Role of Antibody in Primary and Recurrent Herpes Simplex Virus Infection

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When herpes simplex virus was inoculated into the flank of a BALB/c mouse by scarification, the local replication of virus was followed by the establishment of an acute ganglionic infection. The subsequent centrifugal spread of this virus along nerves to the skin of the whole dermatome led to the development of a bandlike "zosteriform" rash. This represents a highly reproducible system in which virus travels through the nervous system synchronously in large numbers of animals. The transection of peripheral nerves at various times after infection showed that the virus had completed the round trip 60 h after inoculation into the upper flank and was detectable as infectious virus by 74 h postinfection. After the administration of virus, neutralizing but not nonneutralizing antibodies prevented the development of the zosteriform rash. The target epitopes of the protective antibodies were not confined to a single glycoprotein. Neutralizing antibody was effective even when given up to 60 h postinfection and was protective even when administered after sensory neurotomy at this time. Antibody was therefore able to prevent clinically and virologically detectable infection of the skin, presumably by acting peripherally on virus emerging from nerve endings. A quantitative estimate of the action of one of the neutralizing monoclonal antibody preparations, AP7, showed that high titers (several times higher than those normally found in immune mice) were needed to prevent this type of infection. These results are discussed in relation to antibody prophylaxis.

The role of immunity in modifying the course of both primary and recurrent herpes simplex virus infection has received considerable attention in recent years (15). The paradoxical occurrence of recrudescent lesions in humans in the face of immunity, often with no detectable rise in circulating neutralizing antibodies (5), leads one to address the question of whether specific immune processes can play any significant role in the prevention of recurrent disease.

We have recently described a model for investigating the mechanisms of immunity in experimental herpes (23). The infection of mice in the flank led to the development of ganglionic infection, and the subsequent delivery of virus to the skin of the whole dermatome, by nerve fibers. Mice infected in this way develop a band-like "zosteriform" rash several days after the initial inoculation of virus, a phenomenon first noticed by Teague and Goodpasture in 1923 (25). The significance of this model is that the epidermal cells distant from the inoculation site become infected from nerve endings, a process which also occurs during the development of a recrudescent lesion. Although in our model system no reactivation has preceded the delivery of virus to the skin, the peripheral events can be used to study the action of immunological effector mechanisms on the prevention or modulation of infection in the epidermis after centrifugal spread of virus from the ganglion.

We report here the results of further studies in this system, investigating the effect of antibodies on the development and course of zosteriform lesions. The possibility that antibodies may indeed be able to prevent recrudescence is discussed in the context of existing knowledge.

MATERIALS AND METHODS

Mice. Female inbred BALB/c mice, raised in the department animal house, were used throughout. They were infected at 6 to 8 weeks of age.

Virus. A well-characterized clone of a recent oral isolate of herpes simplex virus type 1, strain SC16 (7), was grown in BHK-21 cells and stored in samples at -70°C until required.

Inoculation of mice. The left flank was clipped and depilated with Nair (Carter-Wallace Ltd., Folkestone, United Kingdom). The mice were then lightly anesthetized with ether and inoculated with 5×10^4 PFU of virus, as previously described (23).

Removal of tissue and infectious virus assay. Tissue samples from the inoculation site and lower flank were taken separately as previously described (23). To assay for the presence of infectious virus, tissue was homogenized in 1 ml of Glasgow modified Eagle medium containing 10% calf serum and 10% tryptose phosphate. Ten-fold dilutions were then tested for plaque production with BHK-21 cells (19).

Antibodies. The monoclonal antibodies AP7, LP2, and LP11, LP3 were kindly donated by A. Buckmaster and A. Minson of this department. Antibody 20αD4 was a generous gift from W. Rawls (Department of Pathology, McMaster University, Ontario, Canada). The relevant properties of these antibodies are summarized alongside the experimental results. Polyclonal hyperimmune serum (neutralization titer of 1:150 in the presence of complement) was produced in BALB/c mice by repeated monthly subcutaneous injections of SC16.

Antibody assays. Neutralization was measured by 50% plaque reduction in BHK-21 cells as previously described (10). Nonneutralizing antibodies were quantified in an enzyme-linked immunosorbent assay with a monolayer of infected BHK-21 cells fixed to plastic multiwell plates. Serial dilutions of test antibody were reacted with the monolayer for 45 min at room temperature. Binding was determined by sheep anti-mouse immunoglobulin G linked to β-galactosidase developed with a substrate consisting of o-nitrophenyl-β-D-galactopyranoside in 10^{-3} M magnesium sulfate. After the addition of 1 M sodium carbonate, color

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TABLE 1. Prevention of zosteriform spread by the administration of antibody on day 1 postinfection

Antibody	Neutralization in vitro	Immuno-globulin G sub-class	Target gly-coprotein	Zosteri-form pro-tection
LP2	+	2a	gD[a]	+
AP7	+[b]	2a	gD[a]	+
LP11	+	2a	110K[a,c]	+
Immune serum	+	NA[d]	NA[d]	+
LP3	−	2a	gD[a]	−
20αD4	−	1	gA/B[e]	−

[a] Data from A. Minson (personal communication).
[b] Complement dependent.
[c] Not yet named.
[d] NA, Not applicable.
[e] Data from reference 1.

intensity was read at 410 nm. The antibodies gave a binding titer of 1:100 or greater in this test.

Neurotomy. Sensory denervation of the left flank was performed through a posterior midline incision as previously described (23). The animals were anesthetized with intraperitoneal diazepam and fentanyl. Control animals received a similar operation without division of the nerves.

RESULTS

The effect of neutralizing monoclonal antibody AP7 on the recovery of virus from the skin. The relevant properties of

AP7 are summarized in Table 1. A sample of 100 μl of this antibody was administered intravenously to five groups of three mice on day 1 postinfection, resulting in serum neutralizing titers in excess of 1:200 in each animal. Groups were killed daily, and the skin of the inoculation site and lower flank of each mouse was separately tested for the presence of infectious virus. The titers were compared with controls that received saline instead of AP7 (Fig. 1). The administration of AP7 on day 1 had no significant effect on the amount of virus recovered from the inoculation site on days 2 and 3 postinfection. However, although high titers of virus were isolated from the lower flank of controls on days 4 and 5, no virus was found at this site in antibody-treated groups. This corresponded with a failure of the antibody-treated mice to develop zosteriform rashes.

Comparison of various monoclonal antibodies. Five monoclonal antibodies, each with different properties, and polyclonal hyperimmune serum were screened for the ability to prevent zosteriform spread when administered on day 1 postinfection. The results are indicated in Table 1, along with the properties of each antibody. The only property that correlated with the ability of an antibody to prevent virus spread was the ability to neutralize virus in vitro. Quantitation of the protective effect of one of the neutralizing monoclonal antibodies, AP7, is discussed below.

Time at which infectious virus can be detected in the lower flank. Virus can be consistently found remote from the inoculation site on day 3 postinfection (23). We have analyzed the timing of this event more precisely (Fig. 2). Groups of 10 mice were screened at 2-h intervals, from 68 to 76 h postinfection, for the presence of infectious virus in the lower flank. Virus was not found in any mice at 68 h postinfection but by 74 h, all of the mice in the group were positive.
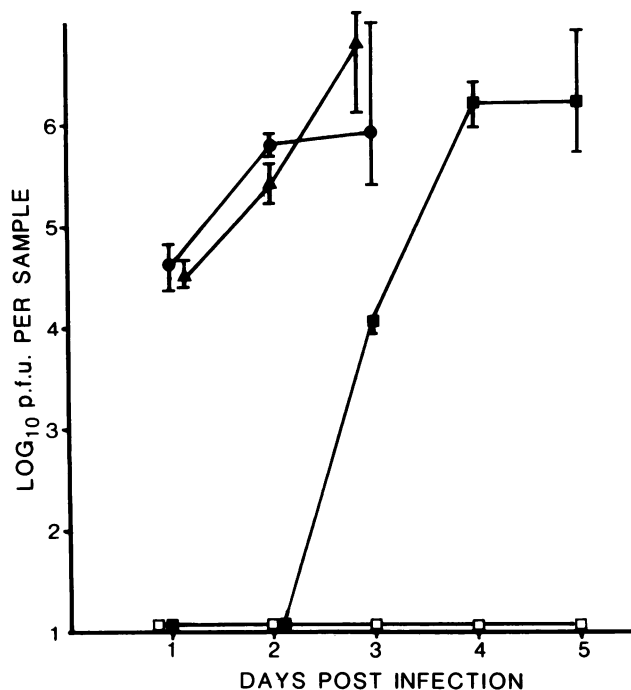

FIG. 1. The effect of antibody given 24 h postinfection on the recovery of virus from the inoculation site and lower flank. Shown are virus isolated from the inoculation site (●) and lower flank (■) of control mice and from the inoculation site (▲) and lower flank (□) of mice receiving AP7 on day 1 postinfection. Each point represents the geometric mean titer obtained from a group of three animals. Vertical bars indicate the range of values.
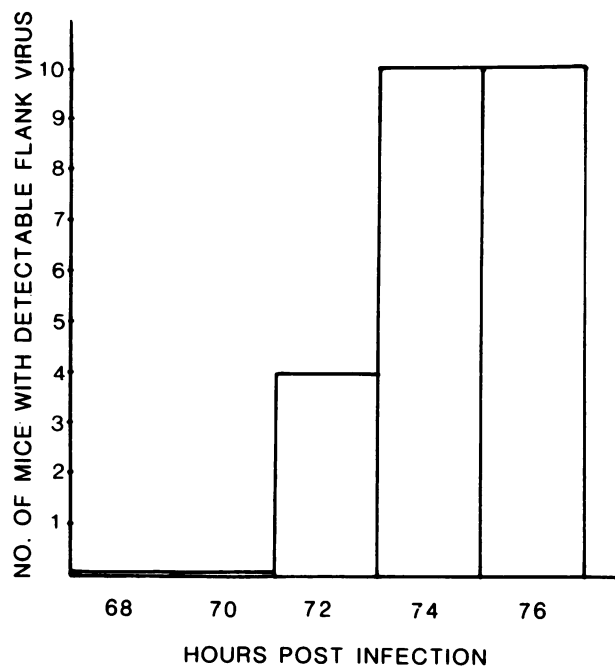

FIG. 2. Time of appearance of virus in the lower flank. Groups of 10 mice were killed at 2-h intervals from 68 h postinfection onwards. Samples of skin from the lower flank were screened for infectious virus. By 74 h postinfection, virus was found in all mice in the group.

TABLE 2. Effect of neurotomy at various times on the
occurrence of zosteriform spread

Neurotomy (h postinfection)	No. of mice with lesions/no. tested on day 5	
	No AP7 after operation	AP7 after operation
54	0/10	0/0
60	9/10	1/0
66	10/10	9/10

Delay of antibody administration. The effect of delaying the administration of antibody (AP7) was also investigated. Groups of 10 mice received antibody at various times postinfection and were examined for the appearance of zosteriform rashes on day 5. AP7 was effective when administered at 54 and 60 h postinfection, after which (at 66 h postinfection and when no antibody was used) its protective effect was lost.

Precise time at which virus arrives in lower flank. It has been previously shown that sensory neurotomy before infection of the flank prevents zosteriform spread (23) because virus is denied access to the nervous system. It was also shown that, with the nerves intact, virus was demonstrable in the sensory ganglia 48 h postinfection. By the division of sensory nerves to the flank at various times after infection (from 48 h onwards), we were able to estimate the time at which virus makes the return trip to the periphery (Table 2). Control mice underwent a similar operation, except that the nerves were left intact. The results indicate that from 60 h postinfection onwards, virus was peripherally located (i.e., in the flank) because at this time neurotomy no longer prevented the development of the zosteriform rash, which appeared between days 4 and 5, in synchrony with controls.

Antibody after nerve section. To demonstrate that an entirely peripheral action of AP7 was sufficient to suppress the formation of the zosteriform rash, additional groups of mice from the previous experiment received AP7 immediately after sensory neurotomy (Table 2). These animals were also examined for the appearance of lesions on day 5, compared with the group that received the operation but no antibody. As previously indicated, at 60 h postinfection, neurotomy alone did not prevent the development of rashes. However, antibody was shown to be effective at this time; AP7 thus remained highly effective at 60 h postinfection, even though it was given after the nerves were cut.

Amount of AP7 required. The ability of AP7 to prevent the formation of the zosteriform lesion when given before infection and at 24 and 48 h postinfection was quantified in terms of the serum neutralization titer that had to be achieved (Fig. 3). At each time point, 100 µl of diluted ascitic fluid was given to each mouse. Groups of five mice were studied, and control mice received saline. Serum neutralization titers were determined 60 h postinfection. The results clearly indicate that high titers of AP7 were required to neutralize the infection.

DISCUSSION

Zosteriform spread of herpes simplex virus, as indicated by the appearance of infectious virus distant from the inoculation site, occurred by 74 h postinfection. Therefore, since the bandlike rash caused by the centrifugal spread of virus includes the site of inoculation, it must be assumed that virus recovered from this site after 74 h is not entirely the result of local replication. This fact must be taken into account when studying agents that accelerate the clearance of virus from the skin. In such instances, an apparent reduction in local virus growth may well result from an inhibition of the zosteriform component without necessarily acting directly on infected peripheral cells. This was found to be the case with the neutralizing antibody AP7, in which zosteriform spread was completely inhibited by intravenous antibody given 24 h postinfection. However, at the inoculation site, early replication (uncomplicated by zosteriform spread) was not significantly different in antibody-treated and control groups.

It has been shown that antibodies are generally unable to affect cell-to-cell spread of herpes simplex virus in infected tissue culture monolayers (9). Our results indicate that this may also be the case in vivo, after an infection in the
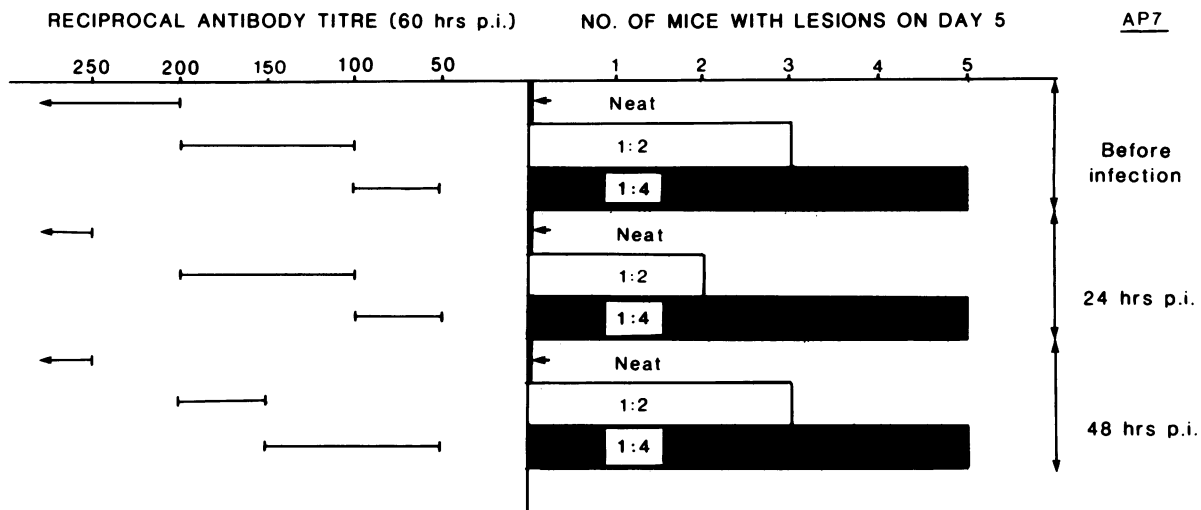

FIG. 3. Amount of AP7, given at various times postinfection, required to prevent zosteriform spread. Samples of 100 µl of neat or diluted ascitic fluid were given to each mouse, producing the range of serum neutralization titers shown on the left-hand side of the figure. A rapid drop in the protective effect was observed with dilution of the antibody preparation.

epidermis has been initiated. The possibility of antibody-dependent cellular cytotoxicity is recognized, but as yet there is no evidence that AP7 participates in such a mechanism in vivo. We are further investigating the role of this process in the host defense against herpes simplex virus, using both monoclonal and polyclonal antibody preparations.

The efficacy of antibody in preventing the zosteriform lesion was maintained up to and including 60 h postinfection. At this time, virus was already located peripherally, and we therefore conclude that the presence of AP7 in skin alone was sufficient to prevent the appearance of a zosteriform rash. This conclusion is reinforced by the demonstration that AP7 was effective even when given after sensory neurotomy, thereby excluding a role for antibody operating in the peripheral or central nervous systems at these times. Indeed, McKendall (13) has shown that radioactively labeled purified immunoglobulin G only appears in the central nervous system after breaching the blood-brain barrier some 5 to 6 days after infection. McKendall also showed that intravenously administered immunoglobulin quickly infiltrates peripheral tissues in detectable amounts. However, in the absence of inflammation (which is the case during the first 24 to 48 h of mouse flank infection [23]), the diffusion of antibody molecules into tissue would be expected to be rather poor, and this may be the reason why a large amount of AP7 was required to produce the protective effect. The titer needed was at least fivefold higher than the titer we routinely find by the same assay in previously infected mice.

The protective effect was only seen with neutralizing antibodies; no protection was observed with nonneutralizing monoclonal antibody preparations. For this reason, one assumes that virus is neutralized when it passes from nerve ending to epidermal cell. Once virus becomes intracellular (60 to 66 h postinfection), neutralizing antibodies have no effect.

A substantial number of investigations have been made in the past into the role of antibodies in protection against herpes simplex virus infection. Nearly all have been based on passive transfer experiments. Conflicting results obtained in various experimental systems lead to continued confusion over the role of antibodies at the various stages of natural infection. In 1946, Evans et al. (6) showed that when antibody was given to mice soon after infection, access to the nervous system was reduced. More recent studies in a variety of experimental models confirm this (1, 10–12, 14, 16–18, 26). However, this has not been shown to be the case in all systems (4, 27). In any case, the presence of a high level of antibodies does not apply to primary infection. Virus reaches the ganglia early (24 to 48 h postinfection [4, 11, 14, 16]), long before a detectable antibody response has occurred.

Most workers agree that T-cells are required for the clearance of virus from established sites of infection (reviewed by Nash and Wildly [15]), although there remains a possibility that antibody-mediated killing of infected cells plays a supportive role (1, 2, 21, 22). We have, so far, failed to show in vivo that any of the antibodies described in this report can increase the already effective T-cell-mediated clearance of virus from the skin (unpublished data). Antibody alone is not sufficient to cause virus clearance in the absence of T-cell mechanisms either in vitro (16) or in vivo (11), although clinical disease can be delayed (11).

In recurrent disease, virus reactivates in the presence of antibody, both naturally in humans (3, 5, 24) and in experimental models (8, 20). It therefore appears that normally acquired levels of antibodies are insufficient to prevent

recurrent ganglionic infection and, in many instances, recrudescent skin lesions. Our results agree that antibodies are of limited value in clearing virus from the skin in primary and recurrent infection; however, our results suggest that a heightened immune response may be able to interrupt the process of epidermal infection which must occur before a recrudescent lesion can appear. Therapy for various clinical presentations of herpetic infection is anxiously sought. The possibility that high-titer monoclonal antibodies might be useful in prophylaxis against the lethal herpetic infections seen in the immunocompromised host is intriguing and deserves further investigation. In addition, the more common recurrent epidermal infections lead to misery and discomfort for many millions worldwide. The recent suggestion that vaccines may both protect against primary disease and reduce the frequency and severity of recrudescent lesions (G. Skinner, personal communication) has aroused considerable interest. It is possible that such protection could result from an elevated (qualitative rather than quantitative) antibody response, leading to protection mediated by the mechanism described here. Certainly, it is apparent that there is a need for further investigation into the immunotherapy of herpes simplex virus infections.

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