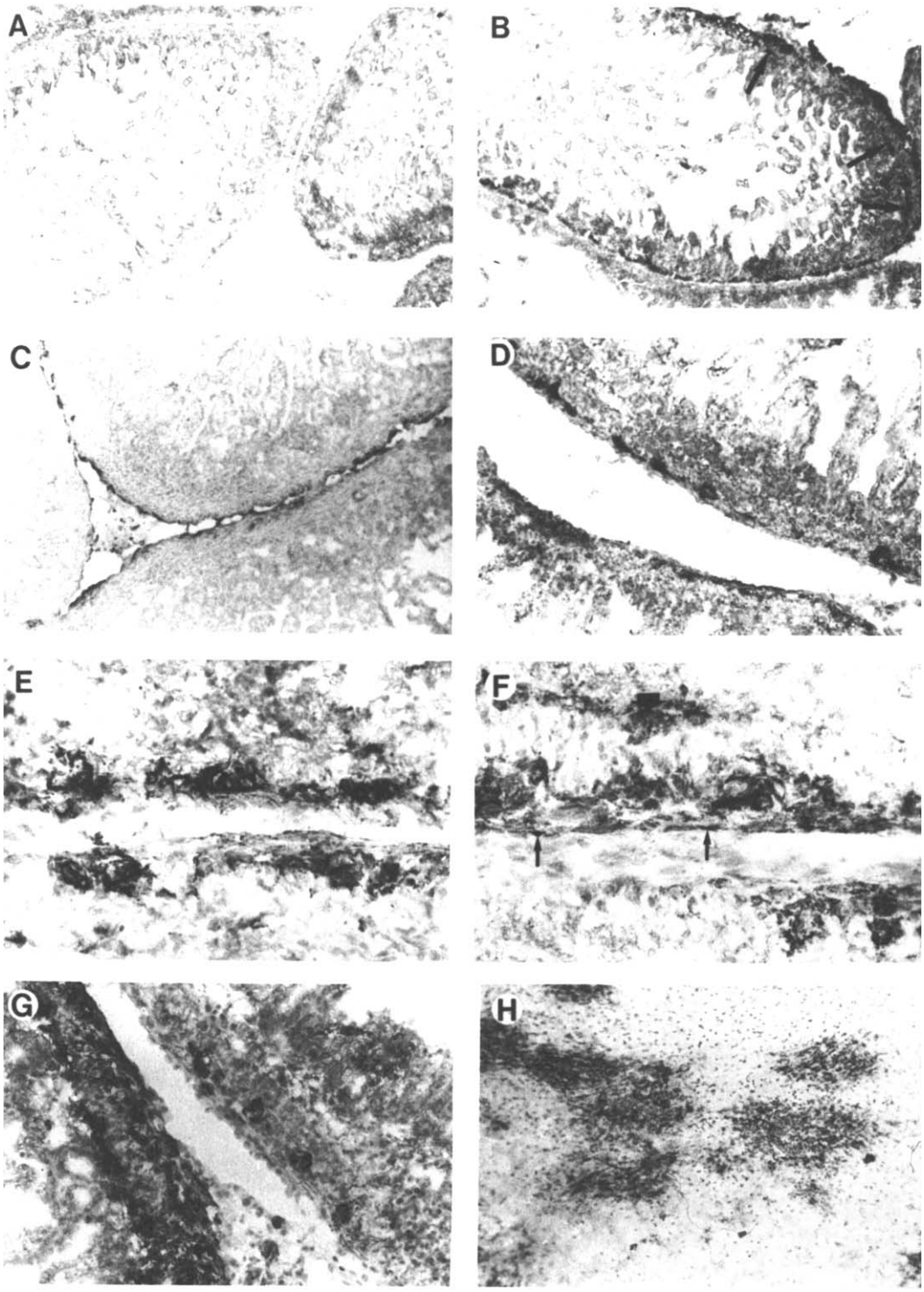

Figure 1. Immunoperoxidase staining of PRV antigen in cryostat sections of intestinal tissue from interferon-treated (A) and untreated infected rats (B–G) at 48 hr PI. Sections have been counterstained with hematoxylin. (A) Low-power view of transverse section of ileum of rRIFN-γ-protected rat showing absence of viral antigen (original magnification ×40). (B) Low-power view of area similar to A in infected rat showing antigen in serosal layer, underlying adventitia, and submucosal plexuses (arrows; original magnification ×40). (C) Viral antigen staining in mesothelial cells covering the intestinal wall (original magnification ×40). (D) Immunoreactivity in myenteric plexuses; note the absence of viral antigen in the serosa and underlying adventitia (original magnification ×100). (E) Viral antigen in ganglion cells of the parasympathetic myenteric plexus of two adjacent intestinal walls. Note that the serosa and underlying adventitia show little antigen (original magnification ×200). (F) Presence of antigen in mesothelial cells (arrows), in the myenteric plexuses of two adjacent intestinal walls as well as in the submucosal plexus (thick arrow) in one of them (original magnification ×200). (G) Viral antigen staining in mesothelial cells, adventitia, and myenteric and submucosal plexuses. In the opposite ileum (right), only myenteric plexuses show immunoreactivity (original magnification ×100). (H) Staining of plaques of infected mesothelial cells in mounted preparation of spread mesenterial membrane (original magnification ×40).

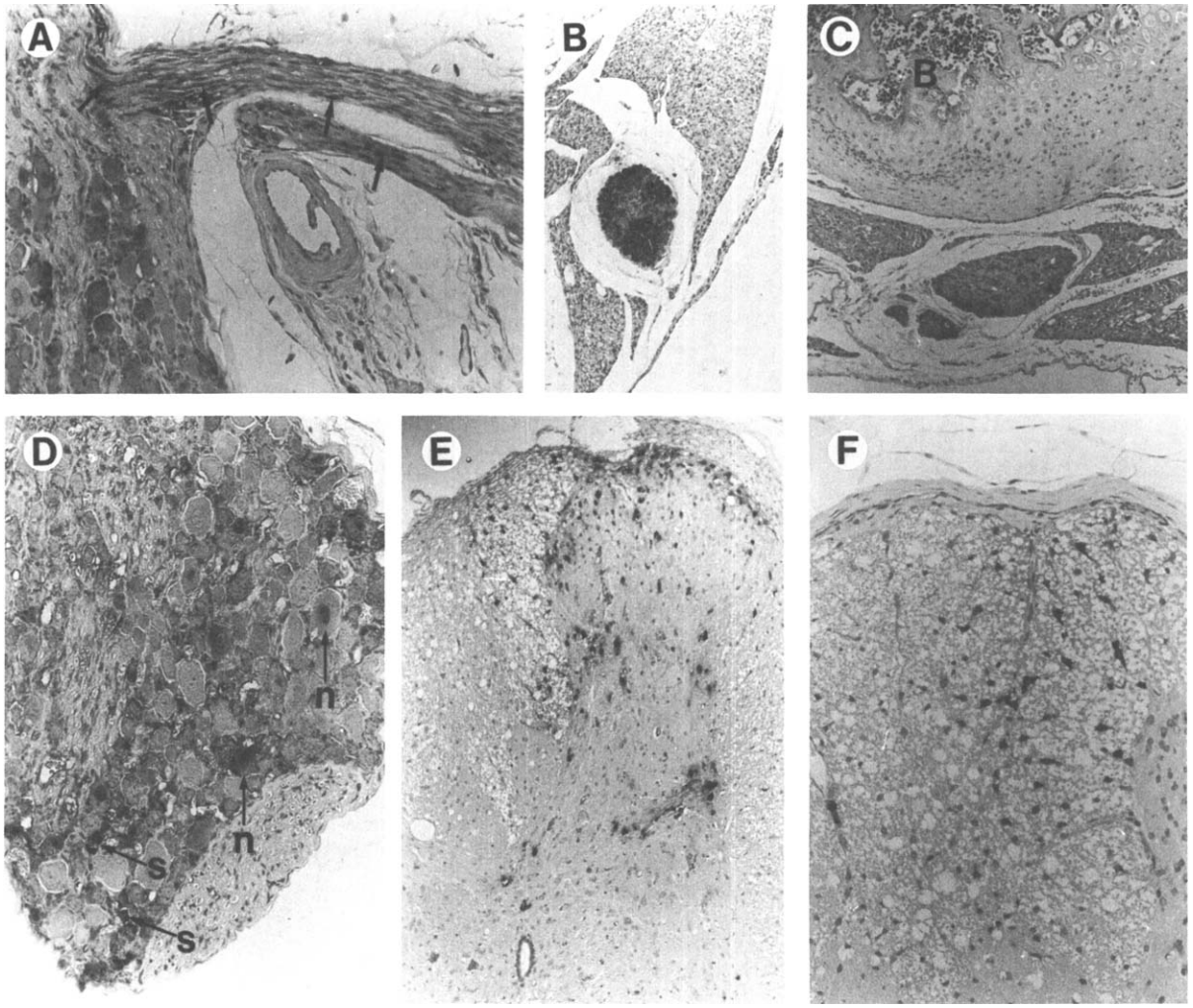

Figure 2. Immunoperoxidase staining of PRV antigen in paraffin sections of neural tissue of PRV-infected rats (60 hr PI). All sections have been counterstained with hematoxylin. (A) Viral antigen in longitudinally sectioned axons (arrows) derived from spinal ganglion cells (original magnification ×320). (B) Antigen staining in transverse-sectioned peripheral nerve bundel (original magnification ×320). (C) Viral antigen in cells of a subvertebral ganglion; bone marrow tissue (B) in the vertebra (original magnification ×40). (D) Antigen staining in the dorsal root ganglion. Specific immunoreactivity in neurons (n) and adjacent satellite cells (S). Note the presence of uninfected neurons (original magnification ×320). (E) Transverse section of thoracic spinal cord showing viral antigen in the right dorsal horn, in the central gray matter, and dorsal white matter (original magnification ×100). (F) More rostral transverse section of the thoracic spinal cord; viral antigen is present only in accessory cells of the dorsal white matter (original magnification ×100).

with 5000 PFU of PRV, isolated at 1 hr PI, also showed no antigen at 18–24 hr after *in vitro* culture and did not infect Ratec cells in a coculture assay. Similar results were obtained with peritoneal adherent cells kept in culture for 5 days.

Effect of rRIFN-γ on PRV Replication *In Vivo*. Application of the IPOX technique to sections of tissues removed from rRIFN-γ-treated, infected rats at 1 or 2 days PI revealed no specific staining of cells in any organ (intestinal tissue, kidney, spleen, heart, lungs, spinal cord, and brain; Fig. 1A).

Effect of rRIFN-γ on PRV Replication in Peritoneal Fibroblasts *In Vitro*. The *in vitro* antiviral activity of rRIFN-γ against PRV infection was determined in 7- to 10-day-old primary fibroblast cultures. These cultures consisted predominantly of large flattened spindle and stellate cells, containing an oval nucleus with two or three nucleoli, which were identified as fibroblasts. These cells may be derived from the adventitia or represent *in vitro* differentiated mesothelial cells. Absence of epithelial cells from these cultures was suggested by immunostaining for cytoplasmic keratin and electron microscopic examination. The fibroblasts were incubated with rRIFN-γ for 24 hr prior to infection with PRV (100 PFU/well). Figure 3 shows that pretreatment with as little as 0.1 unit of rRIFN-γ/ml resulted in about 90% inhibition of plaque formation, whereas after treatment with 1.0 unit/ml complete inhibition was noted.

Surface Expression of MHC Class II Antigens on Peritoneal Cells after rRIFN-γ Treatment. γ-Interferon has been shown to activate macrophages and to enhance the expression of MHC antigens on may cell types, which is one of its prime immunomodulating

effects. We examined the peritoneal cells of rRIFN-γ-treated, uninfected and infected rats for MHC class II (I-a) antigen expression. Flow cytometric analysis showed that in untreated, uninfected rats, peritoneal cells displayed a very low level of I-a expression (mean fluorescence [MF], 64.67; mean fluorescence control staining C, 53.85, Fig. 4A). In infected rats, I-a expression on peritoneal cells was absent (MF, 47.78; C, 51.11, Fig. 4B). In both rRIFN-γ-treated, uninfected and infected rats, peritoneal cells exhibited a markedly increased expression of I-a antigens (MF, 84.53; C, 42.98 and MF, 87.87; C, 40.14, respectively, Fig. 4C and D). The results obtained by flow cytometry were confirmed by fluorescence in microscopy on cytospin preparations (results not shown).

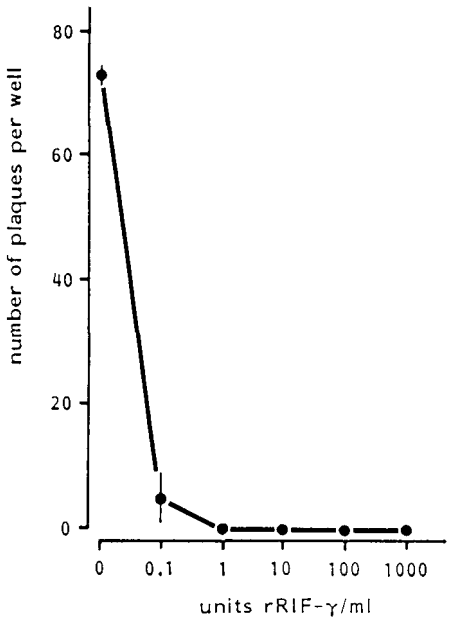

Figure 3. Inhibition of PRV replication in peritoneal fibroblasts by rRIFN-γ. Cells were incubated with rRIFN-γ for 24 hr and infected with 100 PFU/well. Plaque formation was examined at 18 hr PI.

Extrinsic Antiviral Activity of Peritoneal Adherent Cells. The possible involvement of peritoneal macrophages during rRIFN-γ-induced protection was examined by testing for their extrinsic antiviral activity against infected Ratec cells. Table I shows that also

Table I. Extrinsic antiviral activity of peritoneal cells isolated from PBS- and rRIFN-γ-treated rats[a]

	No. of plaques/well	
	Experiment 1	Experiment 2
Virus control	97.0 ± 4.0	74.5 ± 1.5
PBS-treated macrophages	36.0 ± 10.5[b]	25.0 ± 5.6[b]
rRIFN-γ-treated macrophages	13.8 ± 4.6[b,c]	14.3 ± 3.7[b,d]

[a] Data represent the mean of quadruplicate determinations ± SE. Levels of significance were determined by Student's two-tailed *t* test.
[b] P 0.001 as compared with virus control of each experiment.
[c] P 0.025 as compared with PBS-treated macrophages in Experiment 1.
[d] $P = 0.153$ as compared with PBS-treated macrophages in Experiment 2.

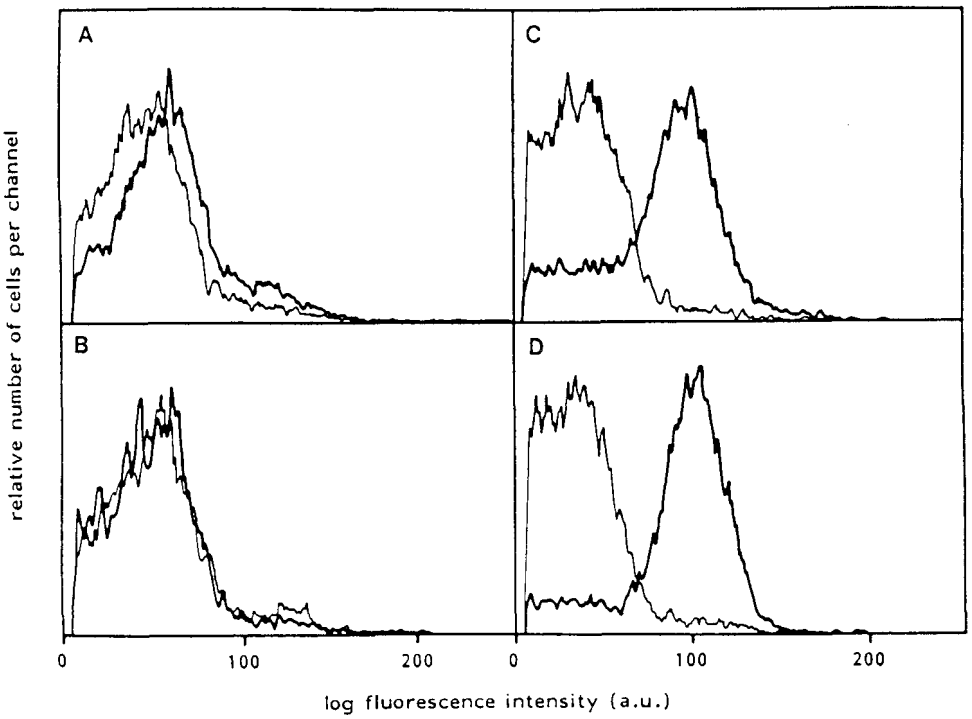

Figure 4. Flow cytometric analysis of I-a expression on peritoneal cells. The fluorescence intensity distribution for untreated, uninfected rats (A), untreated infected rats (B), rRIFN-γ-treated, uninfected rats (C), and rRIFN-γ-treated, infected rats (D) is shown in bold lines. the faint lines represent the background profiles of cells stained only with the FITC-conjugated secondary antibody. The mean fluorescence intensity for I-a expression (in arbitrary units [a.u.] was in A, 64.7 vs 53.9 (control staining); B, 47.8 vs 51.1; C, 84.5 vs 43.0, and D, 87.9 vs 40.1.

unstimulated peritoneal adherent cells reduced plaque formation (63–66%); *in vivo* rRIFN-γ-treated peritoneal cells reduced plaque formation by 80–85%. These results suggest that rRIFN-γ treatment of peritoneal macrophages slightly increases their extrinsic antiviral activity which, however, does not result in complete inhibition of viral replication.

Discussion

In many model systems, exogenous interferon has been shown to protect animals against experimental virus infections (25). The underlying mechanism has been identified in most cases as an inhibition of virus replication in the target cell; however, activation of the host's defense system has often been considered responsible (26–28). Recently, we have found that rRIFN-γ can protect rats against a lethal PRV infection. The antiviral effect was equally pronounced in immunologically incompetent neonatal rats and T cell-deficient nude rats. Treatment with high doses of silica and carrageenan, substances which are cytotoxic to macrophages, did not abolish the rRIFN-γ-induces protection and neither did sublethal whole body irradiation (14).

This study was designed to identify the primary target cell for PRV in the rat peritoneal cavity and to study the effect of a protective dose of γ-interferon on viral replication in these cells. Histologic examination of peritoneal membranes and sections of intestinal tissue showed that after intraperitoneal infection, initial PRV replication occurs in the serosal cells of the peritoneum. Subsequently, the virus gains access to the nerve plexuses of the intrinsic nervous system of the gastrointestinal tract (Fig. 1). Here, viral antigen was detected in the myenteric (Auerbach's) nerve plexus, which contains postganglionic parasympathetic ganglion cells (24). These are supplied by vagus nerve fibers of the parasympathetic division of the autonomous nervous system. Axons given off by these ganglia excite muscular activity and secretion of the gastrointestinal tract (24). The myenteric plexus, which is connected with the submucous (Meissner's) plexus by intrinsic neural fibers, also showed viral antigen. The myenteric fibers are chiefly derived from the superior prevertebral mesenteric plexus and pass directly to endings upon blood vessels and muscle cells of the intestinal wall. In the same rats antigen was also observed in peripheral nerves crossing the peritoneal cavity, in abdominal ganglia (Fig. 2C), in spinal ganglia (Fig. 2A and D), and in the spinal cord (Fig. 2E and F).

It may be assumed that after multiplication in the myenteric and submucosal plexus of the intestine, the virus travels centripetally along peripheral nerves to the abdominal sympathetic ganglia, prevertebral ganglia ultimately reaching the spinal cord. The neural spread of PRV to the central nervous system has been described before (17, 18, 29) and is very similar to the spread of herpes simplex virus after peripheral infection (30). In contrast to other authors (29), however, we were unable to demonstrate PRV antigen in the brains of infected rats; we presume that they succumb before the virus has reached the brain. Failure of autonomous nerve functions, like innervation of the heart and the musculi intercostales may be responsible for death.

In rRIFN-γ-treated rats, antigen could be detected neither in the serosal cells nor in any other target cells. This observations indicates that the infection had been blocked before the virus could accomplish a single cycle of replication in the serosal cells. *In vitro* treatment of peritoneal fibroblasts resulted in complete inhibition of PRV replication which shows that rRIFN-γ is able to confer an antiviral state to the target cell of PRV in the absence of an immune system. *In vivo,* this phenomenon may be sufficient to block primary viral replication, thereby preventing infection to peripheral nerves.

The role of the peritoneal macrophage was further examined. In many mouse model systems this cell has been shown to play an important role in the pathogenesis after intraperitoneal infection (31); its permissiveness may determine the outcome of the infection (32, 33). By phagocytosis and inactivation of viral infectivity, or by exerting extrinsic antiviral activity, macrophages are able to inhibit viral spread (34, 35). In contrast, permissiveness of the peritoneal macrophage results in replication in this abundantly present target cell, eventually followed by virus dissemination in the body (31). Our results show that neither freshly isolated nor 5-day-old cultures of peritoneal macrophages allow PRV multiplication. Nonpermissiveness of the peritoneal macrophage has been described for other viruses (31, 33), and was found to correlate with the age of the host (34, 36), its genetic background, receptors on the plasma membrane (37), or low amounts of endogenous α/β-interferon (37, 38). The resistance of the macrophage in our model system, however, does not correlate with resistance of the host, since Wag/Rij rats are susceptible to intraperitoneal infection with about 1 PFU of PRV (14).

In the presence of γ-interferon macrophages are activated (38, 39), which means that they become more actively phagocytic and more destructive to virus-infected cells. In agreement with other reports (40), we demonstrated that, in rRIFN-γ-treated rats, peritoneal cells including macrophages show enhanced class II expression (Fig. 4C and D), which is another characteristic of activation (41). We also demonstrated that *in vivo*-treated adherent peritoneal cells exert enhanced, but not completely protective, extrinsic antiviral activity against PRV-infected Ratec cells. In our animal model, however, this event is probably only marginal to the rRIFN-γ-induced antiviral state in the target cells, because of the complete inhibition of viral replication in peritoneal fibroblasts. Presence of less than 2% contaminating peritoneal macrophages in these cultures is very unlikely to contribute to the observed

inhibition. In agreement with this hypothesis is the observation that the protective effect is not abolished in rats treated with silica or carrageenan. Furthermore, the passive transfer of *in vivo* rRIFN-γ-treated unseparated peritoneal cells did not protect syngeneic recipients against a lethal PRV infection (14).

Although it is impossible to exclude an indirect mechanism of rRIFN-γ-mediated protection, the presented results in combination with previous findings indicate that the protective effect is most likely based on direct induction of an antiviral state in the target cell. In addition, rRIFN-γ induces macrophage activation and enhanced expression of MHC class II antigens. This immunomodulating feature is essential for optimal antigen recognition which may contribute to the previously reported immunity of surviving rRIFN-γ protected rats to challenge (14).

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