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Antibody to cloned HSV glycoproteins B and D plus adult human leukocytes protect neonatal mice from lethal HSV infection

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Summary

Antisera produced by HSV infection or following vaccination of guinea pigs with the cloned herpes simplex virus (HSV) glycoproteins gB and gD were compared for in vitro antibody-dependent cellular cytotoxicity (ADCC) activity and for in vivo protection. Antibody from guinea pigs was able to participate in ADCC with human mononuclear cells in vitro, anti-gBgD serum being equivalent to HSV convalescent sera. In vivo, each of the guinea pig sera was able to protect neonatal mice from a fatal HSV-1 infection when given with human mononuclear cells but not when given alone. The anti-gBgD serum was the most effective in vivo, protecting 15 of 17 (88%) neonatal mice when given at a 10^{-4} dilution with human mononuclear cells and was the only guinea pig serum protective at a 10^{-6} dilution (5 of 7 neonatal mice).

Herpes simplex virus; ADCC; HSV glycoprotein

Introduction

Herpes simplex virus (HSV) infections remain a serious problem for neonates despite advances in antiviral chemotherapy (Whitley et al., 1980a, 1985). Neo-

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Note: Animals used in this study were maintained in accordance with the guidelines of the IACUC's of the CHRF, Cincinnati, Ohio and University of Texas, Houston.

nates appear to be particularly susceptible to infection because of a number of immunologic defects including both cell mediated and antibody mediated mechanisms (for review, see Kohl, 1984). In mice, an age dependent delay in the ability to produce anti-HSV antibody following infection has been reported (Kohl and Loo, 1982; Koyama and Kasahara, 1975; Kohl et al., 1986b) but whether antibody is an important factor in protection of human neonates from HSV infection is ill defined. Thus, although a collaborative multicentered study (Whitley et al., 1980b) failed to demonstrate a protective role for transplacental antibody, other studies (Yeager et al., 1980; Sullender et al., 1987) have demonstrated a correlation between high titers of transplacental antibody and less severe HSV infection in human neonates.

It has recently been shown that small amounts of antibody plus human adult mononuclear cells when given simultaneously can protect neonatal mice from a lethal HSV infection (Kohl and Loo, 1982). Human xenogenic leukocytes or antibody alone were unable to provide protection from lethal HSV infection but, the combination of nonimmune leukocytes and subneutralizing doses of antibody was protective. This in vivo demonstration of antibody-dependent cellular cytotoxicity (ADCC) may be an important defense mechanism because HSV may be transferred directly from cell to cell and ADCC destroys HSV-infected cells prior to viral release (Shore et al., 1976).

Recent reports have shown that the HSV glycoproteins gB and gD were effective immunogens when given prior to HSV inoculation of animals (Stanberry et al., 1987; Berman et al., 1984; Long et al., 1983; Dix et al., 1985; Waschsman et al., 1987, Cantin et al., 1987). Immunization induced neutralizing antibody titers that were higher than detected following infection (Stanberry et al., 1987) and reduced local viral replication more effectively than prior genital HSV-2 infection (Bernstein et al., 1986; Stanberry et al., 1987). We were thus interested in determining whether guinea pig antiserum produced by vaccination with HSV gB and gD could perform in ADCC using human leukocytes and to compare its protective ability to that provided by antibody produced after infection using the neonatal mouse model.

Materials and Methods

Serum

A representative pre- and 30-day convalescent serum from a 350 g female Hartley guinea pig (Charles River Breeding Labs, Wilmington, MA), inoculated intravaginally with $10^{5.7}$ PFU of MS strain HSV-2 (Bernstein et al., 1986) and a representative 30-day convalescent serum from a 350 g female Hartley guinea pig inoculated following abrasion of the upper lip with $10^{5.0}$ Shealy strain HSV-1 (Bernstein et al., 1988) were employed as control, HSV-2 and anti-HSV-1 sera, respectively. For production of anti-gBgD serum, Hartley female guinea pigs 350–400 g were immunized in the hind footpad twice at 30-day intervals with 20 μg of cloned HSV-1 glycoprotein B (gB) and 10 μg of cloned HSV-1 gD in com-

plete Freund's adjuvant (kindly supplied by Chiron Corporation, Emeryville, CA) as previously described (Stanberry et al., 1987). Briefly, gB was derived from a stable Chinese hamster ovary cell line that constitutively secretes a truncated gB protein of 680 amino acids in length from HSV-1 strain Patton; gD was derived from *Saccharomyces cerevisiae* containing a partial gD gene encoding amino acid 20-364 of the native protein from HSV-1 strain Patton. Animals were bled 30 days following the second immunization. Two sera, one with the highest and one with the lowest neutralization titers, were selected for these studies. The human immune serum globulin used was commercially available (Cutter Biologics, Berkeley, CA). The human serum was a pooled serum from volunteers with anti-HSV antibody. All sera were stored at −70°C prior to use.

Human mononuclear cells

Venous blood from healthy adult humans was drawn into 30 ml plastic syringes containing 30 U of heparin (Upjohn, Kalamazoo, MI) per ml of whole blood. An aliquot of whole blood was centrifuged for 10 min at 300 × *g* then sedimented into 10% (vol/vol) dextran (3%, Sigma Chemical Co., St. Louis, MO). The cells in the leukocyte-rich plasma upper layer were separated by Ficoll-hypaque (Pharmacia, Piscataway, NJ) centrifugation as previously described (Kohl and Loo, 1982). Mononuclear cells were then washed 4 times in Hanks' balanced salt solution (Gibco) before use.

Antibody assays

Neutralization and ELISA titers were measured as previously reported on heat inactivated sera (Bernstein et al., 1986; Stanberry et al., 1987). Neutralization to HSV-1 strain McIntyre was measured with and without the addition of a 1:15 dilution of rabbit complement (Cappel, West Chester, PA) and are reported as the highest dilution that reduced the plaque count by 50%. The ELISA used a lectin purified mixture of HSV-1 glycoproteins as the capture antigen. Immunoblot analysis of anti-gBgD sera were also performed according to published methods (Bernstein et al., 1986).

ADCC assay

As described previously (Kohl and Loo, 1982), the ADCC assay was performed using Chang liver target cells that were infected with HSV type 1 strain HE and radiolabeled with ^{51}Cr. Human mononuclear effector cells from a single healthy adult donor were used at an effector target ratio of 30:1. The incubation period was 18 h. The percentage of antibody activity detected by the ADCC assay was calculated as follows: (the percentage of ^{51}Cr released from target cells, effector cells, and test serum) minus (the percentage of ^{51}Cr released from target cells, effector cells, and negative serum) divided by [100 minus (the percentage of ^{51}Cr released from target cells, effector cells, and negative serum)] multiplied by 100. ADCC activity > 10% represents demonstrable activity.

Neonatal mouse protection

As previously described in detail (Kohl, and Loo, 1982), one day prior to viral inoculation 7-day old C/57/B1/6 mice (Timco Breeding Co., Houston, TX) were injected i.p. with either 10^{-4} or 10^{-6} dilutions of each serum or medium alone with or without the addition of 5×10^6 human mononuclear cells. Mice were then inoculated i.p. with 0.1 ml containing 10^4 PFU of HSV-1 strain HE and observed for 21 days for survival.

Results

Antibody titers

Guinea pigs immunized with the cloned HSV-1 gB and gD produced antibody directed only against these proteins (Fig. 1). The response to homologous HSV-1 glycoproteins appeared to be more intense than that directed to the heterologous

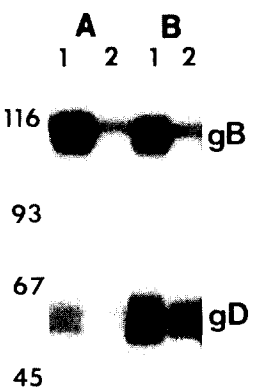

Fig. 1. Immunoblot analysis of sera obtained from guinea pigs immunized with cloned glycoproteins B and D from HSV-1. Lanes A were reacted with serum No. 801 which had the lower neutralization titer while lanes B were reacted with serum No. 804 which had a higher neutralization titer. Lanes with a 1 were reacted with HSV-1 polypeptides and those with a 2, HSV-2 polypeptides.

TABLE 1

Antibody titers of sera used in neonatal mouse protection studies

Antibody source	Log_{10} neutralization	Log_{10} neutralization with complement	Log_{10} ELISA titer
Pre	< 1.0	< 1.0	< 1.0
Convalescent HSV-1[a]	2.5	ND	ND
Convalescent HSV-2[b]	2.5	2.8	3.2
Anti-gBgD[c]	2.8	3.4	6.6
Anti-gBgD[c]	3.4	4.7	6.5
Human Immune Serum Globulin[d]	4.0	3.7	ND

[a]Thirty day convalescent serum from oral infection with 5.0 log_{10} PFU HSV-1.
[b]Thirty day convalescent serum from intravaginal infection with 5.7 log_{10} PFU HSV-2.
[c]Serum obtained 30 days following two immunizations of 20 μg gB and 10 μg gD derived from HSV-1, given 30 days apart in CFA.
[d]HISG commercially available from Cutter Biologics.
ND, Not done.

HSV-2 gB and gD. Although the response to gD appears to be stronger in the animal with the higher neutralization titer this was not a consistent finding in other sera obtained from animals immunized with gBgD (data not shown).

Neutralization titers to HSV-1 were higher in animals immunized with gBgD than those obtained from animals 30 days after HSV-1 or HSV-2 infection (Table 1). The addition of complement increased the neutralization titers in the vaccinated animals 4-fold and 20-fold, while titers in infected animals increased only 2-fold. ELISA antibody titers to HSV-1 glycoproteins were also higher in vaccinated than in infected animals.

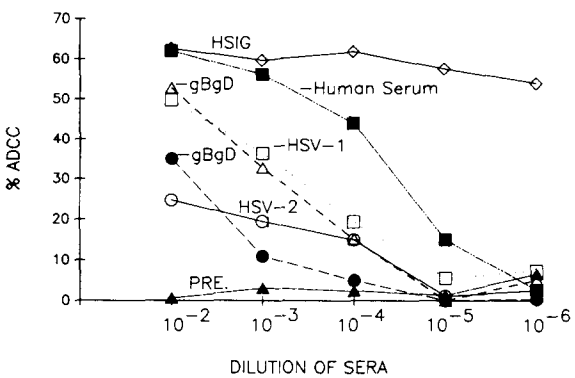

Fig. 2. In vitro antibody dependent cellular cytotoxicity (ADCC) of guinea pig and control (human) sera. The assay employs human mononuclear effectors and HSV-1 infected ^{51}Cr-labelled Chang liver cells at a ratio of 30:1 and an 18 h incubation. ADCC is calculated as described in Materials and Methods. Antiserum was produced by inoculation with cloned gBgD, △—△ high neutralization titer, ●—● low neutralization titer; □—□ infection with HSV-1, ○—○ infection with HSV-2, ▲—▲ pre-infection guinea pig sera, ■—■ pooled human anti HSV serum, ◊—◊ or commercially available immune serum globulin.

TABLE 2

Protection of neonatal mice by serum at various dilutions with or without human leukocytes[a]

Serum source[b]	Serum dilution	Human mononuclear cells	Animal survival (%)
None		Yes	0/17
Pre	10^{-4}	Yes	0/17
	10^{-6}	Yes	0/7
HSV-1 convalescent	10^{-4}	No	0/7
	10^{-4}	Yes	14/17 (82)
	10^{-6}	Yes	0/7
HSV-2 convalescent	10^{-4}	Yes	11/17 (65)
	10^{-6}	Yes	0/7
Anti-gBgD (low neutralization titer)	10^{-4}	Yes	12/17 (71)
	10^{-6}	Yes	0/7
Anti-gBgD (high neutralization titer)	10^{-4}	No	0/7
	10^{-4}	Yes	15/17 (88)
	10^{-6}	Yes	5/7 (71)
Human immune serum globulin	10^{-4}	Yes	7/17 (41)
	10^{-6}	Yes	7/7 (100)

[a]One week old mice received 0.1 ml of serum at the dilutions indicated with or without 5×10^6 human mononuclear cells intraperitoneally one day prior to inoculation with HSV-1 (10^4 PFU). Animals were observed for survival for 21 days. Number surviving/number studied.

Antibody from guinea pigs was able to participate in ADCC with human mononuclear cells in vitro, although ADCC to HSV-1 infected targets was highest utilizing the human serum immune globulin (Fig. 2). The anti-gBgD serum with the highest neutralizing titer had equivalent activity to the HSV-1 convalescent serum while the activity in the other gBgD antisera was equivalent to that seen in HSV-2 convalescent serum. The titers (when a 10% lysis is used as the endpoint) to these anti-gBgD antibodies, 10^{-3} to10^{-4} dilutions were similar to although less than that observed using the pooled anti-HSV human serum; titer 10^{-4}–10^{-5}. Titers reported for convalescent human serum range from 10^{-3}–10^{-5} dilutions using a similar assay (Kohl and Loo, 1986a).

Neonatal protection

All neonatal mice given either HSV-1 convalescent serum or anti-gBgD serum alone at a dilution of 10^{-4} did not survive after HSV-1 inoculation (Table 2). Similarly, adult mononuclear cells given with either no serum or HSV negative guinea pig serum (preserum) was nonprotective. However, the majority of neonatal mice provided with a high dilution (10^{-4}) of guinea pig anti HSV serum plus adult mononuclear cells survived the HSV-1 inoculation. Protection was greatest in mice the high titered anti-gBgD serum: 88% provided with a 10^{-4} dilution plus human mononuclear cells surviving. At a dilution of 10^{-6} this serum plus human mononuclear cells protected 5 (71%) of 7 neonatal mice compared to 0 of 14 for the two convalescent sera ($P < 0.002$, Fisher's exact test). HSIG was the only other serum that provided protection at this dilution.

Discussion

The prevalence of neonatal herpes virus infection may be increasing (Sullivan-Bolyai et al., 1983) and although antiviral chemotherapy has improved the outcome of these infections, the morbidity remains high (Whitley et al., 1980a,b, 1985). As a consequence, adjunctive treatment such as passive immunotherapy is being considered. In animal studies it is clear that passively administered antibody can provide protection against a low dose HSV challenge (Luyet et al., 1975; Baron et al., 1976; Georgiades et al., 1982) and that protection does not require the direct neutralization of virus (McKendall, 1985; Oakes and Lausch, 1981; Erlich et al., 1987). Thus, it has been shown that the Fc portion of the immunoglobulin molecules was required for protection and that neutralizing F(ab)2 fragments of the immunoglobulin provided no protection (McKendall, 1985; Oakes and Lausch, 1981; Erlich et al., 1987). Protection of newborn mice against higher doses of challenge virus, however, requires both antibody and effector cells (Kohl and Loo, 1982).

In this report, we have shown that guinea pig anti-HSV serum can participate in both in vitro and in vivo ADCC employing human mononuclear effector cells; antibody to both HSV-1 and HSV-2 providing protection against lethal HSV-1 infection of neonatal mice only when given with adult mononuclear cells. Further, antibody derived by immunization of animals with the cloned HSV gB and gD provided greater protection than that produced following HSV-1 or HSV-2 infection. Thus, immunization with these cloned glycoproteins has induced high titered neutralizing and ADCC antibody, the two functional antibodies defined against HSV infection, provided protection from HSV infection in adult animals (Stanberry et al., 1987; Berman et al., 1984; Long et al., 1983; Dix et al., 1985; Waschsman et al., 1987; Cantin et al., 1987) and provided passive protection to neonatal mice when given with human mononuclear cells.

Although transplacental acquisition of neutralizing antibody may (Yeager et al., 1980; Sullender et al., 1987) or may not (Whitley et al., 1980a,b) afford protection to the human neonate with HSV infection, the presence of high ADCC titers in sera has recently been associated with protection against dissemination of HSV infection (Kohl et al., 1988). Because immunotherapy with gBgD has been shown to decrease HSV recurrences (Stanberry et al., 1988) as well as recurrent HSV shedding in animals (Myers et al., 1988), it is possible that immunization of infected women might decrease the risk of transmission of infection to the newborn and provide protection to those newborns that are infected.

In these studies we confirm previous reports (Norrild et al., 1979) that the surface gB and gD are important targets for ADCC and demonstrate their role for protection from HSV infection in vivo. In vitro ADCC and the neonatal protection assay, as well as standard assays (ELISA, neutralization) may provide important methods to evaluate the functional efficacy of specific antisera. The potential for antibody to HSV glycoprotein to serve as adjunct to current therapeutic regimens for neonatal HSV infection warrants further study.

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