Identification of type-specific domains within glycoprotein G of herpes simplex virus type 2 (HSV-2) recognized by the majority of patients infected with HSV-2, but not by those infected with HSV-1

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A combination of phage peptide display library mapping and pepscanning, with both murine monoclonal antibodies and a panel of well-characterized human sera, have been used in order to define type-specific epitopes of glycoprotein G of herpes simplex virus type 2 (HSV-2) (gG2). Both techniques revealed an immunodominant region of gG2, centred around amino acids 525–587 of the uncleaved gG2 molecule. A soluble peptide, equivalent to amino acids 551–570, when used as antigen in an ELISA format was recognized by three out of five murine MAbs and by 20/26 (77%) Western blot anti-HSV-2-positive human sera, but by only 1/63 Western blot anti-HSV-2-negative sera (specificity, 98%). The sensitivity of detection of human anti-HSV-2 antibodies was increased to 90% using a peptide derived from this region, presented on a nitrocellulose membrane. This highly antigenic and type-specific domain of gG2 is located at the junction between the ‘unique’ region of gG2 and its C-terminal end, which has approximately 50% identity with gG1. A second antigenic region of gG2, amino acids 351–427, which lies within the ‘unique’ part of gG2, was also identified by both techniques employed in this study and is recognized by a proportion of anti-HSV-2-positive sera. These findings demonstrate the feasibility of developing a peptide-based type-specific assay for the detection of anti-HSV-2 antibody in human sera based on type-specific epitopes of gG2 and have implications for the understanding of the three-dimensional topography of gG2.

Introduction

Identification of antigenic regions within viral proteins subserves a number of functions. Detailed knowledge of epitopes which induce protective immune responses may allow generation of prophylactic subunit vaccines; synthetically derived antigenic epitopes may be used in diagnostic assays to detect virus-specific antibodies; mapping of epitopes within a whole protein may provide important clues as to the three-dimensional structure of that protein, and may enhance understanding of the mechanisms of immune escape adopted by the virus.

Herpes simplex virus type 2 (HSV-2) is the main cause of recurrent genital herpes (Corey et al., 1983). The majority of individuals infected with HSV-2, however, give no clinical history of disease, and yet these asymptomatic individuals shed virus from epithelial surfaces at intervals, and are therefore an infection risk for their sexual partners (Koutsky et al., 1992). Two potential strategies to reduce transmission of the virus have been cited: establishment of accurate serological assays which can distinguish between antibodies to HSV-1 and HSV-2, allowing identification of sexual partners who are discordant...
for HSV-2 infection (Kulhanjian et al., 1992; Gibbs & Meads, 1992), and development of prophylactic or therapeutic vaccines which could be used to generate protective immunity to the virus (Stanberry, 1995; McKenzie & Straus, 1996).

The former strategy has proved difficult due to the considerably shared antigenicity between the two viruses. The glycoprotein G (gG) molecule of HSV-2 has a large region (over 500 amino acids) which is absent from its counterpart in HSV-1 (Lee et al., 1985), and has therefore attracted much attention as a likely source of type-specific antigens. Indeed, gG2-based assays for the detection of HSV-2 antibodies using *Helix pomatia*-purified gG2 as antigen in immunoblot and ELISA formats have been described (Lee et al., 1985; Ho et al., 1992, 1993; Ashley et al., 1998). An alternative approach to the use of whole gG2 would be to construct an assay using synthetic peptides representing key gG2 epitopes as antigen.

We have used two independent technologies to identify type-specific epitopes within gG2 which are recognized by human sera. Both phage peptide display library technology (Smith & Scott, 1993; Grabowska & Irving, 1996) and pepscanning (Geyser et al., 1984) identified two type-specific regions within gG2 recognized by human sera. The results generated may find application in the development of type-specific peptide-based assays to identify asymptomatic carriers generated may find application in the development of type-specific epitopes of gG2.

Methods

**Monoclonal antibodies (MAbs).** Five anti-gG2 mouse MAbs, O2E10.A3.H5, O1B9.E5, P4A10.F11, O3G11.H7 and O1C5.B2 (abbreviated to H5, E5, F11, H7 and B2 respectively throughout), in the form of culture supernatants were used. All five MAbs are positive against gG2 in ELISA. H5 was used at a dilution of 1:100, and E5, F11, H7 and B2 at a dilution of 1:200, as this was found to be optimal in ELISA against gG2.

**Phage peptide display library.** The library used was a gift from G. Smith (Missouri, USA) containing approximately 10^8 different phage clones based on the filamentous phage fd-tet, which is composed of the genome of the filamentous phage fd and a segment of the transposon Tn10, encoding tetracycline resistance, thus allowing the selection of infected host bacteria by plating out in the presence of tetracycline. In addition to wild-type gene VIII, encoding the major coat protein pVIII, the phage in this library were engineered to express a recombinant form of gene VIII containing a degenerate DNA insert encoding random 15-mer peptides and are, therefore, type 88 vectors (Smith, 1993). The recombinant gene VIII is under the control of a lac promoter; the ratio of the peptide-displaying to wild-type pVIII can, therefore, be altered by varying the concentration of IPTG added to the host bacterial culture.

**Bacteria.** The K91Kan strain of *E. coli*, a λ-derivative of K-38 was used throughout. It is Hfr Cavalli and has chromosomal genotype thi. Bacteria were cultured in LB medium (Sigma), with the addition of kanamycin (50 µg/ml), tetracycline (20 µg/ml) or IPTG (1 mM) where appropriate.

**Infection of bacteria.** Infections were carried out by incubating phage for 30 min at room temperature with an equal volume of K91Kan, grown to exponential phase in LB containing kanamycin. LB containing an ‘inducer’ tetracycline concentration of 1 µg/ml was added and the bacteria were incubated for a further 45 min at 37 °C.

**Preparation of polyethylene glycol (PEG)-precipitated phage.** Phage were purified from the culture supernatants of infected bacteria by addition of 1/5th vol. of 20% PEG–2.5 M NaCl followed by incubation for 1 h at 4 °C. The precipitated phage were pelleted, resuspended in Tris-buffered saline (TBS), and the PEG precipitation was repeated. Phage from a culture supernatant volume of 5 ml were usually resuspended in a final volume of 150 µl TBS. The absorbance was then read at 269 nm and the concentration of the phage preparations was standardized to 150 µg/ml, assuming that an A_{265} reading of 1 is equivalent to a concentration of 3.8 mg/ml.

**Biopanning.** Three rounds of biopanning were carried out with each MAb. During the first round, ELISA wells (Nunc Maxisorp) were used as the solid phase; they were coated with aliquots of MAb overnight at room temperature in a humid atmosphere, washed in TBS, blocked in TBS–1% BSA, then washed in TBS–0.05% BSA. One aliquot of the library containing 10^8 phage in 50 µl TBS–0.05% BSA was added to the antibody-coated well, for 1 h at room temperature. Unbound phage were removed and the wells were washed four times in TBS–0.05% BSA and four times in TBS. 50 µl of elution buffer (0.2 M glycine, 0.1 M HCl, 0.1% BSA, 0.1 mg/ml phenol red, pH 2.2) was added for 10–20 s, then removed and neutralized by addition of Tris–HCl pH 8.8 (Sigma). The phage eluted from each antibody were used to infect exponential phase K91Kan, then grown overnight in LB containing tetracycline. They were then purified by PEG precipitation.

The second and third rounds of biopanning were carried out using a 20 µl aliquot of goat anti-mouse-coated Dynabeads (Dynal) as the solid phase. The beads were washed four times in TBS, incubated with a 50 µl aliquot of the MAb, then washed and blocked. During the second round, a 50 µl aliquot of the PEG-precipitated phage from the first round was incubated with the MAb-coated beads, then washed. Bound phage were eluted, amplified and purified by PEG precipitation as in the first round. During the third round, PEG-precipitated phage from the second round were used. Again, bound phage were eluted, amplified and purified by PEG precipitation. Phage eluted during the third round of biopanning were used to infect bacteria which were then plated out at a low concentration on LB agar–tetracycline plates to allow individual phage clones to be isolated.

**ELISA to identify positive phage clones.** ELISA wells (Nunc Maxisorp) were coated by incubating overnight with rabbit anti-fd antibodies (Sigma) diluted 1:1000 in coating buffer (carbonate–bicarbonate buffer, pH 9.6). After each incubation the wells were washed with PBS–0.05% Tween 20. The plates were blocked by addition of PBS–0.05% Tween 20–1% BSA (blocking buffer). Individual phage clones were grown overnight in LB containing tetracycline and IPTG to maximize expression of the recombinant form of gene VIII containing the peptide insert. The rabbit anti-fd-coated wells were incubated in turn for 1 h at room temperature with supernatant from such cultures, the test MAb diluted in blocking buffer (dilutions as described above) and alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma A1682) diluted to 1:1000 in blocking buffer, pNPP at 1 mg/ml in diethanolamine buffer (10% diethanolamine, pH 9.8, 0.5 mM MgCl_2, 0.02% sodium azide) was used as a substrate for the alkaline phosphatase and the absorbance of each well was read at 405 nm.

**Sequencing.** ssDNA was prepared from 1.5 ml overnight cultures by PEG precipitation followed by phenol–chloroform extraction and ethanol precipitation. Sequencing was carried out using a Sequenase version 2.0 T7 DNA polymerase kit (Amersham) according to the manufacturer’s instructions. The oligonucleotide AGCAGAAGCCTGAGA-
AGAGAGTC, complementary to the genomic DNA of the phage Ψ of the insert, was used as a primer.

Peptides. Peptides were synthesized by standard f-moc methodology as soluble peptides or on derivatized membranes. 15- to 20-mer peptides were a gift from Peptide Therapeutics (Cambridge, UK); 6-mer peptides were synthesized using the Genosys Spots kit. Their sequences and derivation are given in Figs 1 and 2.

Inhibition ELISAs. Wells were coated with Helix pomatia lectin-purified gG2 at a dilution of 1:500 in coating buffer. After blocking, peptides or phage were added simultaneously with the MAb diluted in blocking buffer. The MAb was diluted by a factor of 1:2 compared with the concentration used in the ELISA described above. Binding of the MAb was detected using the same procedure as above.

Human sera. 89 patient sera were collected at the Genito-Urinary Medicine Clinic, City Hospital, Nottingham, UK. The sera were characterized by Western blotting (WB) (Ho et al., 1993) for the presence of IgG reactive with HSV-1 and HSV-2 proteins at the Centre for Microbiology and Infectious Diseases, Westmead Hospital, Sydney, Australia. Briefly, HSV-1 or HSV-2 antigens were separated by SDS–PAGE and transferred to nitrocellulose membranes. Following incubation of the membrane with test serum, binding of type-specific antibodies was detected using a biotinylated anti-human immunoglobulin and a peroxidase–streptavidin conjugate. Extensive cross-reactivity occurs between antibodies elicited by the two subtypes of virus; therefore, reactivity with glycoproteins gC of HSV-1 and gG of HSV-2 was used to define sera with antibodies to HSV-1 and HSV-2 respectively. Sera were classified into four groups according to the results of the WB assay: no antibodies to either HSV-1 or -2; antibodies to HSV-1 only; antibodies to HSV-2 only; antibodies to both HSV-1 and -2.

Peptide ELISAs. ELISA wells (Nunc Maxisorp) were coated by incubating overnight with peptides at 5 µg ml⁻¹ in PBS. After each incubation the wells were washed with PBS–0.05% Tween 20. The plates were blocked by addition of a 1:10 dilution of Boehringer Mannheim ECL blocking solution (cat. no. 1500 694) in PBS. Incubation buffer was a 1:20 dilution of this reagent in PBS. Wells were incubated in turn with serum diluted 1:25 and horseradish peroxidase-conjugated rabbit F(ab)₂ anti-human IgG (Dako P0406) diluted to 1:1000 in PBS–10% normal goat serum. Sigma Fast OPD tablets (Sigma P9187) were used as a substrate for the peroxidase and the absorbance of each well was read at 490 nm after stopping the reaction with 2 M H₂SO₄.

Staining of membrane-bound peptides with human sera. Derivatized membranes, on which peptides had been synthesized as discrete spots, were washed briefly in methanol, washed using TBS containing 0.05% Tween 20 (TBS–0.05% Tween) and then incubated with human sera. Sigma Fast OPD tablets (Sigma P9187) were used as a substrate for the peroxidase and the absorbance of each well was read at 490 nm after stopping the reaction with 2 M H₂SO₄.
Fig. 2. Reactivity of MAbs with membrane-bound peptides. MAbs H5, E5, B2, H7 and F11 were incubated in turn with a series of membrane-bound peptides. Binding was detected using a horseradish peroxidase-conjugated anti-mouse antibody. Reactivity of the MAbs with each peptide is scored as negative (−), weak (+) or strong (++). nt, Not tested. The sequences of the peptides used are given, using the standard single-letter code for amino acids, from the N terminus to the C terminus. The positions of the peptides within gG2 are indicated by subscript numbers.

Results

The strategy adopted for use of the random peptide display library was to identify antigenic regions recognized by a small panel of murine anti-gG2-specific MAbs, and then to assess whether anti-gG2-positive human sera recognized epitopes within the same regions. This approach obviates the difficulties associated with selecting phage using complex polyclonal human sera with reactivity against multiple pathogens, and has previously been used successfully to identify epitopes derived from hepatitis B virus (Motti et al., 1994).

Selection of phage clones

Four of the MAbs (H5, E5, F11, H7) with specificity for gG2 were used to screen the library of phage containing random 15-mer peptide inserts. After three rounds of biopanning, individual phage clones were isolated and screened by ELISA to identify those which bound strongly to the antibody of interest, and those which gave a clear positive signal were sequenced. The sequences of the phage clone inserts are given in Fig. 1.

Identification of motifs amongst the sequences of the phage clone inserts and within the native sequence of gG2

Motifs (indicated in Fig. 1) could be identified amongst the phage clones for MAbs H5, E5 and F11 using Clustal W (1.4) for multi-sequence alignment (http://biology.ncsa.uiuc.edu/BW/BW.cgi), followed by minor manual adjustment. For MAb H5, it can be seen that the motif ([DE]HRS) tended to appear at the N-terminal side of the 15-mer insert. We postulated that adjacent amino acids derived from the natural protein VIII sequence may have contributed to the antibody binding site, and therefore have included these amino acids (PAE) in the alignment. The sequence of gG2 was then scanned using Clustal W to identify regions with sequence similarity to these motifs (native sequence, Fig. 1). The motifs identified for H5 and E5 lie adjacent to each other in the sequence of gG2, while the motif for F11 was found in a separate location. For H7 only two distinct phage clones could be identified and, therefore, no motif could be discerned.

washed three times with TBS–0.05% Tween. Throughout, TBS–0.05% Tween–5% Tween–5% Boehringer Mannheim ECL blocking solution was used as the incubation buffer. The membrane was incubated in turn in a 1:400 dilution of the test serum, and a 1:4000 dilution of horseradish peroxidase-conjugated rabbit F(ab)² anti-human IgG (Dako P0406). The signal was developed using the Boehringer Mannheim developing solution, according to the manufacturer’s instructions and detected by exposure to X-ray film. For each peptide spot the signal was designated as negative (−), weak (+) or strong (++). The membranes were re-used following regeneration by exposure of the membrane in turn to 8 M urea–10% SDS and 50% ethanol–10% acetic acid and thorough washing with TBS–0.05% Tween 20. Exposure of the treated membrane to X-ray film demonstrated complete removal of the signal by this procedure.

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Identification of type-specific domains of gG2

Inhibition of binding of the MAbs to gG2 by phage clones

If the inserts present in phage clones selected by the MAbs truly contained epitopes or mimotopes of the native antigen, then such clones should inhibit binding of the relevant MAb to gG2. To test this hypothesis, two representative phage clones for each MAb were used in an inhibition assay. Each phage clone was used at a range of concentrations. For each MAb, wild-type phage M13 was used as a negative control to ensure that inhibition of binding of the MAb to gG2 was due to the physical presence of the phage. The percentage inhibition, compared with wells to which no phage were added, was calculated. The results for H5, which are representative of the results obtained with all the MAbs, are shown in Fig. 3(a). For each MAb, both phage clones tested were able to inhibit binding of the MAb to gG2. The degree of inhibition varied for different clones, but inhibition of over 50% was achieved with each clone whereas less than 10% inhibition was observed using the wild-type phage M13 over the same range of concentrations.

Inhibition of binding of the MAbs to gG2 by peptides representing phage inserts or the primary amino acid sequence of gG2

Further proof that the epitopes of gG2 recognized by each of the MAbs were indeed represented by the phage clone inserts was sought by testing a number of soluble synthetic peptides for their ability to inhibit binding of the MAbs to gG2. For MAbs H5, F11 and E5, two peptides, with sequences derived from the inserts of phage selected by that MAb, and one peptide derived from the native sequence of gG2 with most similarity to the motif common to phage selected by the MAb (Fig. 1) were tested. For MAb H7, a peptide with the sequence of one of the phage clones selected by this MAb was used. An irrelevant peptide was included in each assay as a negative control. The sequences of the peptides which were used are indicated in Fig. 1. Peptides were added at a range of concentrations from 250 µg/ml to 7.5 µg/ml. The percentage inhibition, compared with wells to which no peptide was added, was calculated.

The results for H5 are shown in Fig. 3(b). Binding of MAb H5 to gG2 was inhibited by both peptides PT73 and Ch16685 with sequences derived from phage clone inserts 3.15 and 2.10 respectively, and by peptide PT71 derived from the sequence of gG2. The inhibition of binding of H5 to gG2 was clearly dependent on the sequence of the peptides as PT72, a scrambled version of PT71, did not have this effect. Similarly, binding of E5 to gG2 could be inhibited by both of the peptides derived from phage 12.17 and 12.18 respectively, and by PT71, derived from the native gG2 sequence; binding of F11 to gG2 could be inhibited by a peptide derived from phage 8.22, and PT173, derived from native gG2, but not by a peptide derived from phage 9.4; and binding of H7 to gG2 could be inhibited by a peptide derived from phage clone 7.17 (data not shown).

Further localization of the epitopes recognized by the MAbs

In order to further characterize the epitopes recognized by the MAbs, a series of membrane-bound (MB) peptides was synthesized, consisting of eight overlapping 6-mer peptides (MB1–8) covering the sequence of PT71 (amino acids 551–570 of gG2), and 16 overlapping 20-mer peptides (MB9–24) extending either side of the PT71 region of gG2, i.e. amino acids 538–588. This set included a membrane-bound equivalent of PT71 (MB15). In addition, a membrane-bound 20-mer peptide with the sequence of PT173 was synthesized (MB25). The membrane-bound peptides were stained with each of the MAbs in turn (Fig. 2). As expected, both MAbs H5 and E5 bound to a number of the peptides with a sequence derived from the PT71 region of gG2, including the membrane version of PT71, MB15. B2, a MAb which had not been used for biopanning, also reacted with a number of peptides derived from the PT71 region (MB12–18). Therefore, all three of these MAbs react with the PT71 region of gG2. Whilst there is overlap between the peptides recognized, the overall pattern of binding for each is distinct, these differences being most evident in the binding of the MAbs to the 6-mer peptides. Interestingly, the epitope of H7, which had not been previously located, appears to lie downstream of the PT71 region, represented here by peptide MB24, amino acids 574–588 of gG2. As expected, F11 did not bind to any of the peptides.
Fig. 4. Reactivity of human sera with soluble peptides. The reactivity of human sera with three different peptides in ELISA is illustrated: PT71, derived from gG2 native sequence containing epitopes recognized by MAb H5 and E5 (a); Ch16687, derived from phage insert selected by MAb F11 (b); and PT173, derived from gG2 native sequence, containing an epitope recognized by MAb F11 (c). The sera were used at a dilution of 1:25 and fall into four groups based on the presence of antibodies to HSV-1 and HSV-2 proteins detectable by WB: (1) antibodies to neither HSV-1 nor HSV-2; (2) antibodies to HSV-1 only; (3) antibodies to HSV-2 only; (4) antibodies to both HSV-1 and HSV-2. The sequences of the peptides are given in Fig. 1.

Table 1. Sensitivity and specificity of ELISAs based on soluble peptides

A panel of 89 sera was tested against each peptide using an ELISA format. For each peptide, a cutoff was calculated using the mean plus three standard deviations of the results for anti-HSV-2-negative sera. The percentage of sera from patients in groups 1 and 2 with reactivities below the cutoff (specificity) and of patients in groups 3 and 4 with reactivities above the cutoff (sensitivity) are shown in Table 1 for each peptide. PT71 is clearly the most useful of these sera in identifying patients who are WB-positive for anti-HSV-2, independent of their anti-HSV-1 status. In addition, sera which were found to be positive for any of the other peptides were also positive for PT71.

Further localization of the epitopes recognized by human sera within the region of gG2 containing the peptide PT71

The membrane-bound peptides MB9–24 were tested for their reactivity with a panel of 10 WB anti-HSV-2-positive sera (Fig. 5). Individual positive sera were reactive with many or all of the peptides in this region of gG2, suggesting that this region of gG2 includes numerous epitopes recognized by patients who have been exposed to HSV-2. Sera 1 and 14 were below the cutoff in the assay with soluble PT71 and were negative with MB15 (equivalent to PT71), as they were with
Identification of other peptides within gG2 reactive with human sera

In order to identify other peptides derived from gG2 containing epitopes with which human sera, and particularly WB anti-HSV-2-positive, PT71-negative sera, react, a set of overlapping 20-mer peptides covering the entire length of gG2 was synthesized in membrane-bound format. They were stained with 10 of the HSV-2 WB-positive sera, including the six sera which were PT71 ELISA-negative (1, 14, 16, 20, 36, 51), and three of the anti-HSV-2 WB-negative sera. The number of positive and negative sera reactive with each of the peptides is illustrated in Fig. 6. Two regions of the molecule could be identified which are frequently recognized by WB anti-HSV-2-positive sera but not by WB anti-HSV-2-negative sera. They are represented by peptides including amino acids 350–427 and 525–587 of gG2 respectively. The first of these regions includes the F11 epitope and the second the PT71 region. It should be noted that nine out of 10 of the WB anti-HSV-2-positive sera, including five out of six of the PT71 ELISA-negative sera, and none of the WB anti-HSV-2-negative sera tested were reactive with peptide 39, the peptide within this set which is most closely related to PT71.

Discussion

We have adopted two different approaches towards identification of type-specific epitopes within gG2 that are recognized by sera from patients who have been infected with
HSV-2. Both technologies identified a region within gG2 located between amino acid residues 525–587 that carries multiple epitopes recognized by a large majority of WB anti-HSV-2-positive, but not by anti-HSV-1-positive, anti-HSV-2-negative sera. This region, also recognized by four out of five murine anti-gG2 MAbs, lies at the junction between the unique region of gG2 and the C-terminal end of the glycoprotein. This was a surprising result in view of the fact that this region bears considerable identity with gG1. Both techniques also revealed a second region, amino acids 351–427, located within the unique region of gG2, recognized by one MAb (F11), and a large proportion of human WB anti-HSV-2-positive sera.

The first approach adopted was to use the phage peptide display library technology to identify antigenic regions of the molecule, and then to look for reactivity of human sera from patients infected with HSV-2 with these parts of the molecule. This approach has previously been successful in the context of hepatitis B virus (Motti et al., 1994). The murine MAbs H5 and E5 were shown to define epitopes which lie outside the region generally thought of as gG2-specific. A motif similar to that found in the majority of the phage clones selected by H5 (LEHRSPAE) could be identified within the native gG2 sequence (Fig. 1), and a synthetic peptide containing this sequence from this region of gG2 (PT71), as well as peptides with the sequence of two phage clone inserts, could inhibit binding of H5 to gG2. The insert of one phage clone (2.4) recognized by H5 had a sequence apparently unrelated to that of the remaining clones, even though it was consistently positive in the ELISA with H5 but not with an irrelevant antibody, or with F11 or E5. This may, therefore, be a ‘mimotope’ which is able to mimic the shape and charge distribution of the native epitope (Geyseren et al., 1996). The motif common to phage clones selected by E5 (PPPEH, Fig. 1) is distinct from that of H5 but was also located within the native sequence of gG2 included in peptide PT71. Binding of E5 to gG2 was inhibited by PT71 and by two peptides with the sequence of phage clones selected by E5.

A third epitope defined by the mouse MAb B2, which was not used in biopanning, is also contained within this region defined by PT71 since B2 was reactive with membrane-bound overlapping peptides covering this region of gG2. Staining of this set of peptides with all three MAbs confirmed that the epitopes recognized by these MAbs are distinct since each showed a different pattern of reactivity. Interestingly, the epitope defined by MAb H7 and represented by peptide MB24 is located between amino acids 569 and 588 of gG2, approximately 20 amino acids further towards the C-terminal end of gG2. Biopanning with this MAb identified only two positive phage clones, making it impossible to identify a common motif (7.17 and 7.25, Fig. 1). Identification of such a match is made difficult by the fact that only a small proportion of the amino acids within the phage insert are likely to be contributing to antibody-binding, and these may not be adjacent within the insert sequence. However, both phage clones, and a peptide with the sequence of the insert of one of these phage clones, was able to inhibit binding of H7 to gG2, suggesting that, as with clone 2.4, these two phage clone inserts represent mimotopes of the H7 epitope. The absence of staining of any of the other peptides within the series of overlapping membrane-bound peptides (Table 1) suggests that important amino acids contributing to this epitope lie at the C-terminal end of peptide MB24.

Thus, four of the five MAb-defined epitopes lie between amino acids 551 and 588 of gG2. This region lies at the junction between the ‘unique’ region of gG2 and its C-terminus, which has a relatively high degree of identity with gG1. Using the alignment constructed by McGeoch et al. (1987), this region has 42% identity with gG1, whilst PT71 has 50% identity. The fifth MAb, F11, recognizes an epitope lying within the ‘unique’ region of gG2 which is missing in gG1 (McGeoch et al., 1987). A short motif (TPL) was found to be common to phage clones selected by this MAb (Fig. 1) and a region of gG2, amino acids 359–378, containing this motif (PT173), as well as two peptides with the sequence of phage clones selected by F11, inhibited binding of F11 to gG2.

These epitopes are defined by murine MAbs. In order to determine whether these epitopes are also antigenic in humans infected with HSV-2, it was necessary to bind the peptide mimics to the solid phase in an ELISA. However, whilst the majority of the soluble peptides tested were able to inhibit binding of their associated MAbs to gG2, only a subset of these peptides (PT71, Ch16687 and PT173) retained reactivity with their cognate MAb when bound to the solid phase. Presumably, in solution, the peptides are free to adopt an appropriate conformation which will allow reactivity with the MAb but when bound to the solid phase, their conformation is restricted and the epitope may be lost.

When tested for their utility in detecting anti-HSV-2-positive human sera, using a panel of WB-characterized sera, PT71 showed the highest sensitivity (77%) and specificity (98%) when a cutoff, based on the mean plus three standard deviations of the reactivities of WB anti-HSV-2-negative sera, was used (Fig. 4 and Table 1). This peptide clearly contains human type-specific epitopes, despite having some identity with the equivalent region of HSV-1. It has previously been demonstrated in the context of HSV-1 gC that type-specific epitopes may be located within type-common regions of a molecule (Dolter et al., 1992). PT173 was reactive with a subset of the sera reactive with PT71.

These results demonstrate that human antibodies do recognize the same regions of the molecule as murine antibodies. However, it seems likely that the specific epitopes recognized by the two species within these regions are different. Ch16687, which, like PT173, contains the epitope defined by the MAb F11, was able to detect very few of the anti-HSV-2 WB-positive sera. In addition, further localization of the human epitopes by staining a series of overlapping peptides covering this region of gG2 suggests that the human
sera are recognizing different epitopes from the MAbs. All of the WB anti-HSV-2-positive sera tested reacted with some or all of the series of peptides, with a tendency towards more frequent recognition of the peptides with sequence derived from nearer the C terminus of the protein, whereas the reactivity of the MAbs was with the peptides closer to the N terminus. The reactivity of the sera with multiple overlapping peptides within this region implies that there are many overlapping human epitopes within this region. This analysis does not allow these epitopes to be defined more precisely.

The second approach to identification of epitopes within gG2 was by use of overlapping membrane-bound peptides (Fig. 6). Eight out of 10 of the positive human sera tested were reactive with at least one of the peptides which covered the 350–427 region of gG2 and nine out of 10 were reactive with the 525–587 region. None of the negative sera were reactive with any of these peptides. The single peptide BL181(39) which most closely resembles PT71 was stained by nine out of 10 of the positive sera and zero out of three of the negative sera tested in this format, thus confirming the immunodominance and type-specificity of the PT71 region of gG2.

Thus, the two antigenic regions identified by this approach were the same as those identified by our first strategy. The three-dimensional structure of gG2 has not yet been elucidated, but this result suggests that these areas of the primary sequence are exposed on the surface of the molecule and may be within regions of local mobility in the protein, allowing induced fit between the epitopes and their complementary paratopes (Van Regenmortel, 1989).

Pepscanning has been used previously to investigate human epitopes within gG2 (Levi et al., 1996). Peptides located at the C-terminal end of the molecule were recognized by human sera in a type-specific manner. The sequences of those peptides are equivalent to BL181(46–48) in this study, which were only infrequently recognized by WB anti-HSV-2-positive sera and were also stained by some negative sera. Differences between the two studies may arise from the assay format used for pepscanning (soluble versus membrane-bound) or from the size of the peptides used. A peptide equivalent to PT71 was not used – the closest one lacked the C-terminal amino acids of PT71. Our own data (Fig. 5) demonstrate the importance of this region in binding human sera. Recent reports by two other groups, using only the technique of pepscanning, have also identified an immunodominant region within gG2 overlapping PT71 (Liljeqvist et al., 1998; Marsden et al., 1998).

Five of six WB anti-HSV-2-positive, PT71 ELISA-negative sera recognized the membrane-bound peptide BL181(39), which contains all but the N-terminal three of the amino acids of PT71. The increased sensitivity of the membrane-bound format may be because of the use of a chemiluminescent detection system or through differences in antigen-presentation (membrane versus microtitre well). Whatever the reason, these encouraging results suggest that it should be possible to design a PT71-based assay with high sensitivity and specificity for the routine detection of anti-HSV-2 type-specific antibodies.

Identification of the type-specific domains of gG2 described in this paper raises the possibility of generating a peptide-based assay for the detection of HSV-2 type-specific antibody in human sera that may find wide clinical application in the identification of asymptomatic carriers of HSV-2, and thus, in prevention of transmission of the virus, and may also inform studies of the three-dimensional structure of this glycoprotein.

This work was supported by grants from the Wellcome Trust and Peptide Therapeutics Ltd, UK.

References


Received 4 November 1998; Accepted 24 March 1999