Identification of Interferon-Resistant Subpopulations in Several Strains of Measles Virus: Positive Selection by Growth of the Virus in Brain Tissue

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Subacute sclerosing panencephalitis (SSPE) is a chronic and usually fatal central nervous system disease caused by a persistent infection with measles virus. The pathogenic mechanisms of the disease are poorly understood, but restricted expression of viral antigens within the infected tissue appears to be involved. We have previously proposed that interferon (IFN) plays a role in the pathogenesis of SSPE by interacting with viral subpopulations that are relatively resistant to IFN-mediated inhibition. Such IFN-resistant viral subpopulations have now been identified in six independent strains of measles virus, two derived from patients with measles and four derived from patients with SSPE. By means of a replicative-plating procedure, these IFN-resistant viruses were found to be heterogeneous with respect to their growth in the presence of high levels of IFN. One viral form replicates fully, with complete destruction of the infected-cell culture, whereas the other form induces a restricted, self-limited form of cytopathic effect, similar to that seen with cell-associated strains of measles virus isolated from SSPE patients. Passage of a virus stock containing both of these viral forms through the central nervous system tissue of newborn hamsters strongly selects for the viral form associated with the self-limiting type of cytopathic effect. The presence of this form of IFN-resistant virus coupled with chronic production of IFN within the central nervous system may account for viral persistence in SSPE patients.

Subacute sclerosing panencephalitis (SSPE) is a chronic and ultimately fatal disease of the central nervous system (CNS) most commonly seen in children and young adults (3). The pathogenesis of SSPE is poorly understood but clearly involves persistent infection of the CNS with measles virus (47). An important question that remains unanswered is why host immunological defenses are unable to clear the virus from the infected tissue. Measles virus-specific (23) as well as general (8) defects in cellular immune function have been found in patients with SSPE, but none have been reproducibly described, and those seen may reflect either the chronic disease state of the patients or sequestration of reactive cells within the CNS. Humoral immune function in SSPE patients appears to be intact, and the elevated levels of measles virus-specific antibodies in the blood and cerebrospinal fluid (CSF) are actually diagnostic of the disease (3). Therefore, a major defect in the cellular or humoral immune response does not appear to play a central role in SSPE, but a subtle or mild one could be involved in conjunction with other viral or host factors. For example, a weak or inefficient cellular immune response could have difficulty in clearing virus from the CNS if viral target antigens were expressed at restricted levels (27).

Such a restriction in viral protein expression appears to be a general feature of SSPE (5, 27, 34, 36). The cause of the restriction is not understood, but may be an interaction between cellular defense mechanisms and specific subpopulations of the infecting virus. We have previously shown that measles virus strains contain biologically distinct subpopulations that can differ in their abilities to replicate in CNS tissues (13-15). One such viral subpopulation, which has been identified in the LEC (6) and HBS (11) strains of SSPE measles virus, has the characteristic property of

inducing a self-limited and cell-associated infection in the presence of interferon (IFN) (15, 16). The similarities between this type of viral replication and the properties of several cell-associated strains of SSPE measles virus (9, 24, 42, 45) led to the hypothesis that the restricted viral protein expression seen in the CNS tissues of SSPE patients results from the chronic production of enough IFN to suppress all of the virus present except the resistant forms such as those identified in the LEC and HBS virus strains. An immediate prediction of this postulate is that IFN-producing cells should be present within the CNS lesions of SSPE patients, and such cells have recently been demonstrated by Traugott and Lebon (43). In an effort to further test our hypothesis, we have evaluated two other predictions, namely, that IFN-resistant forms of measles virus should exist in most, if not all, strains of the virus and that this viral subpopulation should be able to replicate, perhaps preferentially, in CNS tissues.

MATERIALS AND METHODS

Cells and viruses. Vero cells, a continuous line of African green monkey kidney cells, were used in all experiments. Details of their growth and maintenance have been described previously (13). The strains of measles virus used in these studies are summarized in Table 1. Upon receipt in the laboratory, each virus was passaged once at a low multiplicity of infection to prepare a working stock.

IFN. Recombinant DNA-derived hybrid human IFN- α was obtained from Hoffman-LeRoche Inc., Nutley, N.J. Procedures involved in its use were identical to those described previously (15, 16). In summary, cells were treated with medium containing the appropriate concentration of IFN for 24 h prior to infection. The IFN was used at three different concentrations, 10^4 , 10^3 , and 10^2 international units (IU)/ml. To approximate the chronic production of IFN

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TABLE 1. Strains of measles virus evaluated for IFN-resistant viral subpopulations

Virus strain	Origin or type	Titer (TCID ₅₀ /ml)	Source	Reference
HBS	SSPE	2.5×10^5	K. P. Johnson ^a	11
LEC	SSPE	4.0×10^7	K. P. Johnson	13
Halle (HAL)	SSPE	2.5×10^6	ATCC ^b	34
McClellan (MAC)	SSPE	1.0×10^6	ATCC	12
Edmonston (ED)	Measles	5.6×10^6	ATCC	25
Leningrad 16 (LEN)	Vaccine	2.5×10^6	D. L. Camenga ^a	40
Attenuvax (ATT)	Vaccine	1.8×10^5	MSD ^c	29

^a University of Maryland, Baltimore.^b American Type Culture Collection, Rockville, Md.^c Merck Sharp & Dohme, West Point, Pa.

within infected tissues and to increase the longevity of the cultures, we replaced the culture medium every third day with fresh medium containing the appropriate concentration of IFN.

Quantitative assessment of viral CPE in infected-cell cultures. The procedure for quantitative assessment of viral cytopathic effects (CPE) in infected-cell cultures has been described in detail previously (16). Briefly, cell monolayers were divided into quadrants, and at least 10 microscopic fields per quadrant were examined for viral CPE by phase-contrast microscopy. The level of culture involvement with CPE was estimated to the nearest 10% in each microscopic field, and an estimation of total culture involvement was computed as the mean of all observed fields.

Detection of cell-free viral progeny in infected-cell cultures. At the time of medium replacement, a sample of the culture medium was clarified by centrifugation, aliquoted, and frozen at -70°C . In batches, the samples were thawed, mixed with adequate neutralizing anti-IFN- α antibody (Chemicon International Inc., El Segundo, Calif.) to ensure at least a twofold antibody excess, incubated for 1 h at 37°C , and overlaid onto a monolayer of Vero cells. These cultures were then observed daily for at least 2 weeks for the appearance of viral CPE. This procedure provides increased sensitivity compared with the methods used previously (15, 16).

Quantification of IFN-resistant viruses and assessment of the type of CPE induced. The procedure for quantification of IFN-resistant viruses and assessment of the type of CPE induced was similar to a replicative-plating technique used in earlier studies in this laboratory to detect subpopulations present in measles virus-infected CNS cells (17). Monolayers of Vero cells were grown in 24-well tissue culture plates and were pretreated with medium containing 10^4 IU of IFN per ml for 24 h before infection. For each virus strain studied, 48 wells were identically infected with a specific dosage of virus that varied among different experiments. Specific viral inputs are noted when appropriate. Infected cultures were observed daily for the appearance of CPE, and the extent of culture involvement with CPE was assessed by a procedure similar to that described above. The medium of these cultures was replaced every third day with fresh medium containing the appropriate concentration of IFN. On day 24 after infection the concentration of IFN-resistant virus present in a viral stock was calculated by using the formula $M = -(1.0 \times 10^6/N) \times \ln F$, where M is the mean number of IFN-resistant viruses per 10^6 50% tissue culture infective doses (TCID₅₀), N is the viral input per culture well

expressed as TCID₅₀, and F is the fraction of infected culture wells remaining negative for viral CPE.

Passage of measles virus strains in neonatal-hamster CNS tissue. Timed pregnant golden Syrian hamsters were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass., and were housed and fed according to American Association for the Accreditation of Laboratory Animal Care-approved procedures. Newborn animals were inoculated intracerebrally within 24 h of birth with $10 \mu\text{l}$ of the appropriate virus strain or stock. Undiluted viral materials were used in all experiments, and the inoculation procedures involved have been described in detail previously (14). On day 14 after infection, two animals infected with each virus were killed with ether, and their brains were removed, finely minced, and cocultivated with Vero cells (14). No clinical disease was seen in any of the infected animals. The entire brain from each animal was used for viral isolation to avoid any anatomical predilection of various viral subpopulations that might be present. When viral CPE in the cocultivation cultures involved more than 90% of the cells in the culture, the supernatant media were removed, clarified by centrifugation, aliquoted, and frozen at -70°C . In some instances the above procedure was repeated by using a brain-derived virus stock to obtain a second-brain-passage virus. Virus stocks derived from brain passage were designated by the addition of the letter B to the virus abbreviation. For example, the first brain-passaged virus stock derived from the Halle (HAL) virus strain was designated the HALB1 virus, and the stock derived by brain passage of this virus was designated the HALBB1 virus.

RESULTS

Replication of measles virus strains in the presence of IFN.

The hypothesis being tested in these studies centers upon the presence of IFN-resistant viral subpopulations in most or all strains of measles virus. One direct test of this possibility would be to subject several independent strains of virus to the same experimental protocol used previously with the LEC and HBS strains of virus (15, 16). To this end, cell monolayers were pretreated with medium containing various concentrations of IFN and then infected with several strains of measles virus. The input multiplicity of infection was 1 TCID₅₀ per 10 cells with all of the virus strains. Infected cultures were observed daily for viral CPE. Results are shown in Fig. 1.

Three patterns could be distinguished in the induction of viral CPE. First, the Attenuvax (ATT) strain was totally suppressed by even the lowest concentration of IFN. This sensitivity may have been due in part to the highly attenuated growth properties of the virus, since it grew slowly in the control culture containing no IFN and failed to completely destroy it. The second pattern of CPE was that shown by the (HAL), Edmonston (ED), and Leningrad-16 (LEN) strains. Here, the viruses appeared to be only marginally suppressed by the IFN, since they were able to induce progressive CPE in all instances and eventually destroyed the infected cultures. However, the IFN did slow the progression of the CPE in a dose-related manner.

The LEC, HBS, and McClellan (MAC) strains showed the third pattern of CPE, which consisted of the same type of self-limited CPE described earlier (15, 16) for the HBS and LEC virus strains. However, these viruses differed from one another with respect to their sensitivities to the suppressive effects of IFN. The HBS and MAC strains were the most sensitive, since their abilities to induce CPE were com-

FIG. 1. Induction of CPE in Vero cell cultures by different strains of measles virus in the presence of IFN. Cells were pretreated for 24 h with the indicated concentrations of IFN before infection, and the culture medium was replaced every 3 days with fresh medium containing the appropriate level of IFN. The proportion of cell monolayers involved in viral CPE was determined as described in Materials and Methods. The virus strains studied are indicated in the figure, and the virus designations are explained in Table 1. Symbols: $\odot$, cell-free virus present in culture medium; $\ominus$, cell-free virus not detected in culture medium; $\circ$, no IFN present; $\times$, 10^2 IU of IFN per ml; $*$, 10^3 IU of IFN per ml; $\blacktriangle$, 10^4 IU of IFN per ml.

pletely inhibited by the highest IFN concentration, and only transient expression of CPE was observed in the infected cultures containing the intermediate IFN level. At the lowest IFN concentration a persistent but self-limited CPE was seen in the HBS-infected culture, whereas only a transient CPE appeared in the MAC-infected culture. In contrast, the LEC strain was able to induce progressive CPE in the presence of the lowest IFN concentration and ultimately destroyed the infected culture. At the intermediate and highest IFN concentrations, the LEC strain induced a chronic, self-limited CPE identical to that seen with the HBS and MAC strains. Control experiments in which Vero cell cultures were treated with high levels of IFN for 3 weeks prior to infection demonstrated that the above results were not due to an acquired resistance of the cells to the antiviral effects of IFN (data not shown). These control experiments were necessary since it has been reported that cells persistently infected with measles virus can become resistant to the antiviral effects of IFN (21).

Also shown in Fig. 1 are the results of analysis of the culture medium from the various infected cultures for the presence of cell-free viral progeny. In the majority of instances, cell-free virus was present in the medium from the infected cultures containing viral CPE, even in the presence of the highest IFN concentration. However, some significant exceptions were noted. For example, no cell-free virus was detectable in the medium of the HBS-infected cultures containing the low or intermediate IFN levels during the first 9 days after infection or in the MAC-infected culture with the intermediate level of IFN on day 9 after infection when viral CPE was present. Also, in some instances, cell-free virus could be detected in a culture supernatant in the absence of detectable CPE in the culture, e.g., the LEC-infected cultures containing the intermediate and highest IFN levels on days 6, 9, and 12 after infection.

One major difference with respect to the production of cell-free virus was seen between the viruses that produced the self-limited form of CPE in the presence of IFN and those that induced progressive CPE, i.e., a longer delay after infection before the cell-free virus appeared. For example, the HBS, MAC, and LEC strains produced no free virus before day 9 after infection in the cultures treated with the highest IFN level, whereas the HAL, ED, and LEN strains had produced detectable amounts of free virus by day 6 after infection.

These findings confirmed the presence of IFN-resistant virus subpopulations in all of the viruses tested except for the highly attenuated ATT strain. Furthermore, from the existence of the different patterns of CPE induction and the overlap among those patterns (e.g., the pattern of the LEC strain compared with those of the HBS and LEN strains), it was postulated that at least two IFN-resistant viral populations were involved. One of these appeared to induce progressive CPE capable of destroying the infected culture in concert with the prompt production of detectable cell-free virus, while the other seemed to induce a self-limited form of CPE associated with delayed or no production of cell-free viral progeny.

Quantification and characterization of IFN-resistant viral subpopulations. If each of the viral strains contained discrete populations of IFN-resistant viruses, it should be possible to separate and quantify those subpopulations. To accomplish this, we subjected the viruses to a replicative-plating procedure in the presence of a high concentration of IFN. Results are summarized in Table 2. The levels of IFN-resistant virus present in the different virus strains varied widely, ranging

TABLE 2. Quantification by replicative plating of IFN-resistant virus present in different strains of measles virus

Virus strain	No. of positive wells/ total no. infected ^a	No. of IFN-resistant viruses/10 ⁶ TCID ₅₀
HBS	4/48	42
LEC	27/48	397
HAL	7/48	76
MAC	2/48	20
ED	27/48	397
LEN	35/48	627
HAL	44/48 ^b	96

^a Virus input was 2,100 TCID₅₀ per culture well except when indicated otherwise.

^b Virus input was 26,000 TCID₅₀ per culture well.

from more than 600 viruses per 10⁶ TCID₅₀ for the LEN strain to approximately 20 viruses per 10⁶ TCID₅₀ for the MAC strain. No relationship could be seen between the concentration of IFN-resistant virus in a strain and the type (self-limited or progressive) of CPE induced by that virus in the presence of IFN. However, the detection of low levels of IFN-resistant virus in the HBS and MAC strains suggested that their failure to induce CPE in the presence of high IFN concentrations in the experiment shown in Fig. 1 was a statistical artifact. In fact, in subsequent experiments these two virus strains were found to occasionally induce self-limited CPE in cultures treated with high IFN concentrations (data not shown).

Confirmation that the viruses in these experiments were sorting independently and that the quantification procedure was valid was obtained by repeating the experiment with the HAL strain, with a different and much higher viral input. The value for the IFN-resistant virus concentration agreed quite closely in the two determinations (Table 2). Therefore, the IFN-resistant viruses were behaving as discrete entities, and the values obtained appeared to accurately reflect their concentrations.

The proportion of the IFN-resistant viruses producing self-limited or progressive CPE was assessed by observing the infected cultures on day 24 after infection and quantifying the fraction of the cell monolayer involved in CPE. Results are shown in Fig. 2. As expected, two extremes of viral behavior were observed. To facilitate comparisons among the different virus strains, we established the following observations and definitions. Infected cultures showing less than 10% involvement with CPE contained, in general, a single nonprogressive focus of CPE and were therefore considered to be prototypical of one extreme of viral behavior. Similarly, viruses that ultimately induced at least 80% CPE involvement in the infected cultures also began with a single CPE focus and were considered representative of the other extreme of behavior. As is evident in the figure, some of the strains contained a high proportion of the progressive CPE-inducing viral forms. The LEN strain epitomizes this virus type in that more than half (19 of 35, or 54%) of the IFN-resistant virus present in it induced progressive CPE. It should also be noted that the LEN strain induced the most rapidly progressive infection in the presence of high levels of IFN (Fig. 1). In contrast, the LEC virus illustrates the other extreme of behavior in that 70% (19 of 27) of the viral forms within it induced a restricted, self-limited form of CPE. This finding is also reflected in the growth of the LEC strain (Fig. 1). The IFN-resistant virus profile shown by the ED strain resembled that of the LEC strain except for the presence of a low level of viruses capable of inducing progressive CPE.

FIG. 2. Quantification, by a replicative-plating procedure, of CPE induced by different strains of measles virus in culture wells containing Vero cell monolayers in the presence of IFN. Data were derived from the experiment shown in Table 2. Vero cells were pretreated with 10^4 IU of IFN per ml for 24 h before infection. Each culture well received 2,100 TCID₅₀ of virus, except for the HAL (high-dose) strain, which received 26,000 TCID₅₀ per well. The percentage of cells involved with viral CPE was determined to the nearest 10% on day 24 after infection. The different viruses studied are indicated in the figure. Only the results derived from the high-input dose (26,000 TCID₅₀ per well) HAL sample from Table 2 are shown. The ATT strain is not shown, since it failed to induce CPE in any of the infected culture wells.

Therefore, the ED strain clearly illustrates the heterogeneous nature of the IFN-resistant viruses present in the various stocks. Finally, some of the other viruses illustrated in Fig. 2 (e.g., the HBS and MAC strains) showed even more extreme behaviors than the LEC or LEN strains, but the sample sizes were too small for conclusions to be drawn.

It is also evident from Fig. 2 that the degree of CPE involvement in the infected cultures was frequently intermediate between the two extreme values defined above (e.g., the HAL strain). In some instances this probably reflected inadequate time for the CPE to fully manifest in the cultures. However, culture wells were occasionally observed in which the CPE appeared to establish a dynamic equilibrium in which the rate of cell destruction was balanced by outgrowth of new cells. Such CPE patterns may reflect the existence of viral forms that are truly intermediate between the two extremes defined above.

Interactions between IFN-resistant viruses and CNS tissues.

At this point it was of interest to determine how the different types of IFN-resistant virus interacted with CNS tissues. That such interactions may contain an element of specificity was suggested by the fact that all of the viruses inducing a restricted pattern of CPE in the presence of IFN in cell culture (i.e., the HBS, LEC, and MAC strains) were derived from CNS tissues of patients with SSPE, whereas, in contrast, two of the viruses inducing progressive CPE in the presence of IFN (i.e., the ED and LEN strains) were derived from non-CNS tissues of patients with measles. The HAL strain, which induced a progressive CPE in the presence of IFN, was originally isolated from a patient with SSPE, but the specimen used was a lymph node and not CNS tissue (31). Therefore, it was postulated that growth of measles virus in CNS tissue may enrich it for the self-limiting CPE-inducing form of IFN-resistant virus.

In an initial series of experiments aimed at testing this possibility, the HAL strain was passaged twice through the

FIG. 3. Induction of CPE in Vero cell cultures by hamster brain passage-derived stocks of the HAL measles virus strain. Procedures were identical to those described in Fig. 1. The viral input was 1 TCID₅₀ per 10 cells for each of the viruses. The IFN concentration present in the cultures is shown in each panel. Viral stock designations and origins are explained in Materials and Methods. Symbols: ×, parental HAL strain; ○, HALB1 strain; ▲, HALB2 strain; ●, HALBB1 strain; □, HALBB2 strain.

CNS tissues of newborn hamsters. Stocks were prepared from the viruses isolated from the infected CNS tissues, and these stocks were analyzed by a method identical to that used for the virus strains in Fig. 1. The parental HAL strain was included for purposes of comparison. Results obtained are shown in Fig. 3. Although brain passage of the HAL strain did not appreciably alter its replication in the absence of IFN, it did result in increased sensitivity of the virus to inhibition by IFN. Replication of the brain-passaged viruses was slowed to a greater extent by the lowest concentration of IFN than was replication of the parental strain, with the greatest effect being seen with the HALBB1 virus stock. In the cultures containing the intermediate IFN concentration, the same general trend was apparent although the effect was larger and the HALBB1 virus produced only a transient and self-limited CPE. In the presence of the highest IFN concentration the same trend continued, since two of the brain-derived viruses produced restricted and self-limited types of CPE that were identical to those seen with the HBS, LEC, and MAC strains. Paradoxically, the HALBB1 virus was not one of the viruses producing the highly restricted CPE, although it did so occasionally in subsequent experiments (data not shown). When the brain-passaged HAL stocks were subjected to the same replicative-plating procedure used previously with the other virus strains, significant changes were found with respect to the parental virus. These results are shown in Table 3. The levels of IFN-resistant virus present in the stocks varied between 20 viruses per 10⁶ TCID₅₀ for the HALBB2 virus and >200 viruses per 10⁶ TCID₅₀ for the HALBB1 stock. As before, no correlation

was found between the concentration of IFN-resistant virus in a stock and the growth characteristics of that stock in the presence of IFN. Also, the concentration of IFN-resistant virus seen in the parental HAL strain was comparable to that observed in previous experiments. However, all of the brain-passaged viruses showed an enrichment for IFN-resistant viral forms that produce a restricted CPE when compared with the parental virus (Fig. 4). This change is most easily seen with the HALBB1 strain owing to its higher content of IFN-resistant viruses. However, the consistency of the other brain-passaged viruses in their behaviors, as well as their similarities to the HBS and MAC strains in Fig. 2, is also worthy of note. Therefore, as predicted from the results obtained in the earlier experiments, growth of a virus in CNS tissue appeared to enrich for viral forms more sensitive to partial inhibition by IFN, with the inhibition manifesting as the restricted and self-limited CPE.

TABLE 3. Quantification by replicative plating of IFN-resistant virus present in brain-passaged stocks of the HAL strain

Virus stock ^a	Virus input per well	No. of positive wells/total no. plated	No. of IFN-resistant viruses/10 ⁶ TCID ₅₀
HAL	2.1 × 10 ⁴	39/48	80
HALB1	4.6 × 10 ³	7/48	34
HALB2	6.7 × 10 ³	9/48	31
HALBB1	3.3 × 10 ³	27/48	251
HALBB2	5.2 × 10 ³	5/48	21

^a Designations of brain-passaged viruses are explained in Materials and Methods.

FIG. 4. Quantification by a replicative-plating procedure, of the CPE induced by hamster brain passage-derived stocks of the HAL measles virus strain in culture wells containing Vero cell monolayers in the presence of IFN. Data were derived from the experiment shown in Table 3. Procedures were identical to those described for Fig. 2.

To confirm these observations and to test whether CNS passage had similar effects on virus strains obtained from measles patients, we passaged the ED and LEN strains through neonatal hamster brain by methods identical to those used for the HAL strain. Growth of these viruses in the presence of IFN is shown in Fig. 5. Both of the viruses showed growth patterns similar to those seen with the brain-passaged HAL strain, although the magnitude of the IFN inhibition varied between them. The LEN strain was the least altered by brain passage, since even in the presence of the highest IFN concentration it was able to induce a very slowly progressive CPE. However, the time required for the destruction of the infected cultures containing the lower and intermediate IFN concentrations was prolonged substantially compared with that for the parental virus (Fig. 1). The

ED strain showed a greater degree of inhibition, although at the lowest IFN concentration it was able to destroy the infected culture. However, in the presence of the intermediate and highest IFN concentrations, a self-limited CPE was seen which was identical to that observed in previous experiments with other virus strains.

DISCUSSION

After two decades of intensive study, the pathogenesis of SSPE remains poorly understood. Many of the basic questions raised by the identification of measles virus as the etiological agent of the disease (20) and its subsequent isolation from the CNS tissues of patients (7) still have not been answered. For example, it is unknown how the virus

FIG. 5. Induction of CPE in Vero cell cultures by hamster brain passage-derived stocks of the ED and LEN measles virus strains. Procedures were identical to those described in Fig. 1. Viral input was 1 TCID₅₀ per 10 cells for each of the viruses. Symbols used are identical to those in Fig. 1.

persistently infecting the brain tissue of SSPE patients differs from the measles virus that originally invaded the CNS. Also unexplained is how a virus that exists in a cell-associated state in CNS tissue can rapidly revert to a productive form upon isolation in cell culture (47). The inference that a mechanism must exist within the infected CNS tissue to restrict the expression of viral genes has been supported by a number of investigators after the original idea was proposed by Haase et al. (27). The mechanism of this restriction remains unknown.

The purpose of the work described here was to further explore the hypothesis that endogenous production of IFN within the brains of SSPE patients is intimately involved in the restriction of viral gene expression and, therefore, in the establishment and maintenance of the persistent CNS infection. Central to this postulate is the existence of naturally occurring forms of measles virus that are relatively resistant to IFN-mediated inhibition and that assume, in the presence of IFN, a restricted and self-limited type of growth. Such viral forms were demonstrated previously in the LEC and HBS strains of virus derived from SSPE patients (15, 16). When several other virus strains, derived from both measles and SSPE patients, were subjected to procedures designed to detect IFN-resistant viruses, it was found that only the highly attenuated ATT vaccine virus did not contain appreciable levels of viruses capable of replicating in the presence of IFN. It is possible that this property is directly associated with the attenuated character of the virus. In contrast, the LEN strain, which is also a measles vaccine strain, contained a high level of IFN-resistant virus, and this may, in part, explain its retention of significant neurovirulence (39).

Although the presence of IFN-resistant virus in the various virus strains was consistent with the prediction made by the hypothesis being tested, the progressive nature of the growth exhibited by several of the strains in the presence of IFN contrasted with the results obtained in earlier studies (15, 16) and in the experiments described here with the HBS, MAC, and LEC strains. These findings suggested that the IFN-resistant virus was heterogeneous and that at least two viral subpopulations were involved. Analysis of the IFN-resistant viruses by a replicative-plating procedure, which provided a compositional picture of the different viral subpopulations present in each virus strain, clearly indicated that this was indeed the case. Furthermore, the facts that these IFN-resistant viruses can be reproducibly quantified

and that they sort independently according to the Poisson distribution support the conclusion that they represent discrete forms of the virus.

The origin of these viral forms is unknown, but since they can be detected in virus stocks derived from clones of the HBS, HAL, LEC, and ED strains (data not shown), it appears likely that they arise spontaneously during viral replication. Negative-stranded RNA viruses such as measles virus are known to have high rates of spontaneous mutation (30), and a high rate of mutation has been observed for measles virus genes in cell culture (4, 18) and in human CNS tissues (19). Whatever their origin, the existence of viruses capable of inducing a self-limited pattern of growth in infected cultures in the presence of IFN in two independent strains of measles virus derived from measles patients (ED and LEN) and in four independent strains of measles virus derived from SSPE patients (LEC, HBS, HAL, and MAC) supports the postulated role of IFN in SSPE.

A second test of the hypothesis was formulated from the observation that three of the four virus strains derived from SSPE patients showed a self-limited growth pattern at some IFN concentrations, whereas both of the viruses derived from measles patients induced progressive CPE at all IFN levels tested. This suggested that the two types of IFN-resistant virus may not occur randomly in different virus strains but, rather, that their relative levels are dictated by selective pressures encountered by the virus. One possible source of such selective pressure on a virus population would be growth in CNS tissue. The hypothesis being tested would predict that the IFN-resistant virus inducing the self-limited type of CPE should grow preferentially in brain tissue, since its affinity for that tissue would be important in the establishment and maintenance of the persistent infection associated with SSPE. Using the same replicative-plating procedure as before with brain-passage-derived viruses, we proved the postulated predilection of the self-limited CPE-inducing IFN-resistant viruses for CNS tissue. A single passage of a virus in CNS tissue was reproducibly shown to alter the growth pattern of the virus in the presence of IFN in cell culture. This change in viral behavior was seen with all three strains of virus tested, i.e., HAL, LEN, and ED, although the magnitude of the change varied both among the different strains and among individual brain-passaged viruses derived from the same parental strain. By incorporating these findings into the basic hypothesis, the

following scenario can be proposed for the establishment and maintenance of viral persistence in the CNS tissues of patients with SSPE. First, virus infection of the brain is established as part of the viremia accompanying acute measles virus infection. The neuroinvasiveness of measles virus is well established, and subclinical CNS involvement in measles is common (28). Second, the dose of virus, i.e., the number of infected cells, introduced into the brain must be adequate to provide a significant load of the IFN-resistant virus that induces a self-limiting pattern of growth. The low level of IFN-resistant virus present in measles virus populations, which is on the order of 1 virus per 10^4 TCID₅₀, would suggest that the viral dose delivered to the brain would have to be large for the infection to become established. It has been shown epidemiologically that patients with SSPE tend to be younger than their siblings (22) and that younger children in a family group are at increased risk of severe complications of measles virus infection (1). These and other observations have been synthesized by Aaby et al., who have proposed that intensive exposure to measles virus, i.e., high dose of infection, is a major risk factor for the development of SSPE (2).

Third, once the IFN-resistant virus and the concomitant IFN-sensitive virus are introduced into the brain, an IFN response is stimulated which suppresses the replication of the IFN-sensitive virus. The IFN-resistant virus would then remain in the CNS, where it would become enriched for the self-limited growth form by a process similar to that demonstrated in the experiments reported here. A partial defect in the production of IFN by the cells of the CNS, such as that postulated previously (16), would enhance the infection process and decrease the initial viral load necessary for the establishment of a persistent infection. At this point, the specific immunological responses would clear most of the remaining virus from the CNS. However, the IFN-resistant viruses producing the restricted, self-limiting patterns of growth may not be easily eradicated by immune effectors as a result of their lower expression of viral polypeptides (D. R. Carrigan and K. K. Knox, unpublished observation). Also, the presence of IFN itself may contribute to a local state of immunosuppression, since this has been demonstrated in cultures of human lymphocytes infected with measles virus (46). This would be especially true if a subtle defect in the host cellular immune response to measles virus (23) was present. Fourth, the IFN-resistant virus remaining in the CNS would slowly replicate and would continually stimulate the production of IFN by the surrounding cells. Cells producing IFN have been detected within lesions in brains of patients with SSPE (43), and IFN has been detected in the CSF of SSPE patients (35). Also, the presence of IFN in the CSF has been used as a marker for viral persistence in chronic canine distemper encephalitis (44), and a role for IFN in the maintenance of measles virus persistence in cell culture has been postulated by others (33, 38). This chronic production of IFN would maintain the virus in a restricted replication state but would not be able to totally suppress it. However, if the magnitude of this IFN response should decrease within the CNS, the virus would become progressively less suppressed and would begin to replicate more vigorously. Such responses of the virus to removal of the IFN in infected-cell cultures have been reported previously (16) and were observed in the course of the work described here (data not shown). These periods of more active viral replication within the CNS could account for the waxing and waning seen in the clinical status of some SSPE patients (3) and would provide repetitive immunological stimulation as

the infected cells became more visible to the immune system. If a state of immunosuppression happened to coincide with such a period of active viral replication, the disease course could dramatically accelerate. This could explain the fulminant disease courses seen in two pregnant patients with SSPE (48). Evidence for a variable pattern of viral replication in SSPE patients can be found in the observations that levels of IFN in the CSF of SSPE patients vary with time (35) and that the concentrations of measles virus-specific antibodies in serum and CSF of SSPE patients can fluctuate in concert with disease progression (26).

The process postulated above would slowly but inexorably lead to progressive tissue damage and to the death of the patient. However, the hypothesis predicts that the CNS infection should be suppressible by IFN if a sufficient concentration of IFN can be attained and maintained. When intrathecal or intraventricular IFN has been used to treat groups of patients with SSPE, both improvement of the clinical status (37, 41) and no change in their conditions (10, 32) have been seen. Although it is difficult to draw firm conclusions from these results, since there were numerous differences in the treatment protocols used in the various studies, the positive results achieved with IFN treatment in some cases support the hypothesis proposed here and justify further testing of it. If the hypothesis proves to be correct, it may be applicable as a general pathogenic mechanism to other diseases associated with persistent viral infections.

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LITERATURE CITED

1. Aaby, P., J. Bukh, I. M. Lisse, and A. J. Smits. 1984. Overcrowding and intensive exposure as determinants of measles mortality. *Am. J. Epidemiol.* **120**:49-63.
2. Aaby, P., J. Bukh, I. M. Lisse, and A. J. Smits. 1984. Risk factors in subacute sclerosing panencephalitis: age and sex dependent host reactions or intensive exposure. *Rev. Infect. Dis.* **6**:239-250.
3. Agnarsdottir, G. 1977. Subacute sclerosing panencephalitis, p. 21-49. *In* A. P. Waterson (ed.), *Recent advances in clinical virology*. Churchill Livingstone, New York.
4. Ayata, M., A. Hirano, and T. C. Wong. 1989. Structural defect linked to nonrandom mutations in the matrix gene of Biken strain subacute sclerosing panencephalitis virus defined by cDNA cloning and expression of chimeric genes. *J. Virol.* **63**:1162-1173.
5. Bacsko, K., U. G. Liebert, M. Billeter, R. Cattaneo, H. Budka, and V. ter Meulen. 1986. Expression of defective measles virus genes in brain tissues of patients with subacute sclerosing panencephalitis. *J. Virol.* **59**:472-478.
6. Barbanti-Brodano, G. S., G. S. Oyangi, M. Katz, and H. Koprowski. 1970. Presence of two viral agents in brain cells of patients with subacute sclerosing panencephalitis. *Proc. Soc. Exp. Biol. Med.* **134**:230-236.
7. Baublis, J. V., and F. E. Payne. 1968. Measles antigen and syncytium formation in brain cell cultures from subacute sclerosing panencephalitis (SSPE). *Proc. Soc. Exp. Biol. Med.* **129**:593-597.
8. Behan, P. O., and W. M. H. Behan. 1983. Immunological abnormalities and immunotherapeutic attempts in subacute sclerosing panencephalitis. *Prog. Brain Res.* **59**:149-162.
9. Burnstein, T., L. B. Jacobsen, W. Zemen, and T. T. Chen. 1974. Persistent infection of BSC-1 cells by defective measles virus derived from subacute sclerosing panencephalitis. *Infect. Immun.* **10**:1378-1382.

10. Bye, A., F. Balkwill, D. Brigden, and J. Wilson. 1985. Use of interferon in the management of patients with subacute sclerosing panencephalitis. *Dev. Med. Child Neurol.* 27:170-175.
11. Byington, D. P., A. E. Castro, and T. Burnstein. 1970. Adaption to hamsters of neurotropic measles virus from subacute sclerosing panencephalitis. *Nature (London)* 225:554-555.
12. Cape, C. A., J. Martinez, J. T. Robertson, R. Hamilton, and J. T. Jabbour. 1973. Adult onset of subacute sclerosing panencephalitis. *Arch. Neurol.* 28:124-127.
13. Carrigan, D. R. 1985. Round cell variant of measles virus: spontaneous conversion from productive to cell-associated state of infection. *Virology* 144:337-350.
14. Carrigan, D. R. 1986. Round cell variant of measles virus: neurovirulence and pathogenesis of acute encephalitis in newborn hamsters. *Virology* 148:349-359.
15. Carrigan, D. R. 1986. Round cell variant of measles virus: mechanisms involved in the establishment of defective infection of the central nervous system. *Virology* 155:614-624.
16. Carrigan, D. R., and C. M. Kabacoff. 1987. Identification of a nonproductive, cell-associated form of measles virus by its resistance to inhibition by recombinant human interferon. *J. Virol.* 61:1919-1926.
17. Carrigan, D. R., and C. M. Kabacoff. 1987. Nonproductive, cell-associated virus exists before the appearance of antiviral antibodies in experimental measles encephalitis. *Virology* 156:185-188.
18. Cattaneo, R., A. Schmid, M. A. Billeter, R. D. Shepard, and S. A. Udem. 1988. Multiple viral mutations rather than host factors cause defective measles virus gene expression in a subacute sclerosing panencephalitis cell line. *J. Virol.* 62:1388-1397.
19. Cattaneo, R., A. Schmid, G. Rebmann, K. Bacsko, V. ter Meulen, W. J. Bellini, S. Rozenblatt, and M. A. Billeter. 1986. Accumulated measles virus mutations in a case of subacute sclerosing panencephalitis: interrupted matrix protein reading frame and transcription alteration. *Virology* 154:97-107.
20. Conolly, J. H., I. V. Allen, L. J. Hurwitz, and J. H. D. Miller. 1967. Measles virus antibody and antigen in subacute sclerosing panencephalitis. *Lancet* i:542-544.
21. Crespi, M., M. Chiu, J. K. Struthers, B. D. Schoub, and S. F. Lyons. 1988. Effect of interferon on Vero cells persistently infected with Sendai virus compared to Vero cells persistently infected with SSPE virus. *Arch. Virol.* 98:235-251.
22. Detels, R., J. A. Brody, J. McNew, and A. H. Edgar. 1973. Further epidemiological studies of subacute sclerosing panencephalitis. *Lancet* ii:11-14.
23. Dhib-Jalbut, S., S. Jacobson, D. E. McFarlin, and H. F. McFarland. 1989. Impaired human leukocyte antigen-restricted measles virus specific cytotoxic T-cell response in subacute sclerosing panencephalitis. *Ann. Neurol.* 25:272-280.
24. Doi, Y., T. Sanpe, M. Nakajima, S. Okawa, T. Katoh, H. Itoh, T. Sato, K. Oguchi, T. Kumanishi, and T. Tsubaki. 1972. Properties of a cytopathic agent isolated from a patient with subacute sclerosing panencephalitis in Japan. *Jpn. J. Med. Sci. Biol.* 25:321-333.
25. Enders, J., and T. C. Peebles. 1954. Propagation in tissue cultures of cytopathic agents from patients with measles. *Proc. Soc. Exp. Biol. Med.* 86:277-286.
26. Friis, B., V. Faber, A. Salmi, and B. Ziola. 1985. Coincidental fluctuations of humoral immunity and clinical progression in a patient with subacute sclerosing panencephalitis. *J. Neurol.* 232:52-54.
27. Haase, A. T., D. Gantz, B. Eble, D. Walker, L. Stowring, P. Ventura, H. Blum, S. Wietgreffe, M. Zupancic, W. Tourtellotte, C. J. Gibbs, E. Norrby, and S. Rozenblatt. 1985. Natural history of restricted synthesis and expression of measles virus genes in subacute sclerosing panencephalitis. *Proc. Natl. Acad. Sci. USA* 82:3020-3024.
28. Hanninen, P., P. Arstila, H. Lang, A. Salmi, and M. Panelius. 1980. Involvement of the central nervous system in acute, uncomplicated measles virus infection. *J. Clin. Microbiol.* 11:610-613.
29. Heymann, D. L., E. L. Smith, J. H. Nakano, J. G. Jato, G. E. Martin, and G. K. Maben. 1982. Further field testing of the more heat-stable measles vaccines in Cameroon. *Br. Med. J.* 285:531-533.
30. Holland, J., K. Spindler, F. Horodyski, E. Grabau, S. Nichol, and S. VandePol. 1982. Rapid evolution of RNA genomes. *Science* 215:1577-1585.
31. Horta-Barbosa, L., R. Hamilton, B. Wittig, D. Fucillo, and J. L. Sever. 1971. Subacute sclerosing panencephalitis: isolation of suppressed measles virus from lymph node biopsies. *Science* 173:840-841.
32. Huttenlocher, P. R., D. L. Picchietti, R. P. Roos, N. R. Cashman, B. Horowitz, and M. S. Horowitz. 1986. Intrathecal interferon in subacute sclerosing panencephalitis. *Ann. Neurol.* 19:303-305.
33. Jacobson, S., and H. F. McFarland. 1982. Measles virus persistence in human lymphocytes: a role for virus-induced interferon. *J. Gen. Virol.* 63:351-357.
34. Johnson, K. P., E. Norrby, P. Swoveland, and D. R. Carrigan. 1982. Expression of five viral antigens in cells infected with wild-type and SSPE strains of measles virus: correlation with cytopathic effects and productivity of infections. *Arch. Virol.* 73:255-262.
35. Joncas, J. H., L. R. Robillard, A. Boudreault, M. Leyritz, and B. J. M. McLaughlin. 1976. Interferon in serum and cerebrospinal fluid in subacute sclerosing panencephalitis. *Can. Med. Assoc. J.* 115:309.
36. Liebert, U. G., K. Bacsko, H. Budka, and V. ter Meulen. 1986. Restricted expression of measles virus proteins in brains from cases of subacute sclerosing panencephalitis. *J. Gen. Virol.* 67:2435-2444.
37. Panitch, H. S., J. Gomez-Plascencia, F. H. Norris, K. Cantell, and R. A. Smith. 1986. Subacute sclerosing panencephalitis: remission after treatment with intraventricular interferon. *Neurology* 36:562-566.
38. Rager-Bisman, B., J. E. Egan, Y. Kress, and B. R. Bloom. 1984. Isolation of cold-sensitive mutants of measles virus from persistently infected murine neuroblastoma cells. *J. Virol.* 51:845-855.
39. Sharova, O. K., E. E. Rozina, N. M. Gordienko, and L. S. H. Shteinberg. 1984. Effect of immunosuppression on morphological changes in CNS of monkeys infected with different measles virus vaccine strains. *Acta Virol.* 28:144-147.
40. Shteinberg, L. S. H., and N. M. Gordienko. 1977. Genetic characteristics of clones derived from measles virus strain L-16. *Acta Virol.* 21:383-390.
41. Smith, R. A., O. Orams, I. Steiner, H. Panitch, and K. Cantell. 1986. The experimental treatment of subacute sclerosing panencephalitis with interferon, p. 505-510. *In* W. E. Stewart and H. Schellekens (ed.), *The biology of the interferon system*. Elsevier Science Publishing, Inc., New York.
42. Thormar, H., G. A. Jervis, S. C. Karl, and H. R. Brown. 1973. Passage in ferrets of an encephalitogenic cell-associated measles virus isolated from brain of a patient with subacute sclerosing panencephalitis. *J. Infect. Dis.* 127:678-685.
43. Traugott, U., and P. Lebon. 1988. Multiple sclerosis: involvement of interferons in lesion pathogenesis. *Ann. Neurol.* 24:243-251.
44. Tsia, S. C., B. A. Summers, and M. J. G. Appel. 1982. Interferon in cerebrospinal fluid: a marker for viral persistence in canine distemper encephalomyelitis. *Arch. Virol.* 72:257-265.
45. Ueda, S., Y. Okuno, Y. Okuno, Y. Hamamoto, and O. Hisashi. 1975. Subacute sclerosing panencephalitis (SSPE): isolation of a defective variant of measles virus from brain obtained at autopsy. *Biken J.* 18:113-122.
46. Vydellingum, S., J. Ilonen, S. Salonen, R. Marusyk, and A. Salmi. 1989. Infection of human peripheral blood mononuclear cells with a temperature-sensitive mutant of measles virus. *J. Virol.* 63:689-695.
47. Wechsler, S. L., and H. C. Meissner. 1982. Measles and SSPE viruses: similarities and differences. *Prog. Med. Virol.* 28:69-95.
48. Wirguin, I., I. Steiner, D. Kidron, T. Brenner, S. Udem, B. Rager, and O. Abramsky. 1988. Fulminant subacute sclerosing panencephalitis in association with pregnancy. *Arch. Neurol.* 45:1324-1325.