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Ability of monoclonal antibody to herpes simplex virus glycoprotein gB to promote healing of herpetic skin lesions in nude mice

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Summary

The effect of monoclonal antibody (MCA) to glycoprotein gB of herpes simplex virus (HSV) was studied in athymic nude mice inoculated with HSV intracutaneously in the midflank. HS1, the MCA used in the study, had a high neutralizing titer (1 : 2048) and had antibody-dependent cell-mediated cytotoxicity. HS1 was injected intraperitoneally at various intervals after HSV infection. HS1 injected 3 h after infection inhibited the development of skin lesions and most mice survived. Administration of HS1 at the time the local skin erosions appeared at the inoculated site (4–7 days after infection) was also effective, and in four of eight mice skin lesions completely healed. Furthermore, in three of the four mice that survived, latent infections in the ganglia were also prevented as evidenced by the failure to detect HSV by co-cultivation with Vero cells. Administration of HS1 after the development of zosteriform skin lesions (5–9 days after infection) reduced virus in the ganglia and prolonged the survival time, though the disease was not completely arrested and all the mice died eventually.

herpes simplex virus; monoclonal antibody; nude mice

Introduction

It is well established that cell-mediated immunity plays an important role in recovery from herpes simplex virus (HSV) infection [12,17]. Virus-specific antibody is also

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involved in resistance to HSV infection [4,7,10,11,22]. However, the precise role of antibody is not yet fully known. We have previously investigated possible resistance mechanisms, using nude mice inoculated intracutaneously with HSV [4,7,11]. This system has the advantage that skin lesions are readily visible and can be assessed quantitatively. Furthermore, because of the complete absence of a mature T-cell population, nude mice provide an excellent model for studying antibody-mediated and cell-mediated protection against HSV infection separately. It has been suggested that passive transfer of hyperimmune serum may be unable to protect immunocompromised animals from lethal infection with HSV [4,7]. However, in a previous study using monoclonal antibody (MCA) with high neutralization titer and strong antibody-dependent cell-mediated cytotoxicity (ADCC), protection from HSV infection was achieved in nude mice [6]. In the present study, we evaluated both the protective and therapeutic effect of MCA against HSV infection in nude mice.

Materials and Methods

Virus and cells

HSV type 1 (HSV-1), strain Hayashida, an isolate from a vesicular skin lesion of herpes labialis, was passaged five times in Vero cells and used for inoculation. HSV-1, strain KOS, was used to immunize mice. Vero cells grown in Eagle's minimal essential medium (MEM) supplemented with 5% calf serum were used for virus propagation and titration.

Mice

6-week-old female nude (nu/nu) mice with BALB/c genetic background (supplied by Central Laboratory of Experimental Animals Co., Ltd., Osaka, Japan) were used throughout the study.

Inoculation of mice

Nude mice were inoculated intracutaneously in the midflank with 1×10^5 plaque-forming units (p.f.u.) of HSV-1 per 0.05 ml as previously described [11]. The development and severity of the skin lesions were scored every day. The scores of skin lesions are as follows: 0, no lesion; 2, local lesion (vesicle or erosion); 6, mild zosteriform lesion; 8, moderate zosteriform lesion; 10, severe zosteriform lesion; and death.

Preparation of MCA

The characteristics of MCA used in this study were described previously [6]. Briefly, BALB/c mice were immunized subcutaneously with HSV-1 strain KOS and the immune spleen cells were fused with cells of the mouse myeloma cell line P3 X63'-AgU1. After selection with hypoxanthine-aminopterin-thymidine (HAT) medium, hybridoma cells producing antibody against HSV glycoproteins were screened. Four hybridomas secreting antibody against gB were injected into the peritoneal cavity of BALB/c mice pretreated with pristane, and the resulting ascitic fluid served as MCA. Of four MCAs against glycoprotein gB of HSV-1, HS1 was used in the present

experiment. HS1 had a high neutralizing titer (1 : 2048) against HSV-1 and possessed ADCC. At various intervals after infection, a single injection of 0.5 ml HS1 was given intraperitoneally (i.p.).

Virus titrations

At various intervals after virus inoculation, three mice from each group were sacrificed and viral titers in their tissues were determined as described previously [23]. Mice were exsanguinated by cutting the femoral vessels before the tissues were removed to minimize the effect of transferred antibody on virus titrations. Tissue samples included the skin (the minimum area encompassing the inoculated site and the whole lesion), dorsal root ganglia from L(lumbar)-1 to L-5 and the brain. Homogenates of the tissues were inoculated into Vero cell monolayers in 24-well tissue culture plates and overlaid with MEM containing 2% calf serum and 2% methyl cellulose. After incubation for 3 days, the plaques were counted. The titers of virus recovered from the various homogenates were expressed as p.f.u. per tissue.

Neutralizing antibody titer

The neutralizing activities of HS1 and mouse serum samples were measured by the microplate method described previously [3], in which the neutralizing antibody titer was expressed as the highest dilution which showed 80% plaque reduction.

Cocultivation of dorsal root ganglia with Vero cells

Mice were sacrificed and the dorsal root ganglia from L-1 to L-5 were removed. The ganglia were put onto monolayers of Vero cells, and the cultures were examined daily for more than 4 weeks for the appearance of viral cytopathic effect.

Results

Clearance of passively transferred HS1

To determine the half-life of the transferred HS1, 0.5 ml of HS1 titering 1 : 2048 was injected intraperitoneally in nude mice. The recipient mice were bled 1, 11, 21, 31 and 41 days after HS1 transfer and the sera were separated. Titers of the sera were determined by the microtiter method. The results are shown in Fig. 1. The half-life of the transferred HS1 was calculated to be approximately 12.1 days.

Effect of passively transferred HS1 on the course of HSV infection

Our previous studies demonstrated that the transfer of hyperimmune serum fails to protect nude mice from death after lethal infection with HSV [4,7,23]. However, transfer of HS1 is able to protect some mice from death when administered as early as 3 h after infection [6]. In the present study, mice were divided into three groups according to the stage of administration of HS1; i.e., the first groups received HS1 3 h after infection, the second group received it at the stage of local skin erosions (score 2) (4–7 days after infection, mean: 5.5 ± 0.8 days) and the third group received it at the beginning stage of zosteriform skin lesions (score 6) (5–9 days after infection, mean: 6.3

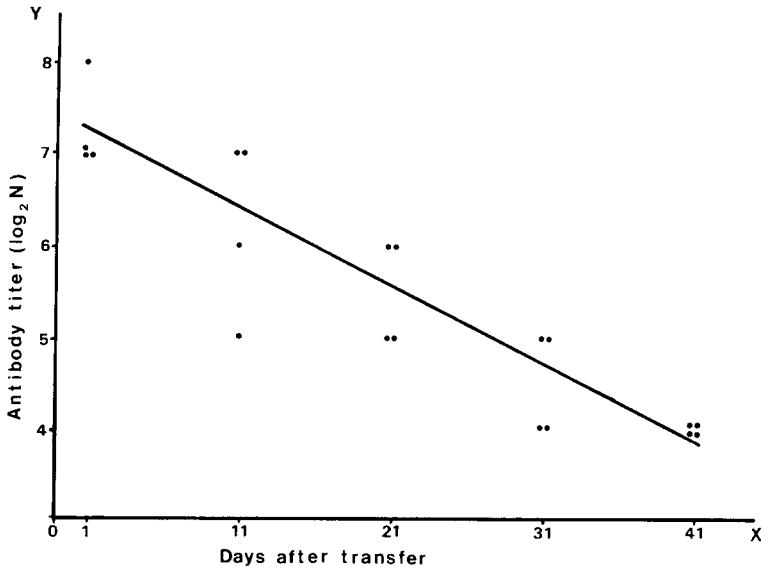

Fig. 1. Clearance of passively transferred HS1. 0.5 ml of HS1 was transferred i.p. to nude mice on day 0. The mice were bled 1, 11, 21, 31 and 41 days after HS1 transfer. The antibody titers were determined by a neutralization assay. The equation line was determined by regression analysis. The calculated equation was $Y = -0.08\,X + 7.2$, where Y is antibody titer expressed as $\log_2$ N, and X is time (in days). The half-life of the transferred antibody was calculated to be approximately 12.1 days.

± 1.3 days). To each group of mice 0.5 ml of HS1 was administered intraperitoneally as a single dose.

The development of skin lesions in each group is shown in Fig. 2. In control mice, which had not received HS1 local skin vesicles appeared on day 3 after infection and developed into local erosions or ulcers. The zosteriform skin lesions began to appear on day 6 and all mice died within 20 days after infection (Fig. 2a).

In the mice given HS1 3 h after infection, the development of skin lesions was inhibited and most mice survived (Fig. 2b), as could be expected from our previous studies [6]. The lumbar ganglia were removed from the mice that survived 85 days after infection and were cocultivated with Vero cells. No virus was recovered from the explant cultures.

Administration of HS1 when local skin erosions (score 2) appeared at the inoculated site was also effective (Fig. 2c). In four of eight mice, the skin lesions completely healed. Furthermore, in three of the four cured mice no virus was recovered from the ganglia by cocultivation with Vero cells.

In the mice that were given HS1 after the skin lesions developed into zosteriform lesions (score 6), the disease was not inhibited and all the mice died (Fig. 2d), though survival time was slightly prolonged.

Effect of HS1 on viral growth in mouse tissues

Fig. 3 presents the virus titers of tissue homogenates from each group at various

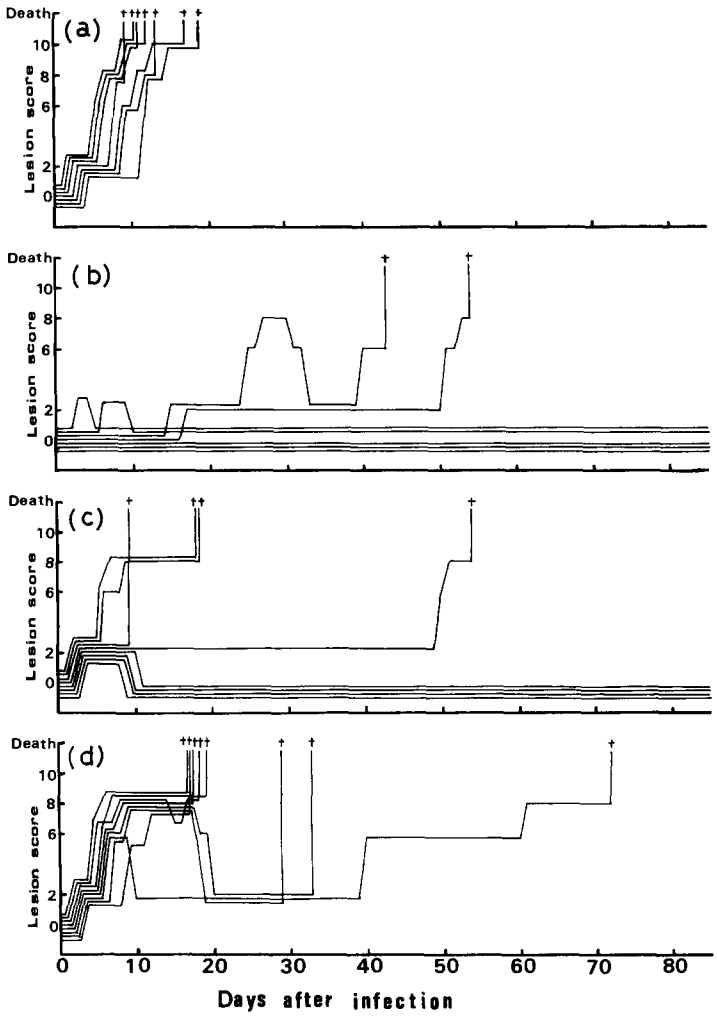

Fig. 2. Effect of transferred HS1 on the development of skin lesions after intracutaneous inoculation of HSV-1 into nude mice. (a) Untreated control mice. (b) 0.5 ml of HS1 was transferred i.p. 3 h after infection. (c) 0.5 ml HS1 was transferred i.p. at the time the local skin erosions appeared at the inoculated site, that is 4–7 days (mean 5.5 ± 0.8 days) after infection (score 2). (d) 0.5 ml of HS1 was transferred i.p. after the skin lesions developed into zosteriform lesions, that is 5–9 days (mean 6.3 ± 1.3 days) after infection (score 6).

intervals after infection. In the control mice, which had not received HS1, the virus titer in the skin rose as the zosteriform lesions began to develop. The virus could be recovered from the dorsal root ganglia as early as 7 days after infection. The virus titers in the dorsal root ganglia rose gradually until death of the mice. The virus could be recovered from the brain only in the case of moribund mice (Fig. 3a).

Tissue samples from the HS1-treated mice showed a striking reduction in virus titer. In the mice which had received HS1 3 h after infection, the virus titer in the skin

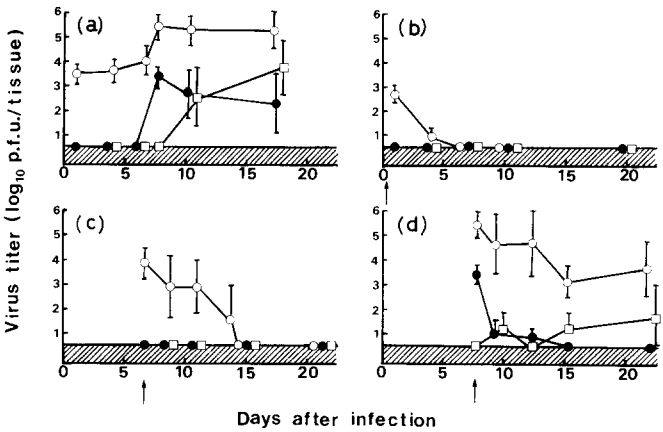

Fig. 3. Effect of HS1 on viral growth in mouse tissues after intracutaneous inoculation of HSV-1. (a) Control mice. (b) 0.5 ml of HS1 was transferred i.p. 3 h after infection (arrow). (c) 0.5 ml of HS1 was transferred i.p. at the time local skin erosions appeared at the inoculated site (arrow). (d) 0.5 ml of HS1 was transferred i.p. after the skin lesions developed into zosteriform lesions (arrow). Symbols: ○, skin; ●, dorsal root ganglia; □, brain. The hatched portion of each graph indicates the threshold of virus detection.

declined and no virus could be recovered after day 7. Furthermore, no virus was recovered from the dorsal root ganglia or the brain throughout the experiment (Fig. 3b).

In the mice given HS1 at the time local skin erosions appeared at the inoculated site, the virus titer in the skin declined gradually and no virus could be recovered from 7 days after the administration of HS1. No virus was recovered from the homogenates of the dorsal root ganglia and the brain throughout the experiment (Fig. 3c).

In the mice which received HS1 after the development of the zosteriform skin lesions, the virus titer in the skin and the dorsal root ganglia declined after the treatment. From 7 days after the administration of HS1, no virus could be recovered from the dorsal root ganglia. However, the virus titer in the skin rose gradually again and in the brain virus could be recovered from moribund mice (Fig. 3d).

Recovery of HSV from dorsal root ganglia by cocultivation with Vero cells

In homogenates of the dorsal root ganglia, no virus could be detected until zosteriform skin lesions began to appear. However, our previous study revealed that, by cocultivation of the ganglia with Vero cells, HSV had reached the dorsal root ganglia as early as 48 h after infection [7]. In the present study, in order to detect the virus in the dorsal root ganglia after the administration of HS1, the ganglia were cocultivated with Vero cells (Table 1). No virus could be recovered from the cultures which received HS1 3 h after infection. The virus was recovered from most cultures that received HS1 at the time local skin erosions appeared, and it was detectable for 10 days after administration of HS1. However, it was undetectable in the cultures on day 15 or later after administration of HS1. The data imply that HS1 is capable to interfere with the multiplication of HSV in the sensory ganglia.

TABLE 1

Recovery of HSV from dosal root ganglia after HS1 transfer by cocultivation with Vero cells

Group[a]	No. positive/No. tested at days after HS1 transfer						
	0	1	4	5	10	15	20
(b)	n.d.	0/3	0/3	n.d.	0/3	0/3	0/3
(c)	2/3	2/3	n.d.	2/3	3/3	0/3	0/5

[a] For (b) and (c), see legend to Fig. 2.
n.d., not done.

Discussion

There have been several reports on the role of antibody in the defense against HSV infections [8], and it has been suggested that serum antibody participates in the control of HSV infections. It has also been shown that antibody alone is not sufficient to clear virus completely in athymic nude mice. In our previous studies using nude mice [4,7,23], the passive transfer of hyperimmune mouse serum or human gammaglobulin (HGG) was effective in prolonging survival time, but all mice eventually died. Antibody could not prevent the colonization of the dorsal root ganglia by HSV after intracutaneous inoculation. Similarly, Openshaw et al. reported [15] that transfer of immune serum was unable to prevent the acute infection of the sensory ganglia by HSV in nude mice.

Recently, the protective effect against HSV infections has been investigated by using MCA to HSV glycoproteins [1,2,5,14,16,18–21]. Kapoor et al., using nude mice inoculated in the ear pinna [5], found that passive transfer of MCA to glycoprotein gD 3 days after infection reduced infectious virus in the ganglia but did not clear virus from the pinna. In the present study, we evaluated the effect of MCA (HS1) by using athymic nude mice infected intracutaneously with HSV and obtained a marked therapeutic effect. HS1 had a high neutralizing titer and ADCC, and the half-life of HS1 was also longer than that of hyperimmune mouse serum or HGG. The passive transfer of HS1 reduced the infectious virus in the inoculated site and the ganglia if administered at the stage of local skin lesions.

As long as the skin lesions were limited to the inoculated site, HS1 was effective and in several mice latent infection of the regional nerve ganglia was prevented. At the time the local skin erosions appeared no virus was recovered from the homogenates of the ganglia, but the virus could be recovered by cocultivation of the ganglia with Vero cells. Our previous study [7] showed that the virus was present in cultures 48 h after infection. From the present results it is evident that HSV had reached the dorsal root ganglia at the time the local skin erosions appeared. It is interesting that, by cocultivation of ganglia with Vero cells, HSV was undetectable on day 15 or later after the transfer of HS1 when it was administered at the stage of local skin lesions (Table 1c). This suggests that HSV was eliminated from the dorsal root ganglia by HS1 therapy.

Furthermore, HS1 could reduce infectious virus in the ganglia (Fig. 3d) even when it

was transferred after the development of zosteriform skin lesions, though HS1 could not protect nude mice from death.

The exact mechanism of action of HS1 is not clear, but there seems to be a close correlation between the in vivo protective capacity and high neutralizing activity or ADCC of HS1. It was indicated in our previous study [4] that the antibody-mediated protection required the Fc portion of the antibody, and that ADCC might play an important role in vivo. A similar conclusion was reached by Oakes and Lausch [13] and McKendall [9].

Although we could not determine the exact mechanism by which HS1 effected protection against HSV infection, the monoclonal antibody clearly showed a therapeutic effect which could not be achieved by the transfer of HGG or hyperimmune serum. Antibody in combination with acyclovir has recently been reported to protect nude mice against HSV infection [23]. Therefore, if human MCA to HSV could be obtained, it should be considered, either as such or in combination with antiviral drugs, for prophylaxis or therapy of HSV infections in immunocompromised patients.

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