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Host Defenses in Herpes Simplex Infections of the Nervous System: Effect of Antibody on Disease and Viral Spread

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BALB/c mice passively immunized with antibody to herpes simplex virus type 1 and challenged in the footpad with $10^{5.7}$ plaque-forming units of herpes simplex virus type 1 were shown to be protected from neurological disease and death compared with control mice treated with normal serum or antibody to Sindbis virus. One hundred percent of untreated mice had virus recoverable from dorsal root ganglia by 48 h after infection. Whereas amputation of the infected limb at 48 h had no effect, antibody administration (resulting in titers of 1:8 and 1:16) was found to prevent acute neurological disease if administered no later than 48 h after infection. Antibody also restricted the extent of latent infection in the lumbosacral ganglia. The data provide strong evidence that antibody is effective in preventing spread of virus both in the peripheral nervous system and in central nervous system (spinal cord) tissue.

Several lines of evidence have suggested that serum antibody is ineffective in the control of herpes simplex virus (HSV) infections: (i) passively transferred antibody fails to protect against HSV infection in some animal models (15, 19, 22); (ii) antibody does not prevent the appearance of virus in the dorsal root ganglion after footpad inoculation (3); (iii) HSV may spread to contiguous cells in tissue culture despite the presence of antibody in supernatant fluids (8); (iv) oral or genital HSV infections recur in patients despite presence of serum neutralizing antibody (2, 4, 21); and (v) HSV encephalitis has occurred in patients with previous history of recurrent cold sores (12).

Under some circumstances, however, it appears that antibody may effectively inhibit spread of virus (1, 13, 17). Therefore, passive transfer studies in mice were undertaken to determine: (i) the titer of prechallenge antibody which is effective, (ii) the range of viral dose which can be influenced by antibody, (iii) the time interval before and after viral challenge during which antibody may be effective, and (iv) the ability of antibody to influence the spread of virus within the nervous system. The passive transfer studies were conducted with a model of peripheral inoculation of the footpad because spread of virus has been well documented to be neural (3, 11) and not viremic as in intraperitoneal (i.p.) models (9, 20); because the resultant disease can be assessed by clinical severity as well as mortality; and because most human infection follows exposure at peripheral sites, either nasopharyngeal, ocular, or genital.

MATERIALS AND METHODS

Virus. HSV type 1 (HSV-1) was isolated from a patient (Heitzman) with pharyngitis (14) and was identified and typed by neutralization kinetics (R. Duff, North Chicago, Ill.) and passive hemagglutination (Center for Disease Control, Atlanta, Ga.). The isolate was passed six times in Hep-2 cells at a multiplicity of infection between 0.1 and 0.01 to produce a stock which contained 1.4×10^7 plaque-forming units (PFU) per ml. Sindbis virus (R. T. Johnson, Baltimore, Md.) was passed three times in Vero cells at a multiplicity of infection of 0.1 to prepare a stock which contained 10^6 PFU/ml.

Animals. Four-week-old BALB/c mice (Charles River, Mass.) were used in viral studies. New Zealand white rabbits, age 6 to 8 weeks, were used as a source of hyperimmune HSV-1 serum.

Hyperimmune sera. Mice were immunized with HSV-1 by an initial footpad dose of $10^{5.7}$ PFU followed by two or three subsequent weekly i.p. doses. Serum was collected and pooled 7 to 10 days after the last i.p. injection. A similar protocol was followed for immunization with Sindbis virus.

Rabbits were immunized i.p. with an initial inoculum of HSV-1 containing $10^{7.6}$ PFU. A second dose divided intramuscularly and i.p. was given 1 week later. At 10 days after a third dose, serum was collected, pooled, and stored at −90°C. An aliquot was tested by microneutralization for antibody titers.

Microneutralization test. Twofold dilutions of sera were treated by standard microtiter assay (6, 16). A viral inoculum containing 50 50% tissue culture infective doses was added to each dilution of serum, and the mixture was incubated for 1 h at 33°C. The serum-virus mixture was then transferred to Vero cell monolayers grown in flat-bottomed microtiter plates (Cooke Engineering, Alexandria, Va.). Control sera consisted of known positive and known negative spec-

imens. The virus preparation was back titrated at the time of each test. The test results were read as the highest dilution sera which produced complete neutralization of virus in four replicate wells.

Passive transfer studies. BALB/c mice were given 0.5 ml of hyperimmune herpes serum i.p. at various times relative to virus challenge. Control mice received normal or hyperimmune Sindbis virus serum. In each experiment, a group of four to six mice which did not receive virus challenge were bled retroorbitally 24 h after passive transfer, and the neutralizing antibody titer was determined by microneutralization. Virus challenge was accomplished by the inoculation into the right rear footpad of a 30-μl volume containing $10^{5.7}$ PFU of HSV-1 from a 1,000-μl syringe (Hamilton Co., Reno, Nev.). Experimental and control groups of mice were coded and were evaluated by two observers at 1- to 2-day intervals for presence and severity of neurological disease. Illness was defined as evidence of definite monoplegia or greater and animals were categorized by two independent observers on coded cages. The code was deciphered and the results were tabulated 1 week after cessation of new illness or death.

Amputation experiments. BALB/c mice were inoculated in the right rear footpad with HSV-1. At 48 h after infection, after ether anesthesia, the inoculated limb was aseptically amputated at the level of the distal calf. Cauterization was used to control bleeding. Control nonamputated mice received 0.5 ml of hyperimmune herpes serum or L-15 medium i.p. 48 h after virus inoculation. Mice were followed for the appearance of neurological illness and death.

Assay for latent infection. At 8 weeks after viral challenge, six lumbosacral ganglia from right and left sides of an animal were removed and individually explanted in multiwell plates (Falcon, Oxnard, Calif.), overlayed with L-15 medium containing 10% fetal calf serum and antibiotic supplements. At 3 days after explant, 1.2×10^5 Vero cells were added to each well, and wells were observed for cytopathogenic effect for 3 to 4 weeks. Questionable cytopathic effect was verified by subsequent passage of culture supernates.

Assay for acute infection of spinal ganglia. At 26, 48, and 72 h after footpad infection, spinal ganglia were removed and explanted. At 2 h after explant, Vero cells were added to the explants as an indicator cell sheet for cytopathic effect. Media consisted of L-15 with 2% fetal calf serum and antibiotic supplements. Cytopathic effect-negative cultures were terminated after 10 days of observation.

RESULTS

Clinical disease. On day 5 after inoculation of HSV-1 into the footpad, the mice developed a very stereotyped evolution of disease (3, 14). In brief, early mild signs of distal weakness progressed on day 6 to dramatic monoplegia ipsilateral to inoculation. The disorder remained static or progressed to flaccid paraplegia and ascending myelitis over the next 2 days. Lethargy, labored respirations, and death ensued if the myelitis was not arrested. This stereotyped pattern was observed in all affected mice.

Effect of viral dose on the incidence and severity of disease. To determine an appropriate dose of virus and assure adequate morbidity without overwhelming mortality, groups of 7 to 11 mice were given $10^{5.7}$, $10^{4.7}$, or $10^{3.7}$ PFU of HSV-1 by footpad inoculation (Fig. 1). All mice receiving $10^{5.7}$ PFU became ill, and 3/11 (27%) died. All eight survivors had residual monoplegia. Of mice receiving $10^{4.7}$ PFU, five of seven (71%) developed neurological disease, and one survived with monoplegia and one survived with paraplegia. At $10^{3.7}$ PFU of virus, four of eight (50%) developed illness, and three survived with monoplegia. In the passive transfer studies described below, $10^{5.7}$ PFU of HSV-1 was the dose of virus utilized.

Effect of passively transferred antibody on the appearance of neurological illness. Groups of seven to eight mice were given 0.5 ml of normal mouse serum or hyperimmune HSV-1 mouse serum i.p. 24 h before virus challenge. A pooled pre-inoculation serum sample from four to six mice was shown by microneutralization to contain a titer of 1:16. The results of challenge with $10^{5.7}$ PFU of HSV-1 are shown in Fig. 2A. All mice (eight of eight) which received normal serum developed neurological disease, whereas only one of eight (12.5%) mice given hyperimmune HSV-1 serum became ill. In a second experiment, mice were given either rabbit hyperimmune HSV-1 serum or mouse hyperimmune HSV-1 serum. Control mice received mouse serum hyperimmune to Sindbis virus. The results, shown in Fig. 2B, indicate that seven of eight (87.5%) mice given Sindbis immune serum became ill after virus challenge

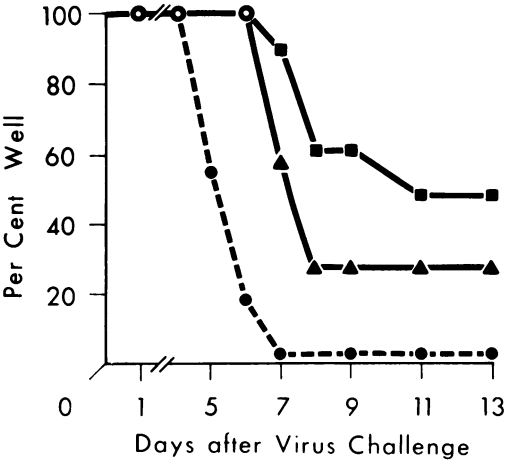

FIG. 1. *Neurological illness in BALB/c mice inoculated in the footpad with $10^{5.7}$ PFU of HSV-1 (●- - -●), $10^{4.7}$ PFU of HSV-1 (▲——▲), or $10^{3.7}$ PFU of HSV-1 (■——■).*

with $10^{5.7}$ PFU of HSV-1, whereas only one of eight (12.5%) mice given mouse hyperimmune herpes serum became ill. None of eight (0.0%) mice receiving the rabbit herpes immune antisera became ill. Both groups of mice receiving the herpes immune sera had prechallenge neutralizing antibody titers of 1:8 by microneutralization. The data show that prechallenge antibody titers of 1:8 to 1:16 were remarkably protective against virus challenges of $10^{5.7}$ PFU.

Effect of antibody on lethal disease. Hyperimmune sera were also effective when judged by mortality criteria. The combined mortality-passive transfer experiments shown in Fig. 3 indicate that 2/24 (8%) of mice treated with herpes immune sera died, whereas 7/16 (44%) of control mice died ($P < 0.05$).

Influence of antibody administered at different times after infection on disease and death. Mice were given 0.5 ml of stock pooled rabbit hyperimmune HSV-1 serum at different times before or after virus challenge. Control mice received normal rabbit serum. Figure 4 shows the results of a viral challenge containing $10^{5.7}$ PFU of HSV-1 by footpad. Of 10 control mice which received normal rabbit serum 2 h before virus challenge, 9 became ill (90%) and 7 (70%) died. Of nine mice treated with hyperimmune HSV-1 serum 2 h before challenge, none (0%) became ill ($P < 0.001$). All eight mice given hyperimmune HSV-1 serum 24 h after challenge remained free of illness. In the groups receiving antibody 48, 72, and 96 h after virus challenge, illness appeared in two of eight (25%), five of eight (62.5%) and six of seven (86%) animals, respectively. The incidence of illness in the group receiving antibody at 48 h after infection was significantly less than the control group ($P < 0.05$) receiving normal rabbit sera. Neither the 72-h ($P < 0.1$) nor the 96-h ($P < 0.1$) group was significantly different from controls. Therefore, antibody effectiveness was limited to a time interval of 48 h after virus infection. The low mortality (12.5%) in the face of high morbidity (62.5%) in the group receiving antibody 72 h after infection is noticeably different compared with the high mortality (70%) observed in control mice ($P < 0.05$).

Figure 5 relates the time of antibody administration to the kinetics of disease appearance.

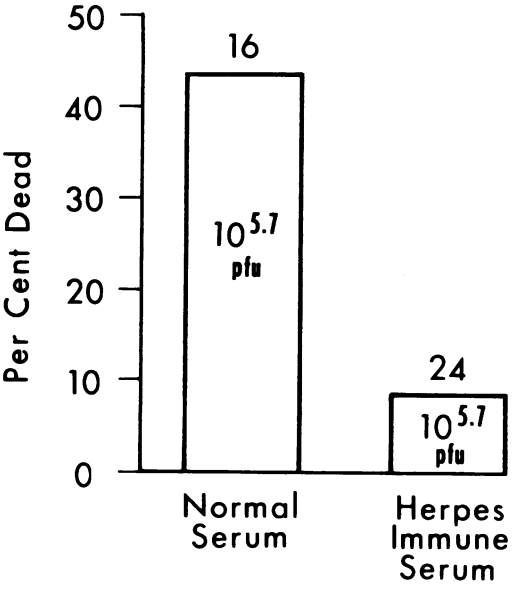

FIG. 3. *Mortality from neurological illness after $10^{5.7}$ PFU of HSV-1 in BALB/c mice treated with herpes immune serum or nonimmune serum.*

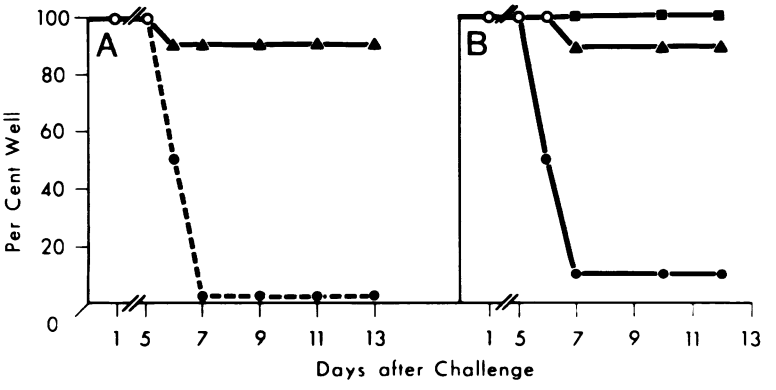

FIG. 2. *(A) Neurological illness in BALB/c mice receiving passively transferred mouse hyperimmune herpes serum (▲——▲) or normal mouse serum (●- - -●) and challenged with $10^{5.7}$ PFU of HSV-1 by footpad. (B) Neurological illness in BALB/c mice receiving passively transferred rabbit hyperimmune herpes serum (■——■), mouse hyperimmune herpes serum (▲——▲), or mouse hyperimmune Sindbis virus serum (●——●) and challenged with $10^{5.7}$ PFU of HSV-1 by footpad.*

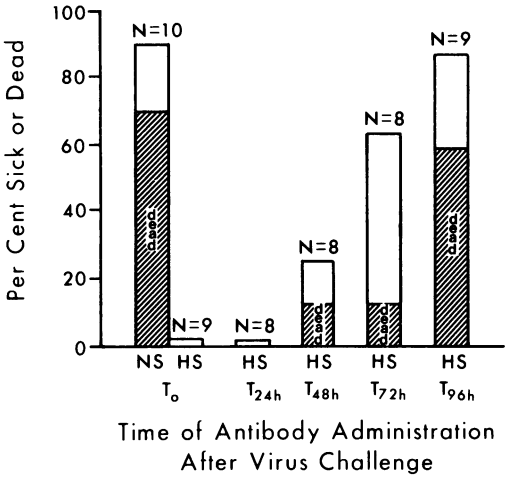

FIG. 4. *Morbidity and mortality from neurological illness in BALB/c mice treated with a single dose of hyperimmune herpes serum (HS) or normal serum (NS) at different times relative to virus challenge. Mice in all groups were challenged in the footpad with $10^{5.7}$ PFU of HSV-1.*

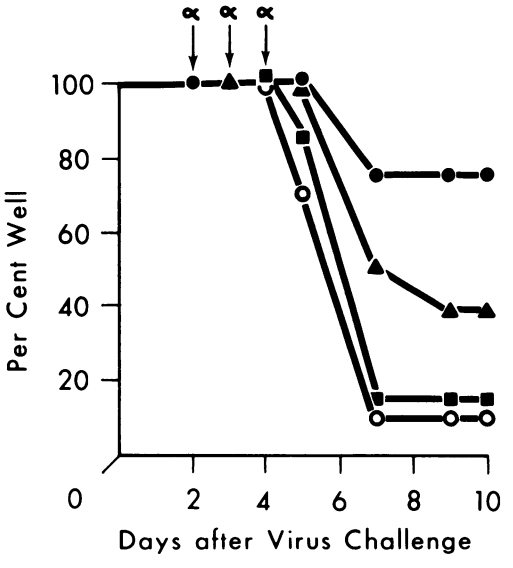

FIG. 5. *Appearance of neurological illness in mice receiving a single dose of hyperimmune herpes serum at different times after virus challenge. Antibody was given to mice intraperitoneally at 48 h (●), 72 h (▲), or 96 h (■), after challenge with $10^{5.7}$ PFU of HSV-1. Control mice (○) received normal serum 2 h before virus challenge.*

The control mice display a time course which has not been modified by antibody and which is remarkably stereotyped for this model of nervous system infection. These mice remained free of illness through the first 4 days after virus challenge. By utilizing the time course in the control mice as the natural history of this infection, it can be seen that antibody administered 96, 72, and 48 h after virus challenge corresponds to 1, 2, and 3 days, respectively, before the anticipated appearance of disease on day 5. The groups clearly protected by antibody received their treatment 3 days before onset of disease. Therefore, antibody prevention of disease was limited to administration times well in advance of the onset of disease.

Distribution of latent infection in mice treated with antibody at different times post-viral challenge. Because clear-cut effectiveness of antibody was limited to a narrow time interval of 48 h after virus inoculation into the footpad, studies were undertaken to determine how widely virus had spread by different times after virus challenge. Survivors of the passive transfer studies described above in Fig. 4 and 5 were studied for the distribution of latent infection of the spinal ganglia as a "footprint" of the acute infection. Mice were sacrificed 6 to 8 weeks after acute disease, and the lumbosacral ganglia from L-1 to S-1 on both sides of the animal were individually cocultivated as explants with a Vero cell monolayer. Figure 6 shows the distribution of latent virus recovered from the groups studied. In mice which had received antibody 2 h before virus challenge, latent virus was recovered only on the side ipsilateral to the virus challenge. Two had latent virus in the L-3 ganglia, four had latent virus in the L-4 ganglia, three had it in the L-5, and one had it at S-1. No virus was recovered at L-2 or L-1 ipsilaterally, and no virus was recovered at any level from the side contralateral to inoculation. Of seven mice which had received antibody 48 h after virus, latent infection was found in seven of seven (100%) at L-3, L-4, and L-5 and in one of seven (14%) at S-1. No animal harbored latent virus on the side contralateral to the inoculated side. Spread across the spinal cord was not observed. The last group of animals shown in Fig. 6 included two mice which had received antibody at 96 h after virus challenge and three mice which had received normal rabbit serum at 2 h before virus challenge. These mice were combined because there was no difference in the distribution of latent virus and because, as shown above in Fig. 4 and 5, there was no difference in disease appearance or severity of disease in these two groups. It can be seen that latent virus was widespread on both the side ipsilateral and contralateral to virus inoculation. Furthermore, it is striking that 9/10 (90%) of L-1 and L-2 ganglia on the R (ipsilateral) harbored latent virus. This is in contrast to

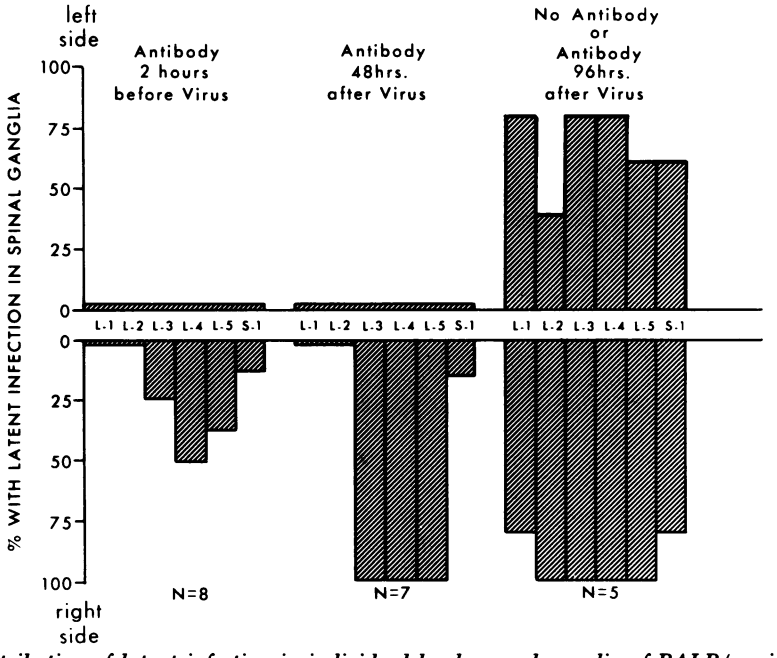

FIG. 6. *Distribution of latent infection in individual lumbosacral ganglia of BALB/c mice treated with antibody at different times relative to HSV-1 challenge. N, Number of animals in each group.*

the two other animal groups studied where 0/32 (0%) ganglia from L-1 and L-2 harbored latent virus ($P < 0.001$). This provides strong evidence that virus spread had extended to much higher spinal cord levels as well as spread laterally across the spinal cord.

Effect of amputation on the disease. Because antibody administration 48 h after infection might act in part by suppressing the well-known distal site of replication in footpad, experiments were done to determine whether anatomical removal of foot would have a similar beneficial effect. At 48 h after footpad inoculation of HSV-1, amputation of the inoculated limb was performed at the distal calf. Of 12 amputated mice, 12/12 (100%) developed paraplegia and died (Table 1). All of 10 nonamputated control mice receiving virus alone died. Of 28 nonamputated mice receiving hyperimmune herpes serum 48 h after virus infection, one (4%) developed paraplegia and death. Hence, antibody must exert an effect higher than the level of the foot.

Appearance of virus in lumbosacral ganglia at times after footpad infection. Because the distribution of virus in the neuraxis at various times after infection is known to vary markedly with the input dose of virus (R. R. McKendall, unpublished data), the level of infection in the neuraxis with time was determined at the dose levels used in the amputation experiments. Table 2 shows the results of virus recovery by ganglia explant at intervals after infection. Virus had reached the level of the spinal ganglia in two of three (66%) mice by 24 h and in four of four (100%) mice at 48 h and 72 h postinfection.

TABLE 1. *Effect of limb amputation or antibody treatment 48 h after HSV-1 infection by footpad*

Treatment	Mortality	% Dead	*P* value
Virus alone	10/10[a]	100	
Virus + amputation	12/12	100	
Virus + antibody	1/28	4	<0.0005

[a] Number of mice dead/number inoculated.

TABLE 2. *Viral recovery from ipsilateral ganglia at intervals after footpad infection*

Time after footpad infection (h)	Viral recovery from spinal ganglia explants[a]
26	2/3 (66)
48	4/4 (100)
72	5/5 (100)

[a] Number of animals positive/number of animals studied. Number in parentheses indicates percentage of positive animals.

DISCUSSION

The passive transfer studies reported here show that antibody to herpes simplex may moderate the appearance and severity of neurologi-

cal illness from HSV-1 inoculation. The titers of antibody required to influence disease have been shown to be relatively modest (1:8 or 1:16), and the doses of virus which can be thwarted are relatively high, although the appearance of illness in 12.5% of animals in both passive transfer experiments shown in Fig. 2A and B indicates that these antibody levels would probably be inadequate if higher viral doses were employed.

In this study, the effectiveness of specific antibody has been shown to be limited to within 48 h of virus inoculation and more importantly to within 3 days before the expected appearance of illness in unprotected control animals. The ineffectiveness of antibody administered later, even though before the appearance of disease, most likely relates to the fact that disease is a poor and late indication of infection. After peripheral inoculation, virus has been seen in spinal cords within 48 h by electron microscopy (10) and has been isolated from dorsal root ganglia within 20 h (3) or from spinal cords within 48 h (11, 22). The appearance of virus in the spinal ganglia by 48 h has been documented in this model. Therefore, the protection afforded mice treated with antibody 48 h after infection must in part relate to suppression of virus which had already appeared in spinal cords and dorsal root ganglia at the time of antibody administration.

The data showing a restriction in the distribution of latent virus demonstrate three points. First, as others have shown (18), latent infection of spinal ganglia may occur despite presence of prechallenge neutralizing antibody. Secondly, antibody restricts the establishment of latent infection to fewer ganglia and also to fewer mice, indicating a peripheral site of action. In comparison to mice which were not given antibody until 48 h later, the number of mice with latent infection was less, as was the number of latently infected ganglia per mouse. Therefore, although in vitro data indicate that HSV may spread in the presence of antibody (5), in vivo spread of virus to ganglia is inhibited by antibody depending upon the titer of antibody, dose of virus challenge, and perhaps other unidentified factors. Thirdly, the data on latent infection indicate that antibody is successful in preventing spread of virus within the spinal cord. Prevention of virus appearance on the side contralateral to inoculation provides evidence for successful antibody intervention to contain virus spread from the dorsal root ganglia across the spinal cord and similarly to prevent ipsilateral spread to higher levels of L-2 or L-1.

The possibility that spread of virus to ganglia was arrested by antibody neutralization of a viremia is unlikely because an extensive literature suggests that viremia is an unimportant or uncommon event after peripheral inoculation of virus (3, 9, 20, 22). In addition viremia would be expected to produce a random distribution of latent infection in ganglia and not a pattern of infection confined to ganglia ipsilateral to inoculation, as shown in this and previous reports from this laboratory (14).

The possibility that antibody, regardless of its time of administration, exerts its sole influence at peripheral levels by suppressing distal replication in footpad and sciatic nerve must be addressed. Several studies already cited show that virus appears in the dorsal root ganglia (3) or in the spinal cord (11, 22) within 24 to 48 h of infection. In this model, virus had reached the spinal ganglia in 100% of mice by 48 h. Amputation at 48 h, however, failed to alter disease, as had previously been observed (5). Therefore, disease is not dependent upon virus generated distally. More significantly prevention of virus spread to the contralateral side by antibody given 48 h after infection must result from intervention at dorsal root or spinal cord levels. It is not clear from these studies what antibody mechanisms are responsible for either its distal or its central effect. Further work will be necessary to determine whether virus neutralization, complement-dependent cell recruitment, interferon production by T-cells stimulated by virus-antibody complexes (7), or other unidentified mechanisms are responsible for the successful inhibition of both acute and latent disease by antibody.

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LITERATURE CITED

1. **Baron, S., et al.** 1975. Post exposure serum prophylaxis of neonatal herpes simplex virus infection of mice. Nature (London) **261**:505–506.
2. **Centefano, Y. M., J. M. Little, and H. E. Kaufman.** 1970. The relationship between virus chemotherapy, secretory antibody formation and recurrent herpetic disease. Ann. N.Y. Acad. Sci. **173**:649–655.
3. **Cook, M. L., and J. G. Stevens.** 1973. Pathogenesis of herpetic neuritis and ganglionitis in mice: evidence for intra-axonal transport of infection. Infect. Immun. **7**: 272–288.
4. **Douglas, R. G., and R. B. Couch.** 1970. A prospective study of chronic herpes simplex virus infection and recurrent herpes labialis in humans. J. Immunol. **104**: 289–295.
5. **Evans, C. A., H. B. Slavin, and G. P. Berry.** 1946. Studies on herpetic infection in mice. IV. The effect of specific antibodies on the progression of the virus within the nervous system of young mice. J. Exp. Med. **84**:

429–447.
6. **Fuccillo, D. A., L. W. Catalano, Jr., F. L. Moder, D. A. Debus, and J. L. Sever.** 1969. Minicultures of mammalian cells in a new plastic plate. Appl. Microbiol. **17**:619–622.
7. **Fujibayashi, T., J. J. Hooks, and A. L. Notkins.** 1975. Production of interferon by immune lymphocytes exposed to herpes simplex virus-antibody complexes. J. Immunol. **115**:1191–1193.
8. **Hooks, J. J., W. Burns, K. Hayaski, S. Geis, and A. L. Notkins.** 1976. Viral spread in the presence of neutralizing antibody: mechanisms of persistence in foamy virus infection. Infect. Immun. **14**:1172–1178.
9. **Johnson, R. T.** 1964. The pathogenesis of herpes virus encephalitis. I. Virus pathways to the nervous system of suckling mice demonstrated by fluorescent antibody staining. J. Exp. Med. **119**:343–356.
10. **Kristensson, K.** 1970. Morphological studies of the neural spread of herpes simplex virus to the central nervous system. Acta Neuropathol. **16**:54–63.
11. **Kristensson, K., E. Lycke, and X. Sjostrand.** 1971. Spread of herpes simplex virus in peripheral nerves. Acta Neuropathol. **17**:44–53.
12. **Leider, W., R. L. Magoffin, E. H. Lennette, and L. N. R. Leanards.** 1965. Herpes-simplex-virus encephalitis. Its possible association with reactivated latent infection. N. Engl. J. Med. **273**:341–347.
13. **Luyet, F., D. Samra, A. Soneji, and M. I. Marks.** 1975. Passive immunization in experimental *Herpes virus hominis* infection of newborn mice. Infect. Immun. **12**: 1258–1261.
14. **McKendall, R. R., N. Vogelzang, and G. G. Jackson.** 1974. Herpes virus latency in spinal ganglia of mice without illness. Proc. Soc. Exp. Biol. Med. **146**: 1093–1096.
15. **Oakes, J. E.** 1975. Role for cell-mediated immunity in the resistance of mice to subcutaneous herpes simplex virus infection. Infect. Immun. **12**:166–172.
16. **Pauls, R. P., and W. R. Dowdle.** 1966. A serologic study of *Herpes virus hominis* strains by microneutralization tests. J. Immunol. **98**:941–947.
17. **Pavan, P. R., and F. A. Ennis.** 1977. The elimination of herpes simplex plaques by antibody and the emergence of resistant strains. J. Immunol. **118**:938–940.
18. **Price, R. W., M. A. Walz, C. Wohlenberg, and A. L. Notkins.** 1975. Latent infection of sensory ganglia with herpes simplex virus: efficacy of immunization. Science **188**:938–940.
19. **Rager-Zisman, B., and A. C. Allison.** 1976. Mechanism of immunologic resistance to herpes simplex virus I (HSV-1) infection. J. Immunol. **116**:35–40.
20. **Renis, H. E., E. E. Eidson, J. Mathews, and J. E. Gray.** 1976. Pathogenesis of herpes simplex virus types 1 and 2 in mice after various routes of inoculation. Infect. Immun. **14**:571–578.
21. **Spruance, S. L., J. C. Overall, Jr., E. R. Kern, G. G. Kreuger, V. Pliam, and W. Miller.** 1977. The natural history of recurrent herpes simplex labialis: implications for antiviral therapy. N. Engl. J. Med. **297**:69–75.
22. **Wildy, P.** 1967. The progression of herpes simplex virus to the central nervous system of the mouse. J. Hyg. Camb. **65**:173–192.