Subacute Sclerosing Panencephalitis: Experimental Infection in Primates

Abstract. *Measles virus isolated from the brain of a 12-year-old boy with subacute sclerosing panencephalitis caused a chronic, progressive encephalitis in experimentally infected rhesus monkeys. The infection was eventually fatal in spite of preexisting measles immunity and a vigorous secondary antibody response in serum and cerebrospinal fluid of the infected animals. The findings provide a basis for studies into the pathogenesis and possible treatment of the human disease.*

Considerable practical interest centers around the question of how some common ubiquitous viruses such as measles virus (*1*), rubella virus (*2*), herpes simplex virus, or influenza virus (*3*) may survive in an infected individual to emerge years or decades later causing debilitating, often fatal disease. Measles virus has been confirmed as the etiological agent in subacute sclerosing panencephalitis (*1*) and is strongly implicated in multiple sclerosis (*4*). The origin and pathogenesis of both diseases are obscure.

Subacute sclerosing panencephalitis (SSPE) is a disease of children and young adults. As a rule it emerges 5 to 7 years after the child had an uneventful measles infection (*5*). The disease of the central nervous system (CNS) starts insidiously with behavioral disorders and deterioration of schoolwork. In approximately 80 percent of SSPE patients the disease terminates fatally in a state of stupor, dementia, and decorticate rigidity 6 to 12 months after the initial clinical symptoms. Acute courses of infection lasting several weeks, or protracted disease with long periods of stabilization or remission, have been reported (*6*). The patients have high titers of measles antibody in their serum and cerebrospinal fluid (CSF) and measles antigen has been demonstrated in the brain by immunofluorescence (*7*). Measles virus has been recovered from brain tissue of SSPE patients (*1*) and transferred to dogs, calves, and laboratory rodents (*8*). Until recently, all attempts to produce an SSPE-like disease in rhesus monkeys failed or led to abortive infection (*9*). The failure to reproduce SSPE in primates has both theoretical and practical implications. Since man and subhuman primates are the only natural hosts of measles infection, the failure to transfer SSPE to monkeys raises questions about the pathogenetic role of measles virus in SSPE (*10*). The lack of a primate model has hampered the study of the mechanisms involved in the progressive character of the disease and possible approaches to its treatment.

Here we report the production of an SSPE-like infection in rhesus monkeys inoculated with a measles virus recently isolated from the brain of an SSPE patient. The experimental infection exhibited the main features of human SSPE, namely a progressive, eventually fatal infection of the CNS in the presence and in spite of high titers of measles antibody in the CSF. The lesions in the brain were those of panencephalitis with characteristic intracellular viral inclusions.

The virus used in this investigation was the strain IP-3 isolated from a brain biopsy of a 12-year-old boy with SSPE (*11*). Trypsinized brain cells were cocultivated with BS-C-1 cells, a line of African green monkey kidney cells. After 17 serial passages from the original coculture and five additional passages in Vero cells the virus was carried through one or two brain passages in suckling hamsters. Rhesus monkeys weighing 3 to 5 kg were inoculated intracerebrally with 1.0 ml of a 10 percent brain homogenate. The titer of the inoculum was 8×10^6 plaque-forming units per milliliter. The hamster-grown virus continued to be cell-associated when titrated in Vero cells.

Measles virus was reisolated from moribund rhesus monkeys by cocultivation with Vero cells. It was again passaged through suckling hamster brain, inoculated intracerebrally into rhesus monkeys and reisolated from moribund animals by cocultivation with Vero cells.

To monitor the integrity of the blood-brain barrier, yellow fever antibody was induced in all animals before they were inoculated with the neurotropic measles virus. The animals were injected intramuscularly with 0.5 ml of the live yellow fever vaccine (strain 17D, Merrell-National Laboratories) and given booster injections 4 to 6 weeks later to raise the serum antibody titers above 1 : 1000. Neutralizing antibodies were determined by a plaque neutralization test in Vero cells. The distribution of yellow fever antibody between the serum and CSF (CSF/serum ratio) was used as an indicator of the permeability of the blood-brain barrier.

The course of the infection and the immune response in infected rhesus monkeys is summarized in Table 1. Infection of animals that were not immune to

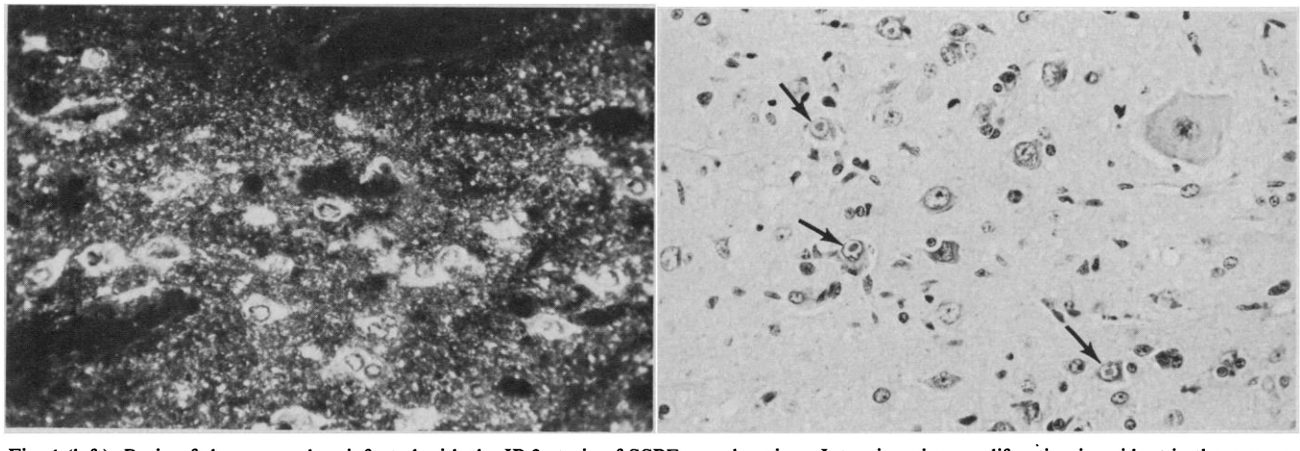

Fig. 1 (left). Brain of rhesus monkey infected with the IP-3 strain of SSPE measles virus. Intensive virus proliferation is evident in the neurons and glia cells. Intranuclear antigen is present in numerous nerve cells. Fluorescent antibody staining with hyperimmune monkey serum and antiglobulin conjugate (× 400). Fig. 2 (right). Brain of rhesus monkey infected with the IP-3 strain of SSPE measles virus. Arrows point to characteristic intranuclear viral inclusions. Hematoxylin and eosin (× 400).

measles virus caused acute, invariably fatal encephalitis 13 to 21 days after inoculation (animals 1 to 6). Several days before death the animals developed persistent and generalized myoclonic jerks (frequency of 1 to 2 per second). No EEG recordings were obtained. No antibodies developed in the serum or CSF in five of six animals. In this respect the infection of measles-negative animals lacked both the clinical and immunological features of SSPE.

In contrast, when rhesus monkeys with a history of natural measles infection were inoculated the neurotropic infection ran a subacute or chronic course (animals 7 to 16). The duration and outcome of the infection appeared to depend upon the level of the preexisting measles immunity and on the degree of brain adaptation of the virus. Thus, the measles-positive animals inoculated with tissue culture-adapted virus have survived the infection thus far for more than 9 months without clinical signs of disease (animals 7 and 8). Their serum and CSF antibody titers increased considerably above the preinoculation levels. Specifically, the CSF antibody titers increased more than 20 and 57 times, respectively, above the level which corresponds to the normal distribution of antibody across the intact blood-brain barrier (see Table 1 for normal CSF/serum antibody ratios).

Passage of the IP-3 virus through monkey brain increased its neurovirulence to a point where the infection became fatal 2 to 4 months after intracerebral inoculation (animals 9 to 12). Clinical disease characterized by ataxia, lethargy, and weakness developed from 1 to 4 weeks prior to coma. Serum and CSF antibody titers increased to extremely high levels, comparable to antibody titers in advanced stages of SSPE in humans. Four other animals with preinoculation antibody titers of more than 1: 2800 are still alive 260 days after inoculation. Two of the animals developed increased CSF/serum antibody ratios indicating the presence of subclinical encephalitis (animals 13 and 16). Based upon the lack of enhanced CSF antibody, the other two monkeys (animals 14 and 15) either eliminated the infection (by neutralization of the intracerebral inoculum?) or the infection may be latent and not stimulating antibody.

In all animals the CSF/serum ratio for yellow fever antibody was considerably lower than the ratio for measles antibody. This was taken as evidence that a major portion of measles antibody was produced in the CNS as a result of local antigenic stimulation. Intracerebral measles antibody synthesis likewise has been shown to be a characteristic feature of the human disease (*12*).

Histologically, the brains of animals with acute encephalitis had predominantly degenerative lesions with sparse inflammatory changes. Brains of animals dying of subacute encephalitis had widely disseminated viral antigen (Fig. 1) and pronounced focal proliferation of glial cells and perivascular cuffs. Long tracks of measles antigen in branching cell processes were evidence for intracellular spread of the infection. Typical intranuclear and cytoplasmic inclusions were found in neurons and in glial cells (*13*) (Fig. 2).

The successful induction of an SSPE-like infection in the rhesus monkey can largely be attributed to the biological properties of the IP-3 strain of measles virus as evidenced by its characterization in vitro (*11*). Since its isolation from human brain, this strain has maintained its cell-restricted, nonproductive character of replication. The infection in vitro spreads by cell fusion in a rather cyclical manner. Repeated bursts of activity are followed by remission and healing of the infected cell sheet. This "on and off" activity is minimally affected by low levels of measles antibody in the cell culture medium. High levels of antibody suppress the spread of infection but effective elimination is only achieved in the presence of complement (*11, 14*).

Low titers of measles antibody in the CSF of experimentally infected rhesus monkeys and of SSPE patients (*15*) presumably reflect low concentrations of antibodies in the brain tissue and may be one of the reasons for the spread of the virus in the early stages of infection. Nevertheless, it is clear that antibody slows the progress of infection dramatically, as evidenced by the acute course of encephalitis in nonimmune rhesus monkeys. A modifying effect of antibody on SSPE virus infection in hamsters has been well documented (*16*). Even though antibody titers in SSPE patients and primates reach high levels as the disease progresses, virus dissemination at later stages may be too advanced to be con-

Table 1. Infection of rhesus monkeys with the IP-3 strain of measles virus isolated from a patient with SSPE. The measles antibody titer was determined by a 50 percent plaque neutralization test. The CSF/serum antibody ratio ($\times 10^3$) in eight normal rhesus monkeys ranged from 0.2 to 0.8. A more than twofold increase in the ratio was considered pathognomonic for a disturbance of the central nervous system (≥ 1.7); ND, not determined.

Animal No.	Immunity to measles prior to inoculation	Virus inoculum (passage level)	Fate of animals	Time from inoculation (days)	Measles antibody titer (reciprocal)				
					Before infection		Late stage of infection		
					Serum	CSF	Serum	CSF	CSF/serum ratio
1 to 3	Negative	TC/22*	Died	15 ± 2†	<8	<4	<8	<4	
4 to 6	Negative	TC/22, RM/1‡	Died	16 ± 4.2	<8	<4	<8 (2/3)§ 10 (1/3)	<4 (2/3) 5 (1/3)	
7	Positive	TC/22*	Surviving	>270	770	<4	4110	82	20
8	Positive		Surviving	>270	4100	<4	11550	658	57
9	Positive	TC/22, RM/1	Died	53	980	<4	ND	ND	
10	Positive	TC/22, RM/1	Died	62	1740	<4	9120	933	102.3
11	Positive	TC/22, RM/2	Died	106	280	<4	43650	851	19.5
12	Positive	TC/22, RM/2	Died	115	2460	<4	112200	4470	39.8
13	Positive	TC/22, RM/2	Surviving	>260	2880	<4	52710	1010	19.2
14	Positive	TC/22, RM/2	Surviving	>260	4070	<4	7220	10	1.4
15	Positive	TC/22, RM/2	Surviving	>260	27540	8.5	30380	21	0.7
16	Positive	TC/22, RM/2	Surviving	>260	34670	32.1	25780	279	10.8

*Virus was passaged 17 times in BS-C-1 and five times in Vero cell cultures. †Mean ± standard deviation. ‡Virus was passed additionally once (RM/1) or twice (RM/2), through rhesus monkey brain. §Of the three animals two had a neutralizing antibody titer of <1 : 8, one had a titer of 1 : 10.

trolled by the immune response. Moreover, the absence of demonstrable lytic complement in the CSF throughout the course of the experimental infection in monkeys (*14*) indicated a lack of an important mechanism for immune control of the infection.

Other factors which are believed to affect the course of infection are fluctuations in the virus-induced antigens on the cell surface (*17*), an excess of antibody against viral nucleoprotein over antibody against viral membrane antigens (*18*), and inhibition of cell-mediated immune lysis by blocking factors in the CSF and serum (*19*).

Studies of the specificity and function of antibody and lymphocyte effector cells in the course of experimental SSPE infection in monkeys should provide results that will serve as a basis in evaluating therapeutic approaches to be used in the primate model.

PAUL ALBRECHT
Bureau of Biologics,
Division of Virology,
Food and Drug Administration,
Bethesda, Maryland 20014
THEODORE BURNSTEIN
Department of Veterinary Medicine,
Purdue University,
West Lafayette, Indiana 47907
MICHAEL J. KLUTCH
JOHN T. HICKS, FRANCIS A. ENNIS
Bureau of Biologics,
Division of Virology,
Food and Drug Administration

References and Notes

1. L. Horta-Barbosa, D. A. Fucillo, W. Zeman, J. L. Sever, *Nature (London)* **221**, 974 (1969); F. E. Payne, J. V. Baublis, H. H. Itabashi, *N. Engl. J. Med.* **281**, 585 (1969).
2. J. J. Townsend, J. R. Baringer, J. S. Wolinsky, N. Malamud, J. P. Mednick, H. S. Panitch, R. A. T. Scott, L. S. Oshiro, N. E. Cremer, *N. Engl. J. Med.* **292**, 990 (1975).
3. E. T. Gamboa, A. Wolf, M. D. Yahr, D. H. Harter, P. E. Duffy, H. Barden, K. C. Hsu, *Arch. Neurol.* **31**, 228 (1974).
4. P. Brown, F. Cathala, D. C. Gajdusek, C. J. Gibbs, Jr., *Proc. Soc. Exp. Biol. Med.* **137**, 956 (1971); E. Norrby, H. Link, J. E. Olsson, M. Panelius, A. Salmi, B. B. Vandvik, *Infect. Immun.* **10**, 688 (1974).
5. J. T. Jabbour, D. A. Duenas, J. L. Sever, H. M. Krebs, L. H. Barbosa, *J. Am. Med. Assoc.* **220**, 959 (1972).
6. J. J. Jabbour, J. H. Garcia, H. Lemmi, J. Ragland, D. A. Duenas, J. L. Sever, *ibid.* **207**, 2248 (1969); J. M. Freeman, *J. Pediat.* **75**, 590 (1969); D. H. Gilden, L. B. Rorke, R. Tanaka, *Arch. Neurol.* **33**, 644 (1975).
7. J. H. Connolly, I. V. Allen, L. J. Hurvitz, J. H. D. Millar, *Lancet* **1967-I**, 542 (1967); J. L. Sever, H. Krebs, A. Ley, L. H. Barbosa, D. Rubinstein, *J. Am. Med. Assoc.* **228**, 604 (1974).
8. M. Katz, Y. M. Kackel, D. Müller, V. terMeulen, H. Koprowski, *Acta Neuropathol.* **25**, 81 (1973); K. P. Johnson, E. Norrby, *Exp. Mol. Pathol.* **21**, 166 (1974); P. Thein, A. Mayr, V. terMeulen, H. Koprowski, M. Kackell, D. Müller, R. Meyerman, *Arch. Neurol.* **27**, 540 (1972).
9. S. Pelc, J. O. Perier, K. Quersin-Thiry, *Rev. Neurol. (Paris)* **98**, 1 (1958); B. R. Adels, D. C. Gajdusek, C. J. Gibbs, Jr., P. Albrecht, N. G. Rogers, *Neurology* **18**, 30 (1968); H. P. Schumacher, P. Albrecht, R. G. Clark, R. L. Kirschstein, N. M. Tauraso, *Infect. Immun.* **4**, 419 (1971); L. H. Barbosa, D. A. Fucillo, W. T. London, J. T. Jabbour, W. Zeman, J. L. Sever, *Proc. Soc. Exp. Biol. Med.* **132**, 272 (1969); S. Ueda, T. Otsuka, Y. Okuno, *Biken J.* **18**, 179 (1975).
10. R. T. Johnson and C. J. Gibbs, Jr., *Arch Neurol.* **30**, 36 (1974).
11. T. Burnstein, L. B. Jacobsen, W. Zeman, T. T. Chen, *Infect. Immun.* **10**, 1378 (1974).
12. R. W. P. Cutler, G. V. Waters, J. P. Hammerstad, E. Merler, *Arch. Neurol.* **17**, 620 (1967).
13. T. Ohya, A. J. Martinez, J. T. Jabbour, H. Lemmi, D. O. Duenas, *Neurology* **24**, 211 (1974).
14. P. Albrecht, J. T. Hicks, M. J. Klutch, in preparation.
15. L. Horta-Barbosa, H. Krebs, A. Ley, T. Chen, M. R. Gilkeson, J. L. Sever, *Pediatrics* **47**, 782 (1971).
16. D. P. Byington, K. P. Johnson, *J. Infect. Dis.* **126**, 18 (1972).
17. M. B. A. Oldstone, V. K. Bokisch, F. J. Dixon, L. H. Barbosa, D. Fucillo, J. L. Sever, *Clin. Immunopathol.* **4**, 52 (1975); B. S. Joseph and M. B. A. Oldstone, *J. Exp. Med.* **142**, 864 (1975).
18. A. A. Salmi, E. Norrby, M. Panelius, *Infect. Immun.* **6**, 248 (1972).
19. A. Ahmed, D. M. Strong, K. W. Sell, G. B. Thurman, R. C. Knudsen, R. Wistar, W. R. Grace, *J. Exp. Med.* **139**, 902 (1974).

14 July 1976; revised 3 September 1976

Lysogeny in Pneumococci Freshly Isolated from Man

Abstract. *Twelve strains of encapsulated pneumococci isolated from patients with pneumococcal disease were examined for the presence of bacteriophage. Four of the strains yielded phage lytic for a noncapsulated indicator strain of pneumococcus. Three of the newly isolated bacteriophages differ serologically from pneumococcus bacteriophages described previously. The ability to yield lytic phage was lost by two of the lysogenic pneumococcal strains on repeated subculture.*

DNA-mediated transformation, first discovered in the pneumococcus (*1*), remains the only reported mechanism of gene transfer in this organism. While results from phage-mediated gene transfer studies have contributed greatly to the understanding of genetic mechanisms in other bacterial systems, no bacteriophages lytic for pneumococci had been described until recently (*2*). The recovery of temperate pneumococcal phages has not been recorded. In the course of testing the sensitivity of various pneumococcal strains to some of the ω phages (*3*), attempts were made to induce lytic phage from 24 laboratory strains of encapsulated pneumococci by growing the organisms in the presence of mitomycin C (MC); the experiments were uniformly unsuccessful. However, it seemed possible that pneumococci freshly isolated from patients in whom the organism was causing disease might be lysogenic. I have examined strains isolated from 12 patients with pneumococcal disease and have recovered active phage from four of the strains.

A pneumococcus, type 8, was recovered from the blood of a patient with bacteremic pneumococcal pneumonia. Colonies from a blood-agar plate streaked with the original blood-broth culture were inoculated into Neopeptone beef-heart infusion broth containing horse blood (2 percent), and the culture was incubated overnight at 37°C. A subculture in casein hydrolyzate (CH) medium (*4*) was grown at 37°C to visible turbidity [2×10^7 to 4×10^7 colony-forming units (cfu) per milliliter] and divided. To one portion, MC was added to a final concentration of 0.1 μg/ml, and both cultures were incubated further. The turbidity of the control culture increased steadily while the turbidity of the culture with MC (protected from light by aluminum foil) increased for about 1½ hours and then began to decrease. Complete lysis did not occur, but at 2½ hours the tubes were chilled and centrifuged to sediment the bacteria. The supernatants were filtered through GA-6 (Gelman) filter disks. Samples (0.1 ml and 0.01 ml) of the filtrates were mixed with 0.1 ml of a young culture (about 2×10^7 cfu/ml) of a noncapsulated pneumococcus, strain 213 [R36A *str-r* (*5*)], grown in tryptic soy broth (Difco). The mixtures were incubated at 37°C for 10 minutes to permit adsorption and plated by the soft-agar overlay technique (*6*). After overnight incubation of the plates at 34°C, examination revealed three possible plaques from samples of the MC-treated culture. Each was picked with a Pasteur pipette into 0.5 ml of MI broth containing 2 percent blood; 0.1 ml of fresh culture of strain 213 was added to each tube, and the mixtures were replated as before. One of them yielded several thousand plaques, and the phage could then be propagated easily in liquid or on solid medium. Attempts to reisolate phage from subcultures of the type 8 pneumococcus were unsuccessful although cultures grown with MC continued to exhibit partial lysis as compared to control cultures. Electron micrographs of phage preparations revealed entities (Fig. 1) resembling the phages described by others (*1*). Electron micrographs of a two-times concentrated preparation of the original MC-treated culture and of a subsequent MC-treated culture showed particles that re-