Antibody Responses and Protection in Mice Immunized with Herpes Simplex Virus Type 1 Antigen Immune-stimulating Complex Preparations

By M. ERTÜRK, R. JENNINGS, D. HOCKLEY and C. W. POTTER

1Department of Virology, Medical School, University of Sheffield, Beech Hill Road, Sheffield S10 2RX and 2National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Hertfordshire EN6 3QG, U.K.

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SUMMARY

The formation of immune-stimulating complexes (iscoms) obtained by mixing the glycoside Quil A with an antigen preparation derived from herpes simplex virus type 1 (HSV-1)-infected cell cultures using a zwitterionic detergent is described. The HSV-1 antigen preparation incorporated into iscoms elicited significantly greater antibody responses in mice than the same preparation administered together with aluminium hydroxide gel, and provided complete protection against HSV-1 or HSV-2 lethal, systemic challenge infection in animals given a single dose containing 5 μg of protein. The HSV-1 iscom preparation also provided significant protection in mice against local reactions following challenge with HSV-1 by skin scarification.

The use of immune-stimulating complexes (iscoms) as an effective means of presenting viral antigens to the immune system was first described in 1984 and consists of a formulation of viral antigens, usually the surface glycoproteins, linked with a lipid adjuvant, Quil A, through hydrophobic interactions producing stable antigen–lipid complexes of about 35 to 40 nm in diameter readily visualized by electron microscopy (Morein et al., 1984). During the past year several groups have reported that when incorporated into iscoms viral glycoproteins derived from respiratory syncytial virus (Trudel et al., 1989), bovine leukaemia virus (Merza et al., 1989), equine influenza virus (Sundquist et al., 1988), measles virus (de Vries et al., 1988) or Epstein–Barr virus (Morgan et al., 1988) are extremely effective in the induction of both antibody and protection in experimental animals. Using iscoms containing the measles virus fusion (F) glycoprotein, one group has also described the induction of delayed-type hypersensitivity and the activation of measles virus-specific T cells in mice (de Vries et al., 1988).

Studies in our laboratory in recent years have been aimed at the isolation and characterization of a mixture of herpes simplex virus type 1 (HSV-1) glycoproteins from HSV-1-infected cells using the zwitterionic detergent Empigen BB and determination of the immunogenicity of these preparations in experimental animals (Mukhlis et al., 1986; Jennings et al., 1988; Ertürk et al., 1989), with a view to using such preparations as vaccines in man. These studies have shown that after treatment of HSV-1-infected cells with Empigen BB followed by dialysis and centrifugation procedures, at least four HSV-1 glycoproteins, gB, gC, gD and gE, can be recovered (Jennings et al., 1988). Gradient centrifugation procedures remove much unwanted protein and the resulting preparations induce good humoral antibody levels and provide complete protection in BALB/c mice against HSV-1, and partial protection against HSV-2, following a single dose of 15 μg total protein in the presence of aluminium hydroxide gel (ALH) (Jennings et al., 1988).

The HSV-1 antigen preparations used in the present study were obtained by a modification of procedures described earlier. Briefly, suspensions of HSV-1-infected Vero cells in phosphate-buffered saline, pH 7.2 (PBS) were treated with 2.5% Empigen for 30 min at 4°C. After sonication and centrifugation at 100000 g for 2 h, the preparations were dialysed against PBS.
and subjected to centrifugation on 5 to 30% sucrose gradients using an SW27.1 Beckman rotor. Gradient fractions were assayed for total protein (Lowry et al., 1951), and for the presence of HSV-1 glycoproteins gB, gC, gD and gE, by enzyme immunoassay (EIA) as described previously (Jennings et al., 1988).

The distribution of protein and HSV-1 glycoproteins gC, gD and gE in the gradient fractions following extraction of the antigens from infected cells using the zwitterionic detergent is shown in Fig. 1. Two protein peaks were obtained, one near the top, the other lower down the gradient in a position corresponding to the peaks of EIA reactivity of HSV-1 gC, gD and gE. Reactivity of these glycoproteins was not associated with the protein peak at the top of the gradient (Fig. 1). Furthermore, EIA reactivity of HSV-1 gB was not detected in the gradient fractions. Earlier studies have indicated that recovery of this glycoprotein following Empigen treatment of HSV-1-infected cells and its reactivity in EIA with the monoclonal antibody used are variable (Mukhlis et al., 1986; Jennings et al., 1988). However, recent studies in our laboratory using SDS–PAGE, immunoprecipitation and a modified EIA have indicated that HSV-1 gB is present in these preparations and peaks at a position similar to that of other HSV-1 glycoproteins in the sucrose gradients (data not shown).

Following gradient centrifugation, fractions with high HSV-1 glycoprotein levels were pooled, dialysed against PBS at 4 °C overnight and mixed with Quil A (kindly donated by Dr B. Morein, National Veterinary Institute, Division of Vaccine Research, Uppsala, Sweden) using a modification of methods described elsewhere (Morein et al., 1984). Briefly, 1.0 mg Quil A was added to 400 μg/ml antigen preparation in a volume of 1.0 ml. To increase the efficiency of iscom formation, HSV-1 glycoprotein–Quil A mixtures were shaken continuously for 1 h at 4 °C. Overnight dialysis against PBS was usually carried out to remove unreacted Quil A. The pooled fractions containing glycoprotein antigens and the glycoprotein–Quil A mixture were examined by negative staining electron microscopy. Both specimens were centrifuged onto glow-discharge-treated carbon films on AEI-type specimen grids using a Beckman Airfuge operated at approx. 100000 g for 10 min; they were stained with 4% aqueous sodium silicotungstate, pH 7.4, and examined using a Philips CM12 electron microscope. The antigen preparation consisted of dispersed granules, 8 to 10 nm in diameter, and a small amount of lipid-like material (Fig. 2a). The Quil A mixture showed typical cage-like iscom structures approximately 40 nm in diameter together with some small granular material (Fig. 2b).

BALB/c mice (from the closed colony, University of Sheffield) or female hairless MF1-Ola mice (Olac) were used in these studies. Methods for administration of HSV-1 antigen
Preparations to mice are described elsewhere (Jennings et al., 1988). Subcutaneous inoculations of these preparations alone, together with ALH or as iscoms were carried out in single doses of 5 to 15 μg total protein into groups of 10 to 20 animals. Control preparations obtained from mock-infected cells by procedures identical to those used for HSV-1 antigen preparation, and inoculated using similar dosages and routes, were included in each experiment. Sera obtained from each mouse after 2 to 3 weeks were tested for EIA antibody to HSV-1 as previously described (Jennings et al., 1988), and in some experiments for HSV-1 neutralizing (NT) antibody by an in vitro test (Mathiesen et al., 1988) using Vero cells and quantification by EIA. Endpoints were taken as the dilution of serum that reduced the EIA absorbance values at 490 nm to 50% of that given by Vero cultures infected by HSV-1 alone.

One to 2 days after the collection of blood samples, the BALB/c mice were challenged with 10 lethal doses (LD_{50}) of either HSV-1 (WAL) or HSV-2 (333) by the intraperitoneal route and observed daily for illness and death. Hairless mice were challenged by skin scarification of the left flank in a standard manner (Blyth et al., 1984), and spreading of HSV-1 (strain WAL) over the scarified area to a dose of 1 × 10^{7} p.f.u. They were observed daily for erythema at the scarification site, and for death, over 14 days.

The results of two typical experiments are shown in Fig. 3 and 4. Fig. 3(a) shows that inoculation of mice with either 15 or 5 μg of HSV-1 iscom preparation elicited significantly greater EIA HSV-1 antibody levels in mice than those induced by equivalent dosages of the same HSV-1 antigen administered either alone or mixed with ALH. Using the Mann-Whitney U test, P values of <0.002 were obtained for these comparisons. Fig. 3(b) shows that following challenge with either HSV-1 or HSV-2, mice receiving 5 μg of the HSV-1 iscom antigen preparation were completely protected. In contrast, animals immunized with 5 μg of HSV-1 antigen preparation either alone or mixed with ALH showed mortality levels ranging from 50 to 90%, the greatest mortality being observed following challenge with HSV-2. Complete protection against both HSV-1 and HSV-2 was also observed in mice immunized with 15 μg of HSV-1 iscom preparation, whereas animals receiving a similar dose of this preparation either alone or mixed with ALH were only partially protected against HSV-2 (Fig. 3b). HSV-1 challenge of animals receiving 15 μg of HSV-1 antigen preparation alone or mixed with ALH was not carried out in this experiment. Fig. 4 is a titration of HSV-1 iscom antigen preparation in BALB/c mice, and shows that doses as low as 1.6 μg induced high EIA and NT antibody levels against HSV-1 in these animals 2 weeks later (Fig. 4a). Furthermore, this dose of HSV-1
iscom preparation induced complete protection against HSV-1 and partial protection (60% of the animals survived) against HSV-2 challenge. Lower doses, however, induced lower EIA and NT antibody responses, and provided only partial protection against HSV-1 and almost no protection against HSV-2 (Fig. 4b).

EIA and NT responses of hairless mice immunized with the HSV-1 antigen preparation, either alone or mixed with ALH, or the HSV-1 iscom preparation are presented in Table 1 which also shows the results of challenge by skin scarification with $1 \times 10^7$ p.f.u. of HSV-1 (strain WAL). Significantly greater EIA and NT antibody levels were observed in sera of animals given

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**Fig. 3.** (a) Comparative EIA HSV-1 antibody levels (log2) in mice immunized with a single dose of Empigen-solubilized, gradient-purified HSV-1 antigen preparations alone, with ALH or as iscoms. (b) Mortality levels in mice following a single dose of HSV-1 antigen preparation either alone, mixed with ALH or as an iscom preparation. Percentage mortality following 10 LD$_{50}$ live HSV-1 (WAL) challenge is represented by the dotted and shaded columns; percentage mortality following 10 LD$_{50}$ live HSV-2 (333) challenge is represented by the unshaded and hatched columns. Inoculation was with 15 µg (unshaded and dotted) or 5 µg (shaded and hatched).

**Fig. 4.** (a) EIA and NT antibody levels against HSV-1 in mice given a single dose of decreasing concentrations of HSV-1 iscoms. EIA antibody levels (log$_2$) (unshaded) and NT antibody levels (log$_{10}$) (hatched). (b) Mortality levels following HSV-1 or HSV-2 challenge in mice immunized with a single dose of decreasing concentrations of HSV-1 iscoms. Percentage mortality after 10 LD$_{50}$ live HSV-1 (WAL) challenge (shaded), percentage mortality after 10 LD$_{50}$ live HSV-2 (333) challenge (hatched). The HSV-1 antigen doses are shown.
## Table 1. Serum EIA and NT antibody levels and responses to HSV-1 challenge by skin scarification in hairless mice immunized with HSV-1 antigen preparations

<table>
<thead>
<tr>
<th>Immunizing materials (dose)</th>
<th>Number of mice</th>
<th>Post-immunization serum antibody levels*</th>
<th>Response to HSV-1 challenge by skin scarification†</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1 antigen preparation alone</td>
<td>11</td>
<td>EIA 10.2 (±0.48)</td>
<td>Day 2: NS (0), Day 3: NS (0), Day 4: NS (0), Day 5: NS (0), Day 6: NS (0), Day 7: NS (0)</td>
</tr>
<tr>
<td>HSV-1 antigen preparation + ALH (1 × 5 μg)</td>
<td>10</td>
<td>EIA 30.0 (±4.3)</td>
<td>Day 2: 10, Day 3: 10, Day 4: 10, Day 5: 10, Day 6: 10, Day 7: 10, Day 8: 10</td>
</tr>
<tr>
<td>HSV-1 antigen preparation + Quil A (1 × 5 μg)</td>
<td>10</td>
<td>EIA 120 (±16.1)</td>
<td>Day 2: 10, Day 3: 10, Day 4: 10, Day 5: 10, Day 6: 10, Day 7: 10, Day 8: 10</td>
</tr>
<tr>
<td>Control antigen preparation (1 × 5 μg)</td>
<td>9</td>
<td>EIA 4.4 (±0.25)</td>
<td>Day 2: 10, Day 3: 10, Day 4: 10, Day 5: 10, Day 6: 10, Day 7: 10, Day 8: 10</td>
</tr>
</tbody>
</table>

*EIA antibody levels expressed as log₂ values (±s.e.); NT antibody levels expressed as log₁₀ values of the reciprocal of the highest serum dilution at which the NT EIA value was reduced to 50% of that of the virus control.
†The values represent the percentage scores for the local skin reactions (degree and intensity of erythema on a scale from 0 to 4) for all the animals in the group shown, out of a total possible score. Figures in parentheses are the numbers of animals surviving on the day shown. --, No skin reactions. NS, No survivors.

A single dose of the HSV-1 iscom preparation, and this material also provided almost complete protection of hairless mice against local skin infection with HSV-1 (Table 1). These results confirm the considerable potential of iscom viral glycoprotein preparations in conferring protection and eliciting high immune responses (Sundquist et al., 1988; de Vries et al., 1988; Trudel et al., 1989).

Both HSV-1 and HSV-2 can induce severe infections in man and are widespread in the human population, causing painful primary and recrudescing lesions in both the oral and genital regions. Although treatment with acycloguanosine (acyclovir) can ameliorate recurrent infection (van Landingham et al., 1988), eradication of an established latent infection using this drug has not been successful (Klein, 1982). Although existing experimental HSV vaccines are also ineffective in eliminating latent virus in both animals and man, subunit vaccines composed of HSV glycoproteins can protect against both primary and spontaneous recurrences in guinea-pigs (Stanberry et al., 1987) and may provide protection against the acquisition of herpes genitalis in man (Skinner et al., 1987). It remains possible that such vaccines administered to susceptible individuals before contact with HSV, may prevent clinical illness and perhaps also modify the establishment of latent infection.

A number of workers have prepared experimental subunit HSV vaccines for use in man (Skinner et al., 1978; Cappel et al., 1982; Ashley et al., 1985; Meignier et al., 1987; Stanberry et al., 1987; Kutinova et al., 1988; Sanchez-Pescador et al., 1988). However, the majority of these vaccines have been inoculated into experimental animals or man at relatively high dosage levels, in multiple doses or in the presence of adjuvants such as ALH or Freund’s complete adjuvant (Skinner et al., 1978; Cappel et al., 1982; Ashley et al., 1985; Stanberry et al., 1987; Kutinova et al., 1988). The studies reported here represent the first description of the incorporation of HSV antigens into iscoms, and illustrate that such preparations elicit complete protection in mice against HSV-1 or HSV-2 challenge infection following a single dose containing 5 μg of protein. However, the extent of association of the different HSV glycoproteins with HSV iscoms remains to be determined.

Other workers have described the immune potential of iscom antigen preparations incorporating glycoproteins derived from viruses as diverse as influenza (Sundquist et al., 1988), measles (de Vries et al., 1988) and the Epstein–Barr virus (Morgan et al., 1988). Although iscom glycoprotein preparations from a number of viruses are undoubtedly highly immunogenic and provide good protection against challenge infections in experimental animals, use of a saponin-derived adjuvant in man may be unacceptable (Allison & Byars, 1986). However, a commercial
vaccine using iscoms has been licensed for use in horses in Sweden and detailed toxicology studies on iscom preparations are in progress (Morein et al., 1988). As further studies on iscom glycoprotein preparations are carried out and information with respect to their ability to promote relevant cellular, as well as humoral, immune responses, together with a detailed knowledge of the associated toxicology of these materials become available, a clearer indication of their practical value and usefulness in humans should emerge.

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REFERENCES


Short communication


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