Identification of a Nonproductive, Cell-Associated Form of Measles Virus by Its Resistance to Inhibition by Recombinant Human Interferon

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Subacute sclerosing panencephalitis (SSPE) is a fatal disease in children and young adults that is caused by persistent infection of the central nervous system (CNS) by a nonproductive, cell-associated form of measles virus. Using an experimental model for SSPE (LEC viral strain in newborn hamsters), we have shown previously that establishment of such CNS infections involves selective elimination from the CNS of productively infected cells by host defensive mechanisms, coupled with the selective sparing of cells carrying nonproductive viral forms. That interferon (IFN) may play a role in this process was suggested by the disappearance of productively infected cells from the CNS tissues prior to the appearance of antiviral antibodies and by the demonstration of cell-associated, IFN-resistant viral variants in the virus stocks that were used. Results of this study support these conclusions by showing that similar IFN-resistant viral variants are present in the HBS strain of SSPE-derived measles virus and that these variants, in the presence of IFN, have properties that are similar to those of naturally occurring cell-associated strains of SSPE viruses, e.g., DR, IP3, and Biken. These IFN-resistant forms of HBS virus were isolated and were shown to maintain their resistance to inhibition by IFN after cloning. However, on removal of IFN, they reverted to productive forms similar to the parental HBS virus. The potential role of such viral forms in the pathogenesis of SSPE is discussed.

Subacute sclerosing panencephalitis (SSPE) is a fatal disease of children and young adults that is caused by persistent infection of the central nervous system (CNS) by measles virus (3). The mechanism by which the persistent infection is established is poorly understood, in spite of almost two decades of intensive investigation. However, it is believed that initial infection by the virus of individuals destined to develop SSPE occurs when they suffer uncomplicated rubeola (3). During this acute infection the virus invades the CNS (18) and, by analogy to experimental models of SSPE (12, 25), undergoes a brief period of complete replication. Then, the virus assumes a persistent state that is characterized by a lack of virion assembly (23) and an overall low level of viral gene product expression (16). The causes for this change in infection state are unclear, but the temporal correlation that has been observed between it and the appearance of viral-specific antibodies in serum suggests that immune mechanisms may be involved (12, 25).

We have previously described an experimental system using newborn hamsters and the LEC strain (5) of SSPE virus that models the induction of nonproductive CNS infections by measles virus (11, 12, 13a, 13b). Findings with this system have suggested that the establishment of the cell-associated infection state arises from the selective elimination of productively infected CNS cells, which leaves behind cells carrying nonproductive, cell-associated virus. Evidence for this conclusion includes the observation that LEC virus spontaneously interconverts between cell-associated and productive forms (11) and the isolation of nonproductive, cell-associated viral variants from infected CNS tissues throughout the course of the infection, including times before the appearance of antiviral antibodies (13a) even in animals subjected to immunosuppression by passive

immunization with anti-immunoglobulin M (IgM) antibodies (13b). The importance of interferon (IFN) in the establishment of these cell-associated CNS infections was suggested by the selective disappearance of cells that were infected with the most productive viral forms during the first several days after infection, before the appearance of antiviral antibodies, and by the identification of IFN-resistant forms of LEC virus with properties that mimic those of defective, cell-associated strains of SSPE virus such as DR (48), BIKEN (51), DOI (15), and IP3 (8), i.e., self-limiting syncytial cytopathic effects (CPEs) and failure to produce cell-free viral progeny (13a).

Therefore, the hypothesis can be made that nonproductive, cell-associated forms of measles virus occur naturally (11), that they commonly infect the CNS during rubeola (18), and that they are usually suppressed by host defensive mechanisms, especially IFN. SSPE may occur when such suppression partially fails, which allows the virus to express low levels of its polypeptides and to slowly spread within the CNS. Results of this study support this hypothesis by demonstrating an IFN-resistant, cell-associated viral form in a second, independent strain of SSPE measles virus, i.e., HBS virus (10), and by showing that IFN-mediated suppression of productive viral forms is dose dependent and reversible.

MATERIALS AND METHODS

Cells. Vero cells, a continuous line of African green monkey kidney cells, were obtained from the American Type Culture Collection (Rockville, Md.) and were used in all studies. Procedures for growth and maintenance of these cells have been described previously (27). This cell line has been reported to be deficient in the production of IFN (30), and this deficiency has been confirmed in our laboratory by using as inducing agents and viral targets the Edmonston strain of measles virus, the LEC strain of SSPE-derived

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measles virus, herpes simplex virus, and vesicular stomatitis virus (data not shown).

Viruses. The HBS strain of measles virus was used in all experiments. This virus is a hamster brain-adapted (10) derivative of the Mantooth strain of SSPE-derived measles virus (21). The viral stocks that were used were derived from virus that had been subjected to two sequential plaque purifications and were prepared as described previously (26). In brief, newborn hamsters were infected intracerebrally, and on day 4 after infection brains were removed and processed into 10% (wt/vol) homogenates. These were then clarified by centrifugation, divided into aliquots, and frozen at -70°C until use. Two related but different stocks of virus were used in these experiments. One was the parental HBS virus (pHBS), which has been described in detail previously in a study from this laboratory (26). The other virus stock was prepared from newborn hamster brains that were infected with virus derived from a single passage of pHBS in a cell culture of primary hamster astrocytes (13). This stock was designated glial HBS, or gHBS. The pHBS and gHBS virus stocks had titers of 1.1×10^6 and 9.0×10^5 PFU/ml, respectively.

IFN. Recombinant hybrid human alpha IFN (rHuIFN-A/D) (31) was provided by M. J. Brunda (Hoffmann-LaRoche, Inc., Nutley, N.J.) and was used in all experiments. The effects of this IFN on the replication of the LEC strain of measles virus in Vero cells has been described previously (13a). IFN concentrations are expressed as international reference units, as determined against National Institutes of Health research reference reagent mouse IFN G-002-904-511 (M. J. Brunda, personal communication). Activity of this IFN in Vero cells was approximately 10% of that shown by it in human WISH cells, as measured by inhibition of vesicular stomatitis virus replication (data not shown).

Quantification of viral CPE induced in infected Vero cells. For each data point, at least 40 phase-contrast microscopic fields (0.02 cm^2 each) were examined, and the extent of CPE involvement was determined. Only syncytial CPE was seen, and for purposes of quantification a syncytium was defined as any body having a clearly defined border and containing more than five discrete nuclei. Such syncytia were not found in uninfected Vero cell cultures (data not shown). Although the microscopic fields that were examined were chosen randomly, care was taken to ensure that 10 fields were examined in each of the four quadrants of the infected cell monolayer.

Quantification of cell-free viral progeny in cell culture supernatants. At appropriate times after infection, the medium of a culture was removed and centrifuged at $800 \times g$ for 10 min at room temperature. The supernatant was then removed, divided into aliquots, and frozen at -70°C . Supernatants to be tested were thawed, and 10- μl samples were added to Vero cell cultures in culture dishes containing 5 ml of medium. This procedure reduced the maximum IFN concentration that was used (10^4 U/ml) to 20 U/ml. At this concentration IFN has no observable effect on the replication of HBS virus (data not shown; see Fig. 1). Twenty-four hours later the medium was removed from the cultures, which were then washed in phosphate-buffered saline (PBS) and overlaid with fresh medium containing 1% agarose. Four days later the number of viral plaques in the cultures was counted after the cells were stained with neutral red. This procedure gave a lower limit of detection of 100 PFU/ml of the original infected culture supernatant, as verified by mixing experiments with pHBS virus and IFN (data not

shown). The maximum number of plaques that could be accurately counted per dish was approximately 500, which gave an upper limit of detection of 5.0×10^4 PFU/ml of the original culture supernatant.

Immunoperoxidase staining of viral antigens in infected Vero cell monolayers. Vero cells were grown on glass slides in tissue culture chambers (Lab-Tek; Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.). Monolayers were then overlaid with medium containing 10,000 U of IFN per ml for 24 h before infection with HBS virus. After infection for 6 h at 37°C , the monolayers were thoroughly washed, and the medium containing IFN was replaced. Identically infected cultures without IFN were included as controls. At daily intervals slides from each experimental group were taken, fixed in cold acetone, air dried, and stored at -70°C . Slides were stained for viral antigens by a peroxidase-antiperoxidase (PAP) procedure. The steps that were used for each slide were as follows: (i) incubation for 30 min at room temperature in 5% normal goat serum (NGS; Dako Corp., Santa Barbara, Calif.) in PBS, (ii) treatment with a 1:2,000 dilution of a rabbit serum hyperimmune for measles virus in PBS containing 1% NGS (PBS-NGS) for 90 min at 37°C , (iii) wash in PBS, (iv) incubation for 30 min at 37°C in a goat anti-rabbit IgG serum (Sternberger-Meyer Immunocytochemicals Inc., Jarrettsville, Md.) at a 1:100 dilution in PBS-NGS, (v) wash in PBS, (vi) treatment with a 1:80 dilution of anti-rabbit PAP (Dako Corp.) in PBS-NGS for 30 min at 37°C , (vii) wash in PBS, and (viii) incubation for 5 to 15 min at room temperature in 3,3'-diaminobenzidine (5 mg/10 ml of PBS with 0.1 ml of 1% hydrogen peroxide; Sigma Chemical Co., St. Louis, Mo.).

Quantification of the number of viral antigen-positive foci in immunoperoxidase-stained, HBS virus-infected monolayers. Twenty-five microscopic fields ($5.8 \times 10^{-3} \text{ cm}^2$ each) that were equidistant from one another in a rectangular array encompassing the entire cell monolayer were examined for each slide. Viral antigen-positive foci that were present in these fields were counted, with distinctions being made between syncytia and individual cells. The calculated lower limit of detection of this method was 69 foci per tissue culture slide (Lab-Tek).

RESULTS

Susceptibility of the replication of HBS measles virus to inhibition by IFN. Vero cell cultures were treated with various concentrations of IFN for 24 h before being infected at high multiplicity (0.5 PFU per cell) with either the pHBS or gHBS virus stocks. Media were replaced every third day with fresh media containing the appropriate amount of IFN. This protocol was chosen to approximate the chronic production of IFN, as has been demonstrated in CNS tissues of dogs that were chronically infected with canine distemper virus (49) and of several patients with SSPE (29). The amount of virally induced CPE that was present in the infected cultures was determined daily, and on selected days after infection the level of cell-free virus in the cultures was quantified. Representative results from one of five such experiments are shown in Fig. 1.

In the absence of IFN, both viruses completely destroyed the infected cultures within the first week after infection. In contrast, all IFN-treated cultures showed some inhibition of viral replication, with the degree of viral suppression being dependent on the IFN concentration. Complete inhibition of viral replication was not observed in any of the infected cultures that were treated with IFN. Rather, three basic

FIG. 1. Effect of recombinant human alpha IFN on the infection of Vero cells by the HBS strain of measles virus. Cells were pretreated for 24 h with the indicated concentrations of IFN before infection at high MOI with either the pHBS (A) or the gHBS (B) virus stock. Culture media were replaced with fresh media containing the appropriate concentration of IFN every third day throughout the experiment. The proportion of infected cell monolayers involved in the CPE and the amount of cell-free virus that was present in culture supernatants were determined as described in the text. Numbers beside the symbols indicate the titer as PFU per milliliter of virus present in supernatants of the cultures at the indicated times after infection. Symbols: x, 0 U of IFN per ml; O, 100 U of IFN per ml; ●, 1,000 U of IFN per ml; +, 10,000 U of IFN per ml.

patterns of viral CPE were seen. The first such pattern was a slowing, sometimes to a considerable degree, of the progression of the CPE (e.g., in the infected cultures that were treated with 100 U of IFN per ml). In other respects, including the production of cell-free viral progeny, this infection pattern was similar to that seen in infected cultures that were not treated with IFN.

Both of the other patterns involved, in one form or another, a distinctive type of viral CPE (Fig. 2). Within a few days after infection of the cultures, various numbers of syncytia were observed which slightly increased in size before they degenerated, leaving holes in the cell monolayer. Then, the remaining cells on the periphery of these areas moved in and replaced the missing portion of the monolayer (Fig. 2). In some instances, within 1 or 2 days new syncytia appeared in the monolayer adjacent to the original CPE focus and repeated the pattern of degeneration and ingrowth of new cells.

The remaining two patterns of infection that were observed in the IFN-treated cultures (Fig. 1) involved different rates and extents of progression of this type of CPE. In the presence of higher levels of IFN (e.g., in the cultures that were infected with either stock of virus and treated with

10,000 U of IFN per ml and in the pHBS virus-infected culture treated with 1,000 U of IFN per ml), the CPE was transitory, with only a few rounds of syncytial appearance and degeneration being seen. On disappearance of the CPE, the cultures assumed an appearance that was indistinguishable from that of uninfected Vero cell cultures. In infected cultures that were treated with intermediate levels of IFN (e.g., in the culture that was infected with gHBS and treated with 1,000 U of IFN per ml), the cycle of CPE appearance and disappearance was more stable and continued for extended periods, with only a small portion of the monolayer being involved at any one time. Cell-free viral progeny were not detected in any culture that showed either of these last two infection patterns. In this regard, and with respect to the appearance-degeneration pattern of CPE in general, the behavior shown by HBS virus in the cultures that were treated with high or intermediate concentrations of IFN was similar to that of the most highly cell-associated strains of SSPE measles virus (8, 15, 48, 51) and of the cell-associated form of LEC virus described previously (13a).

Expression of viral antigens in IFN-treated Vero cell cultures infected with HBS virus. The different types of viral behavior that were observed in HBS virus-infected, IFN-treated Vero cell cultures made it of interest to determine the relationships between viral antigen expression and the pattern of viral CPE induced in the infected cell cultures. Vero cells on glass slides were pretreated with IFN and infected at a high multiplicity of infection (MOI) with either gHBS or pHBS virus. At daily intervals slides were taken and processed to reveal viral antigens by using a PAP procedure with a rabbit antiserum that was specific for the structural proteins of measles virus. Representative examples of the results that were obtained for both viral stocks are shown in Fig. 3 to 5. The syncytia seen in the IFN-treated cultures were approximately equal in size to those seen in untreated cultures, but they contained, on average, fewer nuclei and stained less strongly for viral antigens (Fig. 3). In IFN-treated cultures that were examined several days after infection, syncytia in various stages of degeneration (similar to those shown in Fig. 2) were seen (Fig. 4). Cells that migrated to replace the destroyed portion of the monolayer did not contain detectable levels of viral antigens, and

FIG. 2. Progression of syncytial CPE in a gHBS virus-infected Vero cell culture treated with 10,000 U of IFN per ml. The syncytium was first seen in the culture at 48 h after infection, and it was photographed using phase-contrast optics at the indicated times after infection. Times after infection were 100 h (A), 140 h (B), 190 h (C), and 280 h (D).

FIG. 3. Comparison of viral antigen expression within and structures of syncytia seen in gHBS virus-infected Vero cell cultures in the presence and absence of IFN. Infected cultures were fixed in acetone at 24 h after infection and were stained for measles virus antigens by an immunoperoxidase procedure. (A) Syncytium in an untreated control culture. (B) Syncytium in a culture treated with 10,000 U of IFN per ml.

usually the only remnant of a syncytium to be found was a ring of material that stained intensely for viral antigens (Fig. 4D). Only rarely were viral antigen-positive cells detected within or adjacent to these syncytial skeletons. This finding was in sharp contrast to those for infected cultures that were not treated with IFN, in which the local spread of viral antigens into cells that were adjacent to degenerating syncytia was commonly found.

Viral antigens were also detected in the IFN-treated cultures as foci uniformly spread throughout the infected monolayer (Fig. 5). The majority of these foci appeared to be individual cells, although small groups of two to five cells were not uncommon. These viral antigen-positive foci were similar to the ones that were seen during the first day after infection of non-IFN-treated Vero cells (data not shown). To clarify the relationship between these foci and the syncytia that were seen in the same cultures, the viral antigen-stained slides were subjected to a quantification procedure (Table 1).

With both stocks of HBS virus, the number of viral antigen-positive foci increased to a maximum on about day 3 after infection and thereafter declined steadily. No such foci could be detected on or after day 10 of infection either by systematic quantitative examination or by careful observation of the entire cell culture. In contrast, and consistent with the data shown in Fig. 1, syncytia were seen in the

FIG. 4. Syncytia in various stages of degeneration in a Vero cell culture infected with gHBS virus and treated with 10,000 U of IFN per ml. The culture was fixed on day 8 after infection and was stained for measles virus antigens with immunoperoxidase. The sequence of photographs A to D, while of different syncytia, represent the progression of degenerative changes that were observed for the syncytia in the culture.

majority of the infected cultures, although they often were not detected in the quantitative evaluation. Because it was likely that each focus of viral antigens represented a single infectious virus, it appears that for both virus stocks only about 10% of the input virus is able to initiate infection and that a much smaller proportion is able to actually induce a destructive syncytial CPE. Therefore, the infectious virus that was present in both the pHBS and gHBS virus stocks appeared to exhibit a spectrum of susceptibilities to suppression by IFN, ranging from complete inhibition to the induction of a nonproductive and self-limiting syncytial CPE.

Reversibility of IFN-mediated inhibition of HBS viral replication. The existence of a spectrum of susceptibility of viral subpopulations to inhibition by IFN made it of interest to determine the reversibility of the suppression. To this end, a series of experiments was performed in which Vero cell cultures were pretreated with 10,000 U of IFN per ml for 24 h and infected with gHBS virus at a high MOI. Media were replaced with fresh media containing the same concentration of IFN on every third day after infection. Untreated cultures served as controls. The first experiments involved the study of cultures in which the syncytial CPE had disappeared. The culture media were removed and replaced with media without IFN. The cultures were then observed for the reemergence of CPE, and at various times the media were assayed for cell-free viral progeny. Results of one such experiment are summarized in Fig. 6A. CPE did not reappear in the cultures, even after they were subcultured in the absence of IFN. Furthermore, neither cell-free viral progeny nor viral antigens (data not shown) could be detected in the cultures for at least 30 days. In contrast, removal of IFN from similar cultures during the period when one or more syncytia were present resulted, after a delay of several days, in the CPE becoming progressive and evolving to completely destroy the culture (Fig. 6B). Also, relatively high levels of cell-free viral progeny were produced in concert with the CPE progression. The origin of the viral CPE seen in the IFN-reversed cultures was probably the preexisting syncytia because the local spread of these invariably occurred on removal of the IFN (data not shown) and virus could not be isolated from areas of IFN-treated, infected cultures that were free of syncytia (see below).

Isolation of IFN-resistant substrains of gHBS virus. If the

FIG. 5. Viral antigen-positive foci in a gHBS virus-infected Vero cell culture treated with 10,000 U of IFN per ml. The culture was fixed on day 3 after infection and was stained for measles virus antigens with immunoperoxidase. Arrowheads indicate some of the antigen-positive foci that were present.

TABLE 1. Viral antigen expression in Vero cells infected with HBS measles virus in the presence of recombinant human IFN

Virus stock ^a	No. of viral antigen-positive areas per slide on the following days after infection:											
	1 ^b		1 ^c		3 ^c		5 ^c		7 ^c		10 ^c	
	Foci	Syn-cytia	Foci	Syn-cytia	Foci	Syn-cytia	Foci	Syn-cytia	Foci	Syn-cytia	Foci	Syn-cytia
pHBS	19,000 ^d	1,600	69	<69 ^e	12,300	<69	7,100	69	140	<69	<69	<69
gHBS	9,700	6,800	276	<69	16,000	<69	1,905	69	612	<69	<69	<69

^a Input amounts of virus were 1.1×10^5 and 1.8×10^5 PFU per 6.0×10^5 Vero cells for pHBS and gHBS, respectively.

^b No IFN was present.

^c A total of 10^4 U of IFN per ml was present.

^d Number of designated type of antigen-positive cells per culture. This was calculated from the number of such antigen-positive cells seen in 25 microscopic fields (5.8×10^{-3} cm² each) and the total area of the culture (10.1 cm²).

^e Lower limit of detection.

observations described above were due to the existence of specific subpopulations in the HBS virus stocks, then isolation of IFN-resistant substrains of the virus should be possible. To test this prediction, Vero cell cultures were pretreated with 10,000 U of IFN per ml and infected at a high MOI with gHBS virus. Twenty-four hours later the cultures were thoroughly washed and overlaid with medium containing 1% agarose and 10,000 U of IFN per ml. On day 4 after

infection, the cultures were examined by phase-contrast microscopy, and areas containing syncytial CPE were marked. The agarose overlying each of these areas was carefully removed, and a small volume of PBS containing 0.25% trypsin was added to the well thus formed. When trypsinization of the cell monolayer was complete, the cells were removed and added to fresh monolayers of Vero cells in culture flasks. These were then observed for the appearance of viral CPE. Areas free of CPE in the same infected cultures were taken similarly and processed as controls. The results of two such experiments are shown in Table 2.

No viral isolates were obtained from areas of infected monolayers that were free of preexisting syncytia, even after repeated subpassages of the inoculated Vero cell cultures (data not shown). In contrast, approximately one-half of the attempts to isolate virus from areas of the infected cultures containing syncytia succeeded. This relatively low success rate probably reflects the poor viability of syncytia during the trypsinization procedure. To ascertain the IFN resistance or susceptibility of these viral isolates, cells from the isolation cultures were passaged onto fresh monolayers of Vero cells in the presence and absence of 10,000 U of IFN per ml. Results obtained with the two viral isolates of experiment one (Table 2) are shown in Fig. 7. One of the isolates, designated HBS-IR-1, showed an almost complete resistance to inhibition, with its replication being only slightly slowed by the IFN. In contrast, the other isolate, designated HBS-IR-2, showed a degree of inhibition, but it finally replicated and destroyed the cell culture in the presence of IFN. The CPE induced by HBS-IR-2 also showed a slower rate of progression in the absence of IFN than did that caused by HBS-IR-1. Both HBS-IR-1 and HBS-IR-2

FIG. 6. Reversibility of the inhibition of HBS virus replication by IFN. Replicate cultures of Vero cells were pretreated with 10,000 U of IFN per ml, infected at a high input multiplicity with gHBS virus, and observed daily for the presence and amounts of viral CPE. Culture media were replaced every third day with fresh media containing IFN. At the indicated times the media in selected cultures were replaced with fresh media containing no IFN. Numbers and symbols indicate the titers (in PFU of cell-free virus per milliliter) that were present in the cultures. (A) Representative results when the IFN was removed after the disappearance of the viral CPE; (B) typical results when the CPE was present in the culture at the time of IFN removal. Symbols: X, control culture that was not treated with IFN; O, culture that was maintained in the presence of 10,000 U of IFN per ml; ●, culture from which IFN was removed.

TABLE 2. Isolation of IFN-resistant substrains of HBS measles virus

Expt no.	Original CPE	No. of isolation attempts	No. of isolates that induced CPE
1	— ^a	7	0 ^b
	+ ^c	4	2
2	— ^a	8	2
	+ ^c	5	3
Total	— ^a	15	0
	+ ^c	9	5

^a One square centimeter of an HBS virus-infected Vero cell monolayer containing no viral CPE was passaged onto a new Vero cell culture for each isolate.

^b All cultures were observed for 10 days for viral CPE.

^c Area of monolayer containing and immediately around virally induced syncytium was taken and passaged onto fresh Vero cells for each isolate.

produced low, but detectable, levels of cell-free viral progeny.

DISCUSSION

Results described here and in previous studies with a different strain of measles virus (12, 13a) suggest that IFN may be involved in the establishment of nonproductive, cell-associated CNS infections by measles virus. This suggestion arises from the findings that IFN probably helps to eliminate productive viral forms from the infected CNS tissue (13a) and that selection of variants of two independent SSPE measles virus strains for resistance to inhibition by IFN coselects for the same type of nonproductive, cell-associated viral behavior that characterizes many viral isolates from CNS tissues from patients with SSPE (8, 15, 48, 51). That true viral variants are involved in these effects is shown by the IFN-resistant properties that are shown by the viruses after clonal isolation from the HBS (Fig. 7) and LEC (data not shown) virus strains.

In this study the presence of high levels of IFN during the infection of Vero cells with HBS measles virus led to the marked suppression of viral replication and the eventual eradication of the virus from the infected cell cultures (samples with 10,000 U of IFN per ml; Fig. 1). In contrast, the presence of low levels of IFN slowed but did not completely inhibit viral replication, which led to the eventual destruction of the infected cell cultures (samples with 100 U of IFN per ml; Fig. 1). Intermediate concentrations of IFN gave variable results which depended on, at least in part, the particular stock of virus that was used for the infection (samples with 1,000 U of IFN per ml; Fig. 1). Thus, the outcome of a given infection appeared to be determined by a balance between the replication of relatively IFN-resistant viral forms and suppression of those viruses by IFN. As could be predicted from such a balance, under certain conditions a nonproductive, cell-associated form of the virus could be maintained in the infected cultures (samples with 1,000 U of IFN per ml; Fig. 1B).

An important aspect of this balance can be seen in the comparison between the transient CPE formation by the

virus in Vero cell cultures treated with high levels of IFN (Fig. 1) and the progressive CPE that was observed in cell cultures treated with the same concentration of IFN and infected with the two IFN-resistant viral isolates (Fig. 7). A likely explanation for these seemingly contradictory results is that trypsinization and passaging of the infected cells at least partially abrogated the IFN-mediated suppression of viral replication. Support for this suggestion can be found in the observation that subculturing of IFN-treated cultures containing one or more syncytia, such as those described in Fig. 1, in the presence of IFN led to complete destruction of the new cultures by viral CPE (data not shown). These results, when coupled with the stimulation of cellular metabolism and division that occurs when cultured cells are passaged, suggests that the IFN may suppress viral replication by means of its effects on cellular proliferation (14, 50) or through its interactions with other cellular factors such as cyclic AMP (4, 53). Intracellular levels of cyclic AMP have been shown to have regulatory effects on the replication of measles virus (36, 46).

The molecular mechanisms that are responsible for the effects of IFN on HBS virus replication are unknown. However, suppression of antigen production by approximately 90% of the virus that was present in the viral stocks appeared to be complete (Table 1 and Fig. 3). Most of the remaining 10% of the virus was only able to transiently express viral antigens as foci in the infected cultures. Which of the viral antigens were expressed in these foci is unknown; however, the lack of CPE in those areas suggests that the viral antigens expressed on the outer cell membrane were not among them. Finally, a very small proportion of the virus that was present in the viral stocks (less than 10/100,000 PFU) was able to induce nonproductive, self-limiting syncytia that were associated with the expression of at least some of the viral antigens (Fig. 3). By analogy to similar, defective strains of SSPE virus strains (27), expression of the viral membrane-associated antigens (hemagglutinin, fusion protein, and matrix protein) is probably restricted in these syncytia, but this remains to be confirmed.

In general, the possibility that IFN plays an important role in the pathogenesis of SSPE has received little direct investigational attention. This is in spite of the observations that IFN may be involved in the establishment of persistent infections by measles virus in vitro (24, 43) and that it can serve as a marker for persistent CNS infection by canine distemper virus in dogs (49). Furthermore, in trials of intrathecal or intraventricular IFN as a treatment for SSPE (6, 7, 9, 22, 39-42, 47), clinical improvement has been observed in 9 of 19 (47%) patients, which is in contrast with the approximately 10% of patients with SSPE that show spontaneous stabilization or improvement (44, 45). Furthermore, by combining the results reported with some of these trials with those reported in other studies (6, 7, 9, 22, 29), it can be seen that of 20 patients with SSPE whose cerebrospinal fluid was assayed for IFN by bioassay before treatment with IFN, only 4 (20%) had detectable levels. In contrast, 60% (25 of 41) of patients with herpes simplex encephalitis (2, 20, 33, 34, 38) and over 80% (38 of 47) of patients with mumps meningitis (2, 32, 38) had detectable IFN in their cerebrospinal fluid. Furthermore, the proportion of patients with SSPE that have detectable levels of IFN in their cerebrospinal fluid is approximately the same as that of patients with postinfectious encephalomyelitis following measles virus infection (3 of 10) (33), a disease in which the virus probably does not directly invade the CNS (28). These observations suggest that IFN responses within the CNS

FIG. 7. Infection of Vero cells in the absence and presence of IFN with IFN-resistant isolates of gHBS virus. Vero cells carrying the viral isolates were passaged onto Vero cell monolayers that either had been left untreated or that had been pretreated with 10,000 U of IFN per ml. Media were replaced every third days with fresh media containing the appropriate concentration of IFN. Numbers and symbols show the amounts of cell-free virus present as PFU per milliliter. Symbols: +, HBS-IR-1 virus with no IFN; O, HBS-IR-1 virus with IFN; x, HBS-IR-2 virus with no IFN; ●, HBS-IR-2 virus with IFN.

tissues of patients with SSPE may be lower than those seen in other more acute viral infections of the CNS. When such a possibility is combined with the severe, and fatal, degree of CNS infection that occurs in SSPE (3), with the efficiency of IFN induction shown by measles virus in vitro (19, 35, 52), and with the susceptibility of measles virus replication to inhibition by IFN (37), it can be hypothesized that an abnormally low IFN response within the CNS may be involved in the pathogenesis of SSPE.

From these considerations and those reported previously (11, 12, 13a, 13b), a hypothetical scenario for the induction of CNS infection by measles virus in normal individuals and patients with SSPE can be proposed. First, the CNS is infected via a viremia with the virus and, more specifically, with cell-associated forms of the virus that have been generated spontaneously (11, 13a) either within the CNS or in the periphery. Second, host defense mechanisms, both specific and nonspecific, come into play with the result that, in normal individuals, the majority of virally infected cells are destroyed (12, 13a) and those that remain contain virus whose antigen expression is totally suppressed by IFN. Such viral antigen-negative, but viral genome-positive, cells have been observed in a proportion of normal human brains that were examined by in situ hybridization with measles virus-specific cDNA probes (17). In patients destined to develop SSPE, specific immunological responses destroy the CNS cells that are infected with productive forms of the virus (12, 13a), but production of only low levels of IFN within the CNS allows nonproductive, cell-associated, and relatively IFN-resistant viral forms to continue to express viral antigens and to slowly spread within the tissue. The rate of this spread is, in part, determined by the waxing and waning of IFN production within the infected CNS, such as that described by Joncas et al. (29) for several patients with SSPE, because this allows the virus to shift among different degrees of productivity and destructiveness. This process leads to a relatively slow accumulation of CNS damage and accounts for the extended incubation period that characterizes SSPE (3). Furthermore, a low IFN response would help to increase the density of CNS infection which has been postulated to play a role in SSPE (1, 13a). Finally, when cells from the CNS tissue of a patient with SSPE are cocultivated with virally permissive cells, such as Vero cells, they are released from IFN-mediated suppression in the CNS tissue, and at least the glial cells that are present are metabolically stimulated and begin to divide. This may further release the virus that is present from its suppressed state.

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