Monoclonal antibodies and human sera directed to the secreted glycoprotein G of herpes simplex virus type 2 recognize type-specific antigenic determinants

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Introduction

The herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) genomes are largely collinear in arrangement and closely related (Dolan et al., 1998). The genes encoding glycoprotein G-1 (gG-1) and glycoprotein G-2 (gG-2) are an exception because the gG-2 gene is 2097 nucleotides in length while the gG-1 gene contains only 714 nucleotides (McGeoch et al., 1985, 1987). Glycoprotein G in HSV-2 is the only HSV envelope protein to be cleaved post-translationally during processing. This event generates a secreted amino-terminal protein (sgG-2) and a carboxy-terminal high-mannose intermediate that is further processed by O-glycosylation to constitute the cell-membrane anchored mature gG-2 (mgG-2) (Balachandran & Hutt-Fletcher, 1985; Dall’Olio et al., 1987; Marsden et al., 1984; Olofsson et al., 1986; Roizman et al., 1984; Su et al., 1987, 1993). A unique property among the HSV-2 glycoproteins is that the mgG-2 protein elicits a type-specific antibody response. The mgG-2 protein (Ashley et al., 1988; Ho et al., 1992; Lee et al., 1985; Svennerholm et al., 1984) or mgG-2-derived synthetic peptides (Marsden et al., 1998; Oladepo et al., 2000) have therefore been used as a prototype antigen for type-discriminating serology.

In contrast to the well-described immunogenic properties of mgG-2, no information is available on the ability of sgG-2 to induce an antibody response in HSV-2-infected patients. We have previously presented evidence that the first 22 N-terminally located amino acids of the sgG-2 protein are a signal sequence and are cleaved off (Liljeqvist et al., 1999). The cleavage site of precursor gG-2 has not yet been determined, although it has been proposed that the molecule is cleaved between the amino acids arginine322 and alanine323 as well as between arginine342 and leucine343, where both sites are

Glycoprotein G-2 (gG-2) of herpes simplex virus type 2 (HSV-2) is cleaved to a secreted amino-terminal portion (sgG-2) and to a cell-associated carboxy-terminal portion which is further O-glycosylated to constitute the mature gG-2 (mgG-2). In contrast to mgG-2, which is known to elicit a type-specific antibody response in the human host, information on the immunogenic properties of sgG-2 is lacking. Here the sgG-2 protein was purified on a heparin column and used for production of monoclonal antibodies (mAbs). Four anti-sgG-2 mAbs were mapped using a Pepscan technique and identified linear epitopes which localized to the carboxy-terminal part of the protein. One additional anti-sgG-2 mAb, recognizing a non-linear epitope, was reactive to three discrete peptide stretches where the most carboxy-terminally located stretch was constituted by the amino acids320RRAL323. Although sgG-2 is rapidly secreted into the cell-culture medium after infection, the anti-sgG-2 mAbs identified substantial amounts of sgG-2 in the cytoplasm of HSV-2-infected cells. All of the anti-sgG-2 mAbs were HSV-2 specific showing no cross-reactivity to HSV-1 antigen or to HSV-1-infected cells. Similarly, sera from 50 HSV-2 isolation positive patients were all reactive to sgG-2 in an enzyme immunoassay whilst no reactivity was seen in 25 sera from HSV-1 isolation positive patients or in 25 serum samples from HSV-negative patients suggesting that sgG-2 is a novel antigen potentially suitable for type-discriminating serodiagnosis.

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The GenBank accession number of the nucleotide sequence reported in this paper is AJ303204.

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necessary for correct cleavage (R. Courtney, personal communication). The cleavage and processing pathway has been shown to be independent of other HSV-2 gene products (Su & Courtney, 1988) suggesting that the cleavage events were mediated by a host cell-specific protease.

As sgG-2 contains multiple positively charged residues (McGeoch et al., 1987), the protein was purified from virus-infected cell medium by using a heparin column. The sgG-2 protein was used for production of monoclonal antibodies (mAbs), which were epitope mapped using a Pepscan technique based on peptides coupled to a cellulose membrane support (Frank, 1992; Kramer et al., 1994). The anti-sgG-2 mAbs presented no cross-reactivity to HSV-1-infected cells. Furthermore, since sgG-2 was successfully used as an antigen in ELISA for detection of type-specific antibodies from HSV-2-infected patient sera this protein may be suitable as a novel serological antigen for type-discriminating serology.

Methods

**Cells and viruses.** African green monkey kidney (GMK-AH1) and human epidermoid (HEp-2) cells were cultured in Eagle’s minimal essential medium supplemented with 2% calf serum and antibiotics. A local HSV-2 strain designated B4327UR (Jansson & Molin, 1974) and the HSV-1 strain KOS321 (Holland et al., 1983) were used.

**Purification of sgG-2.** GMK-AH1 cells were infected with HSV-2 and when complete cytopathic effect was seen the medium was centrifuged at 2000 g for 10 min followed by ultracentrifugation at 100,000 g for 1.5 h. The supernatant was concentrated by using microconcentrator tubes (Filtron Skandinavia) before the sample was applied to a HiTrap heparin column (Amersham Pharmacia) and recirculated for 2 h. After washing, a stepwise elution with increasing concentrations of NaCl (0.2–2.0 M) was performed and the fractions were concentrated with microconcentrator tubes until dryness. The proteins were resuspended in 200 µL PBS.

**Production of mAbs.** Heparin-purified sgG-2 (100 µg) was emulsified in Freund’s complete adjuvant for priming and in incomplete adjuvant for booster doses and injected intramuscularly to five female BALB/c mice at 3 week intervals. A sixth mouse was given a third immunization with immunoaffinity chromatography-purified sgG-2 protein. The fusion procedure followed standard hybridoma techniques (Fazekas de St Groth & Scheidegger, 1986) using the myeloma Sp2/O cells as fusion partner. The supernatants of the hybridomas were screened for antibody reactivity by ELISA and positive hybridomas were cloned for antibody reactivity by ELISA and positive hybridomas were cloned by limiting dilution. The mAbs were cultured in dialysis tubing for large-scale production (Sjögren-Jansson & Jeansson, 1985). Subclass specificity was determined by radial immunodiffusion (The Binding Site Ltd).

**Imunoaffinity chromatography purified sgG-2.** The anti-sgG-2 mAb 4G2.10 (5 mg) was coupled to 2 ml of cyanogen bromide-activated Sepharose 4B (Amersham Pharmacia) according to the manufacturer’s instructions. Medium from virus-infected GMK-AH1 cells was harvested as described above and 0.3 M NaCl and 0.5% Nonidet P-40 were added before application to the column. The sample was recirculated for 1 h followed by washing with Tris-buffered saline (TBS) containing 0.5 M NaCl. The proteins were eluted with 0.1 M glycine–HCl buffer (pH 2.8) and neutralized with Tris–HCl (pH 8.0). The protein concentration was measured by ProteinAssay (Bio-Rad).

**Reactivity of anti-sgG-2 mAbs in an ELISA.** Immunoaffinity-purified sgG-2 (2/8 mg/ml) was coated at a 1:10,000 dilution in carbonate buffer (pH 9.6) at 4 °C overnight on Maxisorp microtitre plates (Nalge Nunc). The plates were blocked with 2% skim milk in PBS for 1 h at 37 °C. The antibodies, at an initial concentration of 20 µg/ml, were applied in twofold steps in PBS containing 1% skim milk and 0.05% Tween 20. After washing, peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories) was added at a 1:1000 dilution followed by addition of O-phenylenediamine as the substrate. In addition, the anti-sgG-2 mAbs and the anti-mgG-2 mAb O1.C5.B2 were tested against Helix pomatia lectin-purified mgG-2 as described previously (Liljeqvist et al., 1998). Supernatant from GMK-AH1 cells infected with HSV-1 was clarified and concentrated as described above, coated at a 1:100 dilution on microtitre plates, and the reactivity of the anti-gG-2 mAbs was tested in parallel. The results were given as end-point titres that were expressed as the reciprocal of the dilution giving an absorbance value greater than cut-off. Cut-off was defined as the reactivity to an unrelated antigen (cytomegalovirus) plus three SD.

**Immunoblot.** Immunoaffinity-purified sgG-2 (8 µg) was diluted in 2% SDS including mercaptoethanol, boiled for 5 min. Subjected to PAGE using 4–12% NuPAGE gradient gels (Novex), and electrotransferred to Immobilon-P transfer membrane (Millipore). Strips were incubated with anti-sgG-2 mAbs at a final concentration of 10 µg/ml. Peroxidase-labelled rabbit anti-mouse IgG (Dako) at a 1:100 dilution was used as conjugate with 4-chloro-1-naphthol as the substrate. For one of the mAbs, the reactivity to antigen subjected to SDS–PAGE under non-reducing conditions was tested. In addition, cell lysates of HSV-2-infected GMK-AH1 and HEp-2 cells were subjected to SDS–PAGE under reducing conditions as described earlier (Liljeqvist et al., 1999). The anti-mgG-2 mAb O1.C5.B2 and the anti-sgG-2 mAb 4.A5.A9 were tested for reactivity to the mgG-2 and the sgG-2 proteins. In addition, sera from ten HSV-2 infection proven patients (see below) were tested for reactivity to the sgG-2 protein.

**Indirect immunofluorescence.** Monolayers of GMK-AH1 and HEp-2 cells were cultivated on Lab-Tek chamber slides (Nunc Nalge) and infected with HSV-1 or HSV-2. When 25% of the cells were infected, the cultures were permeabilized and fixed in cold acetone for 5 min, washed in distilled water, dried and kept at −70 °C until use. For detection of membrane fluorescence, the cells were fixed with cold methanol for 10 min after addition of the mAbs. The anti-gG-2 mAbs were diluted in PBS (1 µg/ml) and incubated for 1 h at 37 °C. Fluorescein isothiocyanate-labelled Fab’, goat anti-mouse IgG (Jackson ImmunoResearch Laboratories) was used as conjugate at a 1:40 dilution and incubated for 1 h at 37 °C. The slides were mounted with a glycerol–water solution (4:1), and examined under a Nikon fluorescence microscope.

**Nucleotide sequencing of the gG-2 gene.** As the HSV-2 strain B4327UR was used for the production of sgG-2, the segment of the gG-2 gene encoding the 342 amino-terminally located amino acids was sequenced following PCR amplification using methods and primers described elsewhere (Liljeqvist et al., 1999). The nucleotides were compared with the HSV-2 reference strain HG52 (McGeoch et al., 1987).

**Pepscan analysis of anti-sgG-2 mAbs.** The anti-sgG-2 mAbs were screened for binding using a panel of overlapping peptides (13-mers with 10 amino acid overlaps) spanning amino acids 23 to 342 (Jerini BioTools). Selected 15-mer peptides, overlapping by 14 amino acids, were synthesized for a more precise localization of the epitopes. The cysteine residues were replaced by serines to avoid oxidation during the incubation steps. The peptide sequences were deduced from nucleotide sequence data derived from strain B4327UR. The membranes were
washed once in methanol and three times in TBS containing 0.05% Tween 20, pH 8.0 (T-TBS) and incubated overnight with blocking buffer (Genosys Biotechnologies) diluted 1:10 in T-TBS with addition of 50 mg/ml of sucrose. The mAbs were diluted (0.1 μg/ml) in blocking buffer and incubated for 3 h at room temperature. After triple washing, peroxidase-conjugated rabbit anti-mouse IgG (0.2 μg/ml) (Dako) was added in blocking buffer and incubated for 2 h. After washing, the membranes were developed with ECL detection solution on X-Omat S film.

For mapping of the anti-sgG-2 mAb 4.G2.G10, horseradish peroxidase was directly conjugated to the antibody using EZ-Link maleimide activated horseradish peroxidase (Pierce). After purification, the mAb was incubated, at a concentration of 5 μg/ml, with the Pepscan membrane overnight at 4 °C. After triple washing for 5 min the ECL detection solution was added for development.

- **Competitive indirect ELISA.** 15-mer peptides, comprising the predicted epitopes, were synthesized using 9-fluorenylmethoxycarbonyl chemistry (kindly provided by M. Levi, Karolinska Institute, Stockholm, Sweden). The peptides were incubated at different concentrations with the corresponding mAbs for 18 h at room temperature. The mAb concentrations were selected from the end-point titration curves and close to the inflection points. The mixtures were assayed as described above in an indirect ELISA with sgG-2 as target antigen. The apparent dissociation constant value (K_D) was calculated from the plot and expressed as the concentration of the peptide reducing the reactivity of the respective mAb to sgG-2 by 50% (Friguet et al., 1985) with correction for the bivalence of the mAbs (Stevens, 1987). For the mAb 4.G2.G10, which recognized a non-linear epitope, three peptides including the mapped reactive amino acid stretches were mixed and tested as noted above.

- **Type-specific serology.** Immunoaffinity-purified sgG-2 protein was coated on Maxisorp microtitre plates as described above. Fifty sera from patients with an isolation-proven HSV-2 infection were tested. These sera were characterized by Western blot and identified both the sgG-2 protein and the carboxy-terminal high-mannose intermediate portion of gG-2. In addition, 25 sera from isolation-proven HSV-1-positive patients were included for analysis. These sera presented reactivity to a type-common sodium deoxycholate-solubilized membrane preparation of HSV-1 in an ELISA (Jeansson et al., 1983; Liljeqvist et al., 1998; Svennerholm et al., 1984), but were unreactive to *Helix pomatia* lectin-purified mgG-2. Finally, 25 HSV-negative patient sera were selected based on lack of reactivity to both the type-common HSV-1 antigen and to *Helix pomatia* lectin-purified mgG-2. Sera were tested in duplicate at a 1:100 dilution in PBS containing 0.05 M NaCl, 1% skim milk and 0.05% Tween 20, and incubated overnight at 4 °C. Peroxidase-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories) was added at a 1:3000 dilution, and O-phenylenediamine was used as the substrate. The reactivity was stopped with 1 M sulfuric acid and the absorbance was measured at 492 nm.

**Results**

**Production of anti-sgG-2 mAbs**

Virus-infected cell medium was applied to a HiTrap heparin column and eluted with an increasing concentration of NaCl. Proteins were separated on SDS–PAGE and visualized with a Coomassie blue solution. The sgG-2 band with apparent molecular mass of 40 kDa was clearly visible from the fractions eluted with 0.2–0.7 M NaCl (data not shown). This band was recently confirmed to constitute sgG-2 by using amino-terminal amino acid sequencing (Liljeqvist et al., 1999).

For the production of anti-sgG-2 mAbs, five fusions were performed after using the heparin-purified sgG-2 eluted with 0.5 M NaCl as immunization antigen. With this protocol, the mAbs 4.G2.G10 and 4.A5.A9 were detected. Immunoaffinity-purified sgG-2 (mAb 4.G2.G10) was used for booster immunization of the sixth mouse. In the subsequent fusion, the mAbs 8.A10.F10, 5.C3.C6 and 6.D12.B3 were produced. The mAbs were of IgG1 subclass.

**Identification and localization of sgG-2 and mgG-2**

Four anti-sgG-2 mAbs were reactive in immunoblot when sgG-2 was subjected to SDS–PAGE under reducing conditions (Fig. 1a). MAb 4.G2.G10 showed reactivity to sgG-2 only when SDS–PAGE was performed under non-reducing conditions. This finding suggests that the mAb recognized a non-linear epitope of which at least one disulfide bond is essential to maintain the three-dimensional integrity of the epitope.

In previous reports sgG-2 was shown to be rapidly secreted into the cell-culture medium of virus-infected cells (Su et al., 1987, 1993). The protein was therefore detectable only when the normal Golgi processing and transport functions were inhibited by monensin. In this study substantial amounts of sgG-2 were detected from lysates of HSV-2-infected HEp-2 cells by using the anti-sgG-2 mAb 4.A5.A9 (Fig. 1b). Similar reactivity was seen from lysates of HSV-2-infected GMK-AH1 cells (data not shown). The mAb also recognized a distinct band with apparent molecular mass of 115 kDa which was interpreted to represent the precursor gG-2 protein as it also was recognized by the anti-mgG-2 mAb O1.C5.B2. In addition, the anti-mgG-2 mAb recognized the carboxy-terminal high-mannose intermediate and the mgG-2 with apparent molecular masses of 76 and 120 kDa, respectively.

The cellular localization of the gG-2 proteins was also tested by indirect immunofluorescence with HSV-2-infected GMK-AH1 and HEp-2 cell membranes or permeabilized cells. The reactivity was essentially identical for the two cell-lines. The anti-mgG-2 mAb recognized the mgG-2 protein both on virus-infected cell membranes and in the cytoplasm of permeabilized cells (Table 1). All the anti-sgG-2 mAbs clearly identified the sgG-2 protein, and as judged from the immunoblot experiment to a minor extent the precursor protein (see Fig. 1b), in the cytoplasm of permeabilized HSV-2-infected cells while no reactivity was seen to virus-infected cell membranes.

All of the HSV-2-positive human sera identified, in Western Blot, the mgG-2 protein and the carboxy-terminal high-mannose intermediate but not the sgG-2 protein (data not shown). Similarly, as described for the mAb 4.G2.G10, the sera were clearly reactive to purified sgG-2 subjected to SDS–PAGE on a non-reducing gel but unreactive when reducing agent was included. These data, together with the finding that sera were
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Fig. 1. (a) Immunoaffinity-purified sgG-2 was subjected to SDS–PAGE under reducing (MC+) or non-reducing conditions (MC−) and transferred to membranes. Four anti-sgG-2 mAbs were clearly reactive to sgG-2 antigen prepared under reducing conditions. The mAbs recognized a protein with an apparent molecular mass of 40 kDa (lane 1-4). The anti-sgG-2 mAb 4.G2.G10 was reactive to sgG-2 only when the antigen was prepared under non-reducing conditions. (b) Lysates of HSV-2-infected HEp-2 cells were subjected to SDS–PAGE under reducing conditions. The anti-mgG-2 mAb O1.C5.B2 identified the carboxy-terminal high-mannose intermediate (76 kDa), the precursor gG-2 molecule (115 kDa) and the mgG-2 protein (120 kDa). The anti-sgG-2 mAb 4.A5.A9 recognized the sgG-2 (40 kDa) and the precursor gG-2 protein. The molecular mass markers are marked in kDa on the left.

Table 1. Type-specific reactivity to different antigens in ELISA and in indirect immunofluorescence (IIF) for five anti-sgG-2 mAbs and one anti-mgG-2 mAb

<table>
<thead>
<tr>
<th>mAb</th>
<th>ELISA*</th>
<th>mgG-2 antigen</th>
<th>HSV-1 supernatant†</th>
<th>IIF Cells infected with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sgG-2</td>
<td></td>
<td></td>
<td>HSV-1+</td>
</tr>
<tr>
<td>4.G2.G10</td>
<td>1600</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4.A5.A9</td>
<td>102400</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5.C3.C6</td>
<td>51200</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>8.A10.F10</td>
<td>51200</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>O1.C5.B2§</td>
<td>12,800</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

* Reactivity is expressed as end-point titres. −, End-point titres less than 100.
† Extracellular medium from HSV-1-infected cell cultures.
‡ The left column shows the results for permeabilized HSV-infected cells and the right column results for HSV-infected cell membranes. The fluorescence signal was interpreted as positive (+) or negative (−).
§ Directed to mgG-2.

reactive to purified sgG-2 in an indirect ELISA (see below), indicated that the human antibodies recognized non-linear epitopes within sgG-2.

Type-specific reactivity of anti-gG-2 mAbs

The anti-sgG-2 mAbs were all reactive to sgG-2, presenting variable end-point titres in an ELISA (Table 1). The anti-sgG-2 mAbs were unreactive to mgG-2 and to culture medium from HSV-1-infected cells. By indirect immunofluorescence, all anti-gG-2 mAbs were unreactive to HSV-1-infected cell membranes as well as to permeabilized cells, indicating that the mAbs recognized HSV-2 type-specific epitopes.

Identification of linear epitopes of sgG-2

The synthetic peptides used in the Pepscan analysis were deduced from nucleotide sequence data of strain B4327UR. The following nucleotide differences were found in strain B4327UR as compared with strain HG52 (McGeoch et al.,
Glycoprotein G of herpes simplex virus type 2

Fig. 2. (a) The reactivity of four anti-sgG-2 mAbs to 13-mer peptides with 10 amino acid overlaps using a Pepscan technique. Each spot represents one peptide and each row contains 20 spots. Two reactive regions were identified and the aligned peptide sequences are noted below. —, Deletion of the nucleotides encoding the amino acid valine_{293} detected in HSV-2 strain B4327UR as compared to the reference strain HG52 (McGeoch et al., 1987). (b) Using a Pepscan membrane with 15-mer peptides with 14 amino acid overlaps the anti-sgG-2 mAbs identified linear stretches of residues that were crucial for binding. The epitopes and the localization within the sgG-2 are marked schematically.

For screening purposes, the mAbs were mixed and tested for reactivity to a cellulose membrane with 13-mer peptides with 10 amino acid overlaps spanning amino acids 23 to 342. Two stretches of the sgG-2 protein, localized within the carboxy-terminal part of the protein, were shown to exhibit antibody reactivity (Fig. 2a). The respective mAbs were fine-mapped by using separate membranes covering the reactive regions (15-mer peptides with 14 amino acid overlaps). Four antibodies presented distinct reactivities to the peptides whereby the respective epitopes were determined. The length of the epitopes varied between three to five residues and the sequences and their localization in sgG-2 are illustrated in Fig. 2(b).

Mapping of a non-linear sgG-2 epitope

MAb 4G2.G10 showed no reactivity to any peptide in the Pepscan analysis by using peroxidase-labelled secondary antibodies. To facilitate the detection of multiple linear stretches of sgG-2 residues important for binding, peroxidase enzyme was directly coupled to the mAb. Three discrete stretches of reactive peptides were identified and residues in common within the different peptides were delimited by the residues \(_{240}\text{GLRFRER}_{251}\) and \(_{284}\text{IRMARPT}_{290}\) and \(_{328}\text{RRAL}_{333}\), separated by 32 amino acids and 28 amino acids, respectively (Fig. 3). Note that cysteine_{252} is localized between two of the reactive peptide stretches and adjacent to the most amino-terminally defined stretch. In addition, a weak reactivity was seen to four single peptides. These peptides all contained two to four arginine residues. Peroxidase enzyme was directly coupled to a non-related mAb (an anti-HSV-1 gG antibody) and used as a control. No reactivity was seen to the sgG-2 peptides (data not shown). As the most carboxy-terminally located stretch of the epitope overlapped one of the proposed cleavage sites of the precursor gG-2 molecule, the reactivity of the mAb to this region was fine-mapped by using 15-mer peptides with 14 amino acid overlaps. As described for the 13-mer peptides with 10 amino acid overlaps the reactive amino acid motif was constituted by the residues \(_{328}\text{RRAL}_{333}\) (data not shown).

Calculation of the dissociation constants

Four of the anti-sgG-2 mAbs recognized short epitopes containing three to five residues. To ascertain the binding specificity of the antibodies, selected peptides were pre-incubated with the corresponding mAbs and evaluated in indirect ELISA using sgG-2 as target antigen. Peptides comprising the predicted linear epitope sequences (underlined) are aligned below. The apparent \(K_D\) values (M) of the peptides, expressed as a mean value and SD from three experiments, were as follows; \(20\text{GPGPTAPPQAARR}_{215}\).
Fig. 3. Peroxidase enzyme was directly coupled to the anti-sgG-2 mAb 4.G2.G10 and the antibody was incubated with the Pepscan membrane. Three discrete peptide stretches were recognized, and residues that were important for binding within the reactive peptide sequences are marked in bold. The localization of the reactive peptide stretches in the primary sequence of sgG-2 is illustrated below. Cysteine 252 is marked with an arrow and is suggested to be essential for maintaining the three-dimensional integrity of the epitope via a disulfide bond.

Fig. 4. Enzyme immunoassay reactivity to immunoaffinity-purified sgG-2 expressed as absorbance values from sera from 50 isolation proven HSV-2-infected patients and from sera from 25 isolation proven HSV-1-infected patients as well as from 25 HSV-negative patient sera.

2.7 x 10^{-7} \pm 1.2 (mAb 4.A5.A9); \ 1.4 x 10^{-7} \pm 1.4 (mAb 5.C3.C0); \ 2.6 x 10^{-7} \pm 1.0 (mAb 8.A10.F10); \ 4.1 x 10^{-7} \pm 0.8 (mAb 6.D12.B3). The $K_d$ values are in accordance with the dissociation constants normally seen for mAb–peptide interactions (Geyser et al., 1985; Hönne et al., 1993; Stigler et al., 1995). As Pepscan techniques are biased towards detection of short linear stretches of residues within epitopes (Laver et al., 1990), it seems likely that, at least for some of the mAbs described, additional residues may be involved in the antibody–antigen binding. However, the values for dissociation constants suggest that the blocking peptides contained at least the major parts of the relevant epitopes. The anti-sgG-2 mAb 4.G2.G10 was not significantly inhibited by a mixture of peptides (> 10^{-5} M) encompassing the three reactive amino acid stretches defined with the Pepscan technique (data not shown).

Reactivity of human sera to sgG-2

Imunoaffinity-purified sgG-2 was evaluated as an antigen in an indirect ELISA under specific conditions described in Methods. Fifty sera from isolation proven HSV-2-infected patients presented absorbance values in the range 0.21 to 1.86 (Fig. 4). Sera from 25 isolation proven HSV-1-infected patients and sera from 25 HSV-negative patients presented significantly lower values in the range 0.01 to 0.07.

Discussion

A correct diagnosis of HSV-2 infection is crucial for the physician in counselling of patients attending STD clinics, for seroepidemiological studies, and for HSV vaccine follow-up programs. Type-specific HSV serology is of major importance in the diagnostic arsenal since HSV establishes latency in sensory neurons giving no symptoms during long periods. Furthermore, asymptomatic shedding of HSV-2 may constitute the major route for transmission of infection (Langenberg et al., 1999; Wald et al., 1997) and type-specific serology is therefore the most accurate method to identify silent carriers of HSV-2 infection. mgG-2 has hitherto been shown to be the only HSV-2 protein which induces a type-specific antibody response. However, one problem when using mgG-2 as a type-specific antigen is the recently reported limitations in sensitivity (Schmid et al., 1999). In addition, the antibody response to this
protein develops relatively late after onset of primary HSV-2 infection (up to 6 months) as compared to the antibody response to other envelope proteins, for example glycoprotein B (Ashley et al., 1988; Lopez et al., 1993). This may create a problem during the acute phase of the HSV-2 infection when isolation of the virus is lacking and the diagnosis has to be based on type-specific serology only. For some of these patients repeated testing of serum samples may be necessary to obtain a correct serodiagnosis with a risk that the diagnosis of the HSV-2 infection is delayed.

A major HSV-1/HSV-2 difference in gene length is found between the two gG genes where HSV-1 lacks a stretch homologous to sgG-2. The sgG-2 protein has therefore the potential to elicit an exclusively type-specific B-cell immune response in the host. Here we showed that the anti-sgG-2 mAbs presented a type-specific reactivity to HSV-2 with no cross-reactivity to HSV-1 antigen, and that the sgG-2 protein was immunogenic and evoked a type-specific antibody response in HSV-2-infected patients. This protein may therefore be suitable as an additional serological antigen for detection of anti-sgG-2 antibodies. As sgG-2 is secreted rapidly during replication, the antibody response to sgG-2 may be elicited after infection. However, until the performance of sgG-2 is investigated in large serological studies including early infections, the mgG-2 protein should be considered as the prototype antigen for type-specific serology.

In the mapping of HSV epitopes, antibody reactivity to antigen subjected to SDS–PAGE and immunoblot has been used for discrimination of linear from non-linear epitopes. In antigen subjected to SDS–PAGE and immunoblot has been considered as the prototype antigen for type-specific serology. In studies including early infections, the mgG-2 protein should be considered as the prototype antigen for type-specific serology.

In the mapping of HSV epitopes, antibody reactivity to antigen subjected to SDS–PAGE and immunoblot has been used for discrimination of linear from non-linear epitopes. In the latter case denaturing and reducing agents disrupt the integrity of the epitope and usually no antibody reactivity is detected under these conditions (Muggeridge et al., 1990). The distinction may be valuable for selection of mapping strategy as antibodies recognizing linear epitopes can usually be localized by synthetic peptides in contrast to non-linear or discontinuous epitopes which are unreactive (Isola et al., 1989; Jemmerson, 1987). However, a few studies have recently detected non-linear epitopes by the use of Pepscak techniques based on peptides coupled to Immobilon (Gao & Esnouf, 1996) or to cellulose membranes (Korth et al., 1997; Reineke et al., 1995, 1998).

The non-linear epitope described for mAb 4.G2.G10 was composed of three discrete peptide stretches of sgG-2. In a previous study, Su et al. (1993) produced a rabbit hyperimmune serum against a peptide derived from the sgG-2 sequence. This peptide sequence overlapped with one of the reactive peptide stretches described here for the non-linear epitope $^{235}$GLFR $^{231}$. These observations indicate that this region of sgG-2 is exposed and immunogenic in both mouse and rabbit. The epitope contained mostly positively charged arginines and hydrophobic residues compatible with data obtained from X-ray crystallography analysis of antibody–antigen interactions showing that non-linear epitopes usually are composed of highly charged amino acids flanking a central core of hydrophobic residues (Kwong et al., 1998; Padlan et al., 1989; Smith et al., 1996). The most carboxy-terminal reactive peptide stretch of the epitope described for mAb 4.G2.G10 was amino acids $^{326}$RRAL $^{323}$. This motif overlapped with the amino-terminally localized cleavage site which is proposed to occur between arginine $^{324}$ and alanine $^{322}$. One explanation for this finding might be that small amounts of the gG-2 precursor protein are secreted into the extracellular medium or that the cleavage site is not utilized in the cell line used here.

Several mammalian alphaherpesviruses sequenced to date code for positional gG homologues which are secreted (Crabb et al., 1992; Keil et al., 1996; Rea et al., 1985; Telford et al., 1992). The gG-2 gene has been shown to be non-essential for replication of HSV-2 in vitro (Harland & Brown, 1988) and in vivo for isolates from the recurrent HSV-2 lesions of some rare patients (Liljeqvist et al., 1999). Furthermore, mutants of pseudorabies virus lacking the gene encoding the secreted gG homologue (gX) were shown to be fully virulent in pigs with induction of a complete protective immunity (Kimman et al., 1992). Thus, the functions of these gene products have hitherto been elusive. An interesting observation is that sgG-2 was not only secreted into cell-culture medium but also accumulated in lysates of HSV-2-infected cells, a finding which suggests that the protein may also have an intracellular function within HSV-2-infected cells. In addition to the potential use of sgG-2 as a novel HSV-2-specific serological antigen, the production and mapping of anti-sgG-2 mAbs described in this study may be a valuable tool facilitating future studies addressing possible intra- and extracellular interactions of sgG-2.

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References


Glycoprotein G of herpes simplex virus type 2


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