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Peptide Sequences of Glycoprotein G-2 Discriminate between Herpes Simplex Virus Type 2 (HSV-2) and HSV-1 Antibodies

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The complete herpes simplex virus type 2 envelope glycoprotein G was represented by overlapping synthetic peptides. Herpes simplex virus type 2-specific human seroreactivities were mainly seen against three peptides, peptides G2-64, G2-69, and G2-70, located in the C-terminal part of glycoprotein G. This is, interestingly, a region which has strong homology between herpes simplex virus types 1 and 2. G2-69 was the most herpes simplex virus type 2-specific peptide, reacting with 93% (13 of 14) of herpes simplex virus type 2 immunoglobulin G-positive human serum samples ($n = 14$) and none of the type 2 negative serum samples ($n = 16$) tested. The epitope of peptide G2-69 was mapped to the amino acid sequence RYAHPS, and the antibody binding to G2-64 could be increased by chemical oxidation of the peptide. The three reactive peptides were used, alone or combined, together with herpes simplex virus type 2 antigen in an attempt to discriminate anti-herpes simplex virus type 2 from anti-herpes simplex virus type 1 immunoglobulin G. Inclusion of the herpes simplex virus type 2 glycoprotein G peptides increased the specificity compared with the use of glycoprotein G alone. Combinations of peptides with whole glycoprotein also showed a greater discriminative capacity than single peptides. We conclude that these synthetic glycoprotein G peptides may be useful for herpes simplex virus type 2 serology based on peptides or combinations of peptides and antigens. This is the first study describing the possibility of discriminative herpes simplex virus serology by using synthetic peptides combined with small amounts of whole glycoprotein G.

The two closely related herpes simplex viruses (HSVs), types 1 (HSV-1) and 2 (HSV-2), are usually acquired early (HSV-1) or later (HSV-2) in life. A primary HSV infection is followed by an established latent infection. During the latent period, recurrent infections may occur. Apart from the orofacial lesions characteristic of HSV-1 infection and the genital blisters characteristic of HSV-2 infection, both primary and reactivated HSV infections may cause severe and sometimes fatal encephalitis and meningitis (11). Severe, generalized infection is particularly seen in neonates and immunocompromised patients. It is therefore important to distinguish HSV-2 from HSV-1 infections in order to optimize treatment of the disease. Identification of asymptomatic HSV-2 infections can prevent transmission to sexual partners and neonates. Diagnostic methods include virus isolation and antigen and DNA detection. Detection of antibodies in the sera of HSV-infected patients is simple, but distinguishing between antibodies against HSV-1 and HSV-2 is more difficult because of cross-reactivity (3). The analysis of type-specific virus proteins or peptides should facilitate type-specific serology (5, 10, 12).

Analysis of HSV-1 and HSV-2 DNAs shows a close relation between the two serotypes, and an overall homology of 50% is found. HSV virions carry seven glycoproteins, glycoproteins B, C, D, E, G, H, and I. Previous studies have indicated that HSV-2 glycoprotein G (gG-2) is type specific (1, 10). Its US4 gene is located in the short unique U_S region of HSV-2. gG-2 and its corresponding HSV-1 protein, gG-1, show limited similarities in the N-terminal region but are clearly homologous in the C-terminal region. In between these regions there is no similarity, and the gG-2 protein is about 460 amino acids longer than gG-1 (6). Different diagnostic seroassays have been developed with purified gG-2 protein (3). In the study described here, we used synthetic peptides for epitope mapping of the gG-2 protein in order to investigate the possibility of improving the sensitivity and specificity of HSV-2 serology.

TABLE 1. Synthetic overlapping peptides used for epitope mapping

Short peptide	Original peptide	Amino acids
	G2-59	576–590
SP1		623–632
SP2		625–634
	G2-64	626–640
SP3		627–636
SP4		629–638
SP5		631–640
SP6		633–642
	G2-68	666–680
SP7		673–682
SP8		675–684
	G2-69	676–690
SP9		677–686
SP10		679–688
SP11		681–690
SP12		683–692
SP13		685–694
	G2-70	686–699
SP14		687–696
SP15		689–698
SP16		691–700

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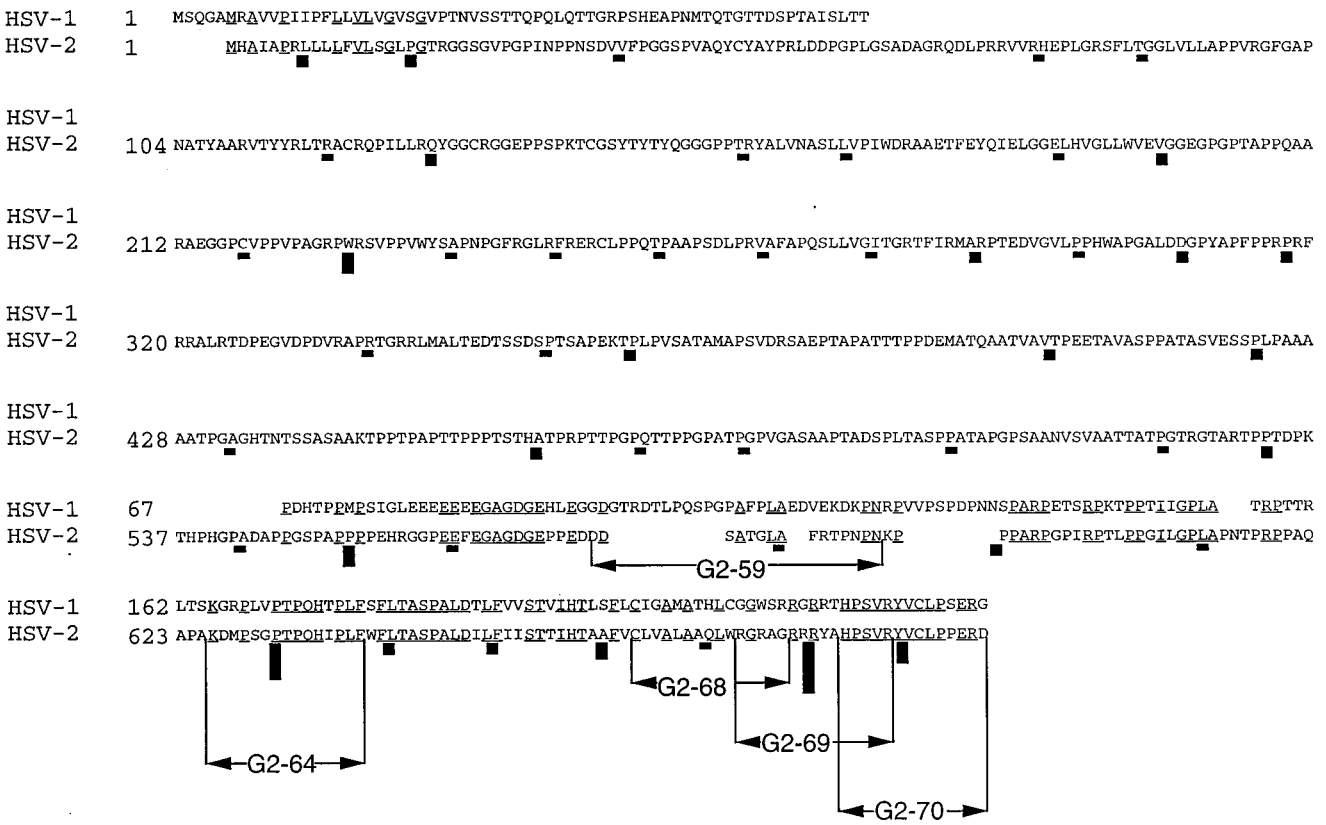

FIG. 1. Sequences of HSV-1 and HSV-2 gG proteins. Human seroreactivities to different regions are indicated by black bars. A tall bar corresponds to a large number of HSV-2-positive serum samples reactive with a certain sequence, as seen for the regions represented by peptides G2-64, G2-69, and G2-70. We showed that mouse and rabbit B epitopes are located in regions represented by peptides G2-59 and G2-68/G2-69, respectively. HSV-1 and HSV-2 sequence homologies are underlined.

MATERIALS AND METHODS

Antigens and peptides. HSV-1, isolated from a patient, was used to infect baby hamster kidney-21 cells. After cultivation, the virus was characterized by immunofluorescence with monoclonal antibodies (MAbs) specific for HSV-1 (Syva Co., Palo Alto, Calif.). For the production of HSV-2, baby hamster kidney-21 cells were infected with virus strain F95760, which was characterized with MAbs specific for HSV-2 (Syva). Specific virus glycoproteins, glycoprotein C (gC; HSV-1) and glycoprotein G (gG; HSV-2), were purified from extracts of the infected baby hamster kidney-21 cells by affinity chromatography with *Helix pomatia* lectin coupled to Sepharose 6MB (Pharmacia, Uppsala, Sweden) as described previously (8, 9). The glycoproteins bound to the lectin and were eluted with *N*-acetylgalactosamine. They were used as antigens in enzyme immunoassays (EIAs) to identify HSV-1 and HSV-2 antibodies.

Synthetic peptides, covering the HSV-2 protein gG-2, were produced in the multiple peptide synthesizer SyRo (Multisyntech, Bochum, Germany) by using Fmoc methodology. Amino acids (Millipore, Sundbyberg, Sweden) were activated with diisopropylcarbodiimide (Sigma, St. Louis, Mo.) in the presence of hydroxybenzotriazole (Novabiochem, Läufelfingen, Switzerland). The peptides were 15 amino acids long, with 5 overlapping amino acids. Some additional 10-amino-acid peptides with an 8-amino-acid overlap were synthesized for further analysis of reactivities (Table 1). Cyclization of some peptides was performed by inducing disulfide bridges between cysteines oxidized with iodine. Terminal cysteines, not present in the natural sequence, were introduced when necessary in order to allow the cyclization.

Sera. A total of 143 HSV-2 immunoglobulin G (IgG)-positive and -negative human serum samples obtained from Swedish or Colombian patients or laboratory personnel were used (7). All sera had previously been screened for HSV IgG antibodies by using purified HSV antigens in an EIA (7) and Western blot (immunoblot) (1) analysis (see Table 2).

Rabbit anti-HSV-1 antibodies and anti-HSV-2 antibodies (DAKO, Glostrup, Denmark), diluted 1/20 to 1/500, were tested for their reactivities with nine of the C-terminal gG-2 peptides in an EIA. The rabbit antibodies react with both type-specific antigens and antigens common to HSV-1 and HSV-2. The antibodies react with all of the major glycoproteins present in the viral envelope and at least one core protein, as determined by crossed immunoelectrophoresis.

Mouse MAbs (Advanced Biotechnologies Inc., Columbia, Md.) specific for HSV-1 gG-1 and HSV-2 gG-2 proteins were tested against all gG-2 peptides and were used to characterize our purified gG-2 antigen coated onto EIA wells.

EIA. Human sera were tested for their reactivities with HSV-2 peptides and antigen in EIAs. A total of 10 μg of gG-2 peptides per ml was coated onto 96-well microtiter plates (Nunc, Odense, Denmark) in 0.05 M sodium carbonate buffer (pH 9.5) at room temperature overnight. The plates were stored for at least 24 h at 8°C until they were used. After washing, the plates were dried overnight at 37°C. Sera, diluted 10^{-2} in phosphate-buffered saline with 0.5% bovine serum albumin–0.05% Tween 20–0.01% Merthiolate and 2% goat serum, were then added to the plates, and the plates were incubated for 2 h at 37°C. After washing, alkaline phosphatase-conjugated goat anti-human IgG (Sigma) was added, and the plates were incubated for 2 h at 37°C. Finally, the substrate *p*-nitrophenyl phosphate (Sigma) was added, the plates were incubated for 30 min at 37°C, and the reaction was stopped by adding 1 M NaOH. The A_{405} was measured. Cutoff absorbance values were calculated as the mean for the HSV-2-negative sera plus two standard deviations. Alkaline phosphatase-conjugated goat anti-rabbit IgG (Southern Biotechnology Associates Inc., Birmingham, Ala.) was used as the conjugate for rabbit antibodies, and horseradish peroxidase-conjugated goat anti-mouse IgG (DAKO) was used as the conjugate for the mouse MAb.

Before running an EIA including HSV-2 antigen, each new batch of antigen was titrated to determine the amount necessary to coat the EIA plates. The incubation times for the sera and the conjugates were 1 h each. The remaining parameters were the same as those for the peptide EIA described above.

Peptide inhibition. For mapping of epitopes, a modified peptide inhibition EIA was performed. Three reactive HSV-2 peptides were first coated onto EIA plates. HSV-2-positive sera were added to the plates together with 100 μg of HSV-2 peptides per ml; the peptide was either the same as the coated peptide, a different peptide, or buffer. The EIA was then performed as described above. The addition of a reactive peptide in solution reduced the reactivity of the EIA because of competition with the coated peptide for specific serum antibodies.

RESULTS

Unique HSV-2 peptides. Human HSV-2 IgG-positive and -negative sera were tested against 70 peptides derived from the

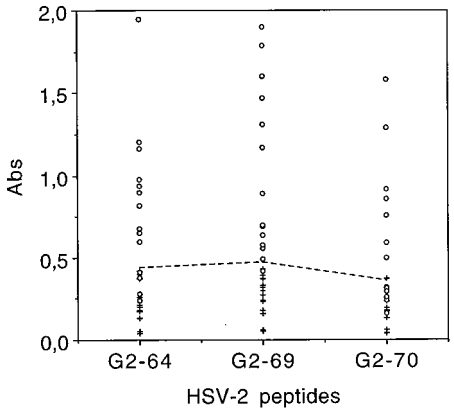

FIG. 2. Reactivities of 14 HSV-2 IgG-positive (circles) and 16 HSV-2 IgG-negative (plus signs) human serum samples with three synthetic peptides derived from the C-terminal part of the HSV-2 protein gG-2. The dotted line shows the cutoff, which is the mean for negative sera plus two standard deviations. Abs, absorbance.

HSV-2 protein gG-2. Specific seroreactivities in the EIA were mainly seen against three peptides, peptides G2-64, G2-69, and G2-70, located in the C-terminal end of gG-2 (Fig. 1). The most reactive peptide was G2-69, with which 13 of 14 (93%) of HSV-2 antibody-positive serum samples reacted (Fig. 2).

A more accurate epitope analysis of the three peptides was performed by using shorter overlapping peptides in an inhibition EIA (Fig. 3). Peptides SP10 to SP12, which all have the common sequence RYAHPS, inhibited HSV-2-positive sera from binding to coated G2-69. As a control, the peptide G2-69 in solution inhibited the binding of HSV-2-positive sera to immobilized G2-69 peptide. Figure 3 thus demonstrates that a short peptide sequence, RYAHPS, may be the common epitope for many HSV-2-positive sera.

In other experiments, inhibition of binding to coated G2-64 or G2-70 peptide was seen only with a few of the reactive sera. Inhibition of serum reactivities with coated G2-64 was seen with peptides SP4 and SP5, while peptide SP16, located at the very C terminus of gG-2, inhibited sera from binding to coated G2-70 (data not shown).

Since the sequences of the three reactive peptides, G2-64, G2-69, and G2-70, are rather homologous with the HSV-1

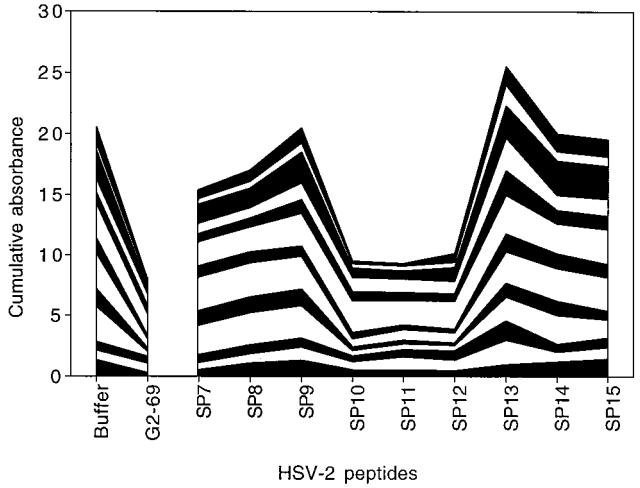

FIG. 3. Inhibition of binding of HSV-2 IgG-positive human sera (n = 13) to coated peptide G2-69 by 100 μg of gG-2 peptides per ml in solution.

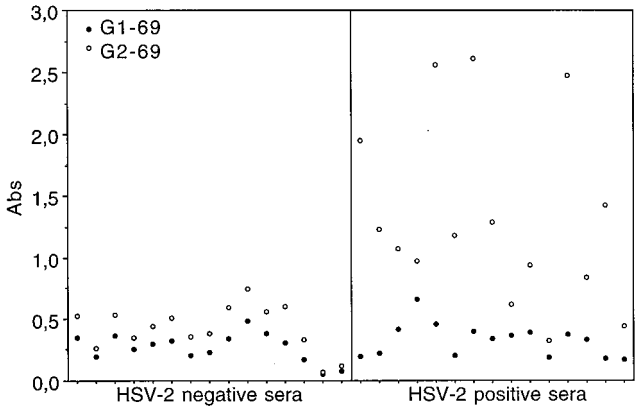

FIG. 4. Seroreactivity of HSV-2 IgG-negative (n = 15) and -positive (n = 15) human sera with the HSV-2 gG peptide G2-69 and the analogous HSV-1 gG peptide G1-69. Abs, absorbance.

C-terminal sequence (Fig. 1), the corresponding HSV-1 peptide analogs were compared for their seroreactivities. HSV-2-positive sera reacted more strongly with gG-2 peptides than with the analogous gG-1 peptides, as seen for the G2-69 epitope in Fig. 4. No significant reactivity was seen against either the gG-1 or the gG-2 peptide with HSV-1-positive sera.

Cyclic peptide analogs of the three reactive epitopes were synthesized in an attempt to improve the conformation of the epitope. The linear peptides were included in the same synthesis as the cyclic ones for accurate comparisons. Cyclization clearly improved the binding of HSV-2-positive sera with the G2-64 epitope (Fig. 5), while no significant effect was seen with the cyclic G2-69 peptide or the cyclic G2-70 peptide (data not shown).

To further evaluate the antigenicity of the C-terminal gG-2 region, we analyzed rabbit anti-HSV-1 and anti-HSV-2 antibodies for binding to all peptides between G2-62 and G2-70. Rabbit anti-HSV-2 antibodies specifically reacted with peptides G2-68 and G2-69 (data not shown). This reactivity was similar to the reactivity of human anti-HSV-2 sera (Fig. 1). The rabbit anti-HSV-1 antibodies did not react as strongly with the gG-2 peptides as the anti-HSV-2 antibodies did.

To characterize our HSV-2 antigen, mouse anti-gG-1 and anti-gG-2 MAbs were reacted with gG-2 peptides and antigen in the EIA. The epitope of the anti-gG-2 MAb was mapped to peptide G2-59 (Fig. 1), while the anti-gG-1 MAb did not bind

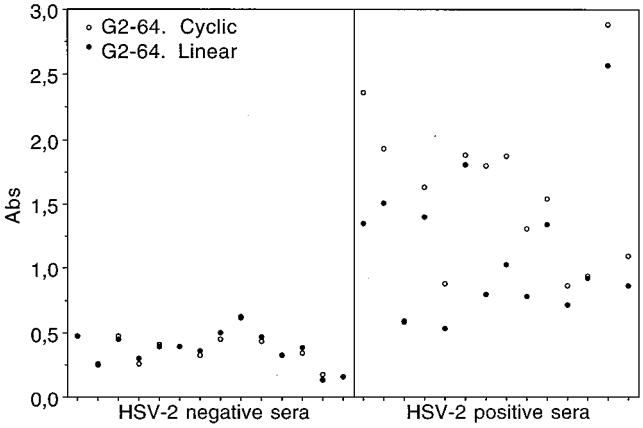

FIG. 5. Reactivities of HSV-2 IgG negative (n = 14) and positive (n = 14) human sera with cyclic and linear G2-64 peptides. Abs, absorbance.

TABLE 2. Sensitivity and specificity for HSV-2 serology of human sera with gG-2 antigen and peptides

No. of serum samples tested	HSV-1/HSV-2 seroreactivity[a]	gG-2 antigen[b]		Peptides G2-64, G2-69, and G2-70		Antigen + peptides[c]	
		No. of serum samples detected	Sensitivity or specificity (%)[d]	No. of serum samples detected	Sensitivity or specificity (%)	No. of serum samples detected	Sensitivity or specificity (%)
6	−/+	6	100 (se)	6	100 (se)	6	100 (se)
24	+/+	24	100 (se)	23	96 (se)	23	96 (se)
63	+/−	3	95 (sp)	2	97 (sp)	1	98 (sp)
50	−/−	0	100 (sp)	0	100 (sp)	0	100 (sp)

[a] Determined by EIA and Western blotting.
[b] High antigen concentration.
[c] Low antigen concentration.
[d] se, sensitivity; sp, specificity.

to any HSV-2 gG peptide (data not shown). The anti-gG-2 MAb specifically reacted with our purified HSV-2 antigen, while the anti-gG-1 MAb was unreactive, verifying the antigenicity of gG-2 in EIA (data not shown).

Antigen-peptide combinations. In attempts to further increase the sensitivity and specificity of the EIA, HSV-2 antigen was used together with different combinations of the reactive peptides, peptides G2-64, G2-69, and G2-70, to coat the EIA plates and for subsequent testing of sera. In total, 143 HSV-2-negative and -positive human serum samples were tested (Table 2). The inclusion of peptides increased both the specificity and the discriminative capacity compared with those obtained with antigen alone (Table 2; Fig. 6). The use of peptide combinations appeared to be more beneficial compared with the use of peptide G2-69 alone. Still higher concentrations of coated peptide did not increase the absorbance signals or the sensitivity.

DISCUSSION

The gG-2 protein of HSV-2, encoded by the US4 gene, is a protein that is larger than the equivalent gG-1 protein of HSV-1 (6). This protein was selected for use in the identification of HSV-2-specific epitopes. We identified three synthetic peptides, peptides G2-64, G2-69, and G2-70, derived from the C-terminal part of gG-2, with which HSV-2 antibody-positive sera specifically reacted. The gG-1 and gG-2 proteins are homologous at the N- and C-terminal ends. Consequently, our three reactive peptides have some homology with HSV-1, especially G2-70, which differs by only two amino acids. G2-69 was the most effective peptide, showing a sensitivity of 93%. Three gG-1 peptides analogous to G2-64, G2-69, and G2-70 were tested in the EIA. Most of the HSV-2-positive sera showed higher reactivities with the gG-2 peptides than with the gG-1 peptide analogs, despite the high degree of homology between these peptides. The HSV-1-positive, HSV-2-negative sera did not show any significant reactivities with the three gG-1 peptides, indicating the absence of HSV-1 epitopes within these regions. This may depend on the difference in size between gG-1 and gG-2, which may lead to different glycosylation and folding patterns by the proteins. Thus, the cross-reactivity of HSV-1-specific antibodies which is seen when whole HSV-2 glycoprotein is used may be avoided by using these peptides.

A peptide inhibition test was performed for further mapping of the gG-2 epitopes. The epitope of peptide G2-69 was shown to mainly consist of RYAHPS. The epitope of peptide G2-64 was located within the sequence GPTPQHIP, which is the central part of the G2-64 peptide. The mapping of the G2-70 region showed a motif of YVCLPPERD, which is the very C-terminal sequence. Mapping of the G2-64 and G2-70 epitopes was not as successful as mapping of the G2-69 epitope, since poor or no inhibition of sera was seen even when the original peptides G2-64 and G2-70 were used (data not shown). The poor inhibition may indicate either that reactive antibodies are of low affinity or that the coated peptide is stabilized in a spatial structure more favorable than the peptide in solution can adopt. It is not clear whether G2-69 and G2-70 represent two separate linear epitopes or parts of the same discontinuous epitope. The latter possibility is supported by the presence of several prolines, which are known to form hairpin turns in protein chains. Such turns could bring amino acids, which are far apart in the primary sequence, in close proximity to form a discontinuous epitope.

Cyclization of the G2-64 peptide improved its binding capacity with HSV-2-positive sera, while no effect was seen with HSV-2-negative sera. The cyclization may have stabilized some important loop conformation, since the sequence contains several prolines. It may also be possible to increase the binding capacities of the peptides by introducing D-isomeric amino acids or amino acids not present in the natural sequence.

Specific reactivities of anti-HSV-2 rabbit antibodies were seen against peptides G2-68 and G2-69. Thus, the rabbit antibodies reacted with one of the three sites detected by human sera. This region has previously been used to produce antipeptide rabbit antisera (6).

A mouse anti-gG-2 MAb showed reactivity with peptide

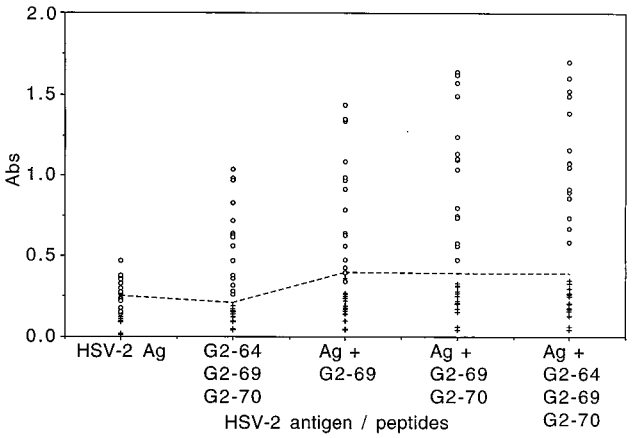

FIG. 6. Reactivities of 15 HSV-2 IgG-positive (circles) and 15 HSV-2 IgG-negative (plus signs) human serum samples with antigen (low concentration), gG-2 peptides or combinations of antigen (low concentration), and peptides. The total peptide concentration used to coat the EIA plates was 10 μg/ml. The dotted line shows the cutoff, which is the mean for negative sera plus two standard deviations. Abs, absorbance.

G2-59. Apparently, the mouse antibody response against gG-2 differs from the responses seen in rabbits and humans.

The most reactive peptide, peptide G2-69, was used alone or in combination with the other reactive peptides, peptides G2-64 and G2-70, together with HSV-2 antigen, in attempts to improve HSV-2 IgG serology. All combinations increased the discriminative capacity. Combinations of peptide G2-69 with peptides G2-64 and G2-70 gave slightly higher signals than those obtained with peptide G2-69 alone. The combination of peptides with whole gG-2 antigen appeared to be superior to only peptides or only antigen. The reason for this additive effect may be that the three peptides bind antibodies better than the antigen, while the antigen catches the remaining gG-2-specific antibodies. It should be noted that the single HSV-2-positive serum sample that was not detected by gG-2 peptides (Table 2) was just barely detected by antigen. This reactivity may have depended on cross-reactive HSV-1 antibodies. In order to detect any effects exerted by the peptides, the concentration of glycoprotein was deliberately chosen to give a rather low absorbance.

We did not study the presence of IgM in the HSV IgG-positive sera. No patient had an acute or reactivated infection. Detection of specific IgM antibodies may be valuable for the diagnosis of acute primary HSV infections, but the levels of these antibodies decline during the convalescent phase. Additionally, type-specific IgM responses do not generally appear in patients with recurrent HSV-2 genital herpes, while IgG antibodies are induced against the gG-2 protein during both primary and recurrent infections (4).

HSV-2 vaccines are being tested, and promising results have been reported (13, 14). Antiviral compounds can be used with good efficacy for the treatment of HSV-induced diseases (2). However, it is important to distinguish HSV-2 infection from HSV-1 in order to optimize treatment of neonates and immunocompromised patients. Our results may be of importance in designing new HSV-2-specific IgG assays. Production and purification of native or recombinant HSV antigens are both expensive and time-consuming. Inclusion of peptides into such tests, and eventually even into vaccines, would then be beneficial, since lower amounts of antigen would be required. Modified peptides may also be more stable and of higher purity than native antigen. As far as we know, this is the first report describing the possible design of HSV serological test systems consisting of synthetic peptides or peptides combined with small amounts of native glycoprotein.

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REFERENCES

1. **Ashley, R. L., J. Militoni, F. Lee, A. Nahmias, and L. Corey.** 1988. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. J. Clin. Microbiol. **26:**662–667.
2. **De Clercq, E.** 1993. Antivirals for the treatment of herpesvirus infections. J. Antimicrob. Chemotherapy. **32**(Suppl. A)**:**121–132.
3. **Field, P. R., D. W. T. Ho, W. L. Irving, D. Isaacs, and A. L. Cunningham.** 1993. The reliability of serological tests for the diagnosis of genital herpes: a critique. Pathology **25:**175–179.
4. **Ho, D. W. T., P. R. Field, W. L. Irving, D. R. Packham, and A. L. Cunningham.** 1993. Detection of immunoglobulin M antibodies to glycoprotein G-2 by Western blot (immunoblot) for diagnosis of initial herpes simplex virus type 2 genital infections. J. Clin. Microbiol. **31:**3157–3164.
5. **Ho, D. W. T., P. R. Field, E. Sjögren-Jansson, S. Jeansson, and A. L. Cunningham.** 1992. Indirect ELISA for the detection of HSV-2 specific IgG and IgM antibodies with glycoprotein G (gG-2). J. Virol. Methods **36:**249–264.
6. **McGeoch, D. J., H. W. M. Moss, D. McNab, and M. C. Frame.** 1987. DNA sequence and genetic content of the HindIII1 region in the short unique component of the herpes simplex virus type 2 genome: identification of the gene encoding glycoprotein G, and evolutionary comparisons. J. Gen. Virol. **68:**19–38.
7. **Munoz, N., I. Kato, F. X. Bosch, S. De Sanjosé, V.-A. Sundquist, I. Izarzugaza, L. C. Gonzalez, L. Tafur, M. Gili, P. Viladiu, C. Navarro, P. Moreo, E. Guerrero, K. V. Shah, and B. Wahren.** 1995. Cervical cancer and herpes simplex virus type 2: case-control studies in Spain and Colombia with special reference to immunoglobulin-G subclasses. Int. J. Cancer **60:**438–442.
8. **Olofsson, S., S. Jeansson, and E. Lycke.** 1981. Unusual lectin-binding properties of a herpes simplex virus type 1-specific glycoprotein. J. Virol. **38:**564–570.
9. **Olofsson, S., M. Lundström, H. Marsden, S. Jeansson, and A. Vahlne.** 1986. Characterization of a herpes simplex virus type 2-specified glycoprotein with affinity for *N*-acetylgalactosamine-specific lectins and its identification as g92K or gG. J. Gen. Virol. **67:**737–744.
10. **Parkes, D. L., C. M. Smith, J. M. Rose, J. Brandis, and S. R. Coates.** 1991. Seroreactive recombinant herpes simplex virus type 2-specific glycoprotein G. J. Clin. Microbiol. **29:**778–781.
11. **Roizman, B., R. J. Whitley, and C. Lopez.** 1993. The human herpes viruses. Raven Press, New York.
12. **Sánchez-Martínez, D., and P. E. Pellett.** 1991. Expression of HSV-1 and HSV-2 glycoprotein G in insect cells by using a novel baculovirus expression vector. Virology **182:**229–238.
13. **Straus, S. E., L. Corey, R. L. Burke, B. Savarese, G. Barnum, P. R. Krause, R. G. Kost, J. L. Meier, R. Sekulovich, S. F. Adair, and C. L. Dekker.** 1994. Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 for immunotherapy of genital herpes. Lancet **343:**1460–1463.
14. **Straus, S. E., B. Savarese, M. Tigges, A. G. Freifeld, P. R. Krause, D. M. Margolis, J. L. Meier, D. P. Paar, S. F. Adair, D. Dina, C. Dekker, and R. L. Burke.** 1993. Induction and enhancement of immune responses to herpes simplex virus type 2 in humans by use of a recombinant glycoprotein D vaccine. J. Infect. Dis. **167:**1045–1052.