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A Subset of Type-specific Epitopes Map in the Amino Terminus of Herpes Simplex Virus Type 1 Glycoprotein B

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SUMMARY

We constructed a recombinant herpes simplex virus (HSV) containing the transcribed coding and non-coding sequences of HSV-1 strain F glycoprotein B (gB) gene, a γ_1 gene, fused to the promoter–regulatory sequences of the HSV-1 α4 gene and inserted into the thymidine kinase gene of RH1G44, an HSV-1 × HSV-2 recombinant that contains an HSV-2 gB gene at the natural locus. Phenotypic analyses of the insertion mutant, R3145, showed that the αgB gene was transcribed in the presence of cycloheximide but underwent partial conversion to the HSV-2 form. Nucleotide sequencing of the gene indicated that the 5′ crossover occurred between nucleotides 107 and 117 upstream from the translation initiation site and that the 3′ crossover occurred between the sequences specifying amino acids 402 and 412 of the HSV-1 gB. The chimeric protein consisted of an N-terminal 405 to 415 amino acids encoded by the HSV-2 gene and a C-terminal 462 to 472 amino acids encoded by the HSV-1 gene. Comparison of the reactivity of the parental and recombinant gB with type-specific monoclonal antibodies indicated that the chimeric gB lost reactivity with four HSV-1-specific antibodies but gained reactivity with three HSV-2-specific antibodies.

Herpes simplex virus type 1 (HSV-1) glycoprotein B (gB) is an essential gene product that was reported to promote fusion of the virion envelope with the cell membrane (Little *et al.*, 1981; Manservigi *et al.*, 1977; Ruyechan *et al.*, 1979; Sarmiento *et al.*, 1979). Previous reports have shown that HSV-1 (Bzik *et al.*, 1984; Pellett *et al.*, 1985) and HSV-2 (Bzik *et al.*, 1986; Stuve *et al.*, 1987) gB genes are homologous but diverge at the 5′ terminus. Insofar as HSV-1- and HSV-2-specific monoclonal antibodies to gB neutralize virus, each gene encodes both type-specific and type-common epitopes (Chapsal & Pereira, 1988; Pereira *et al.*, 1981, 1982). In the present study, we mapped type-specific epitopes on gB using a recombinant that contained a hybrid HSV-1 × HSV-2 gB inserted into the thymidine kinase (TK) locus and regulated as an α gene product.

The recombinant virus R3145 was constructed as illustrated in Fig. 1. Specifically, the 5′ transcribed coding and non-coding sequences of HSV-1 gB were fused to the promoter–regulatory sequences of the HSV-1 strain F α4 gene. This construct was flanked by the short *Bgl*II–*Bam*HI fragment and the larger *Sac*I–*Bam*HI fragment of the *Bam*HI Q fragment containing the TK gene. The construct was cotransfected on rabbit skin cells with intact DNA of RH1G44, a recombinant virus that contains an HSV-2 gB gene at the natural locus (Tognon *et al.*, 1981) and the progeny were plated on 143 TK^- cells in the presence of bromodeoxyuridine (3 μg/ml). Since replacement of the *Bgl*II–*Sac*I subfragment of the *Bam*HI Q fragment with the

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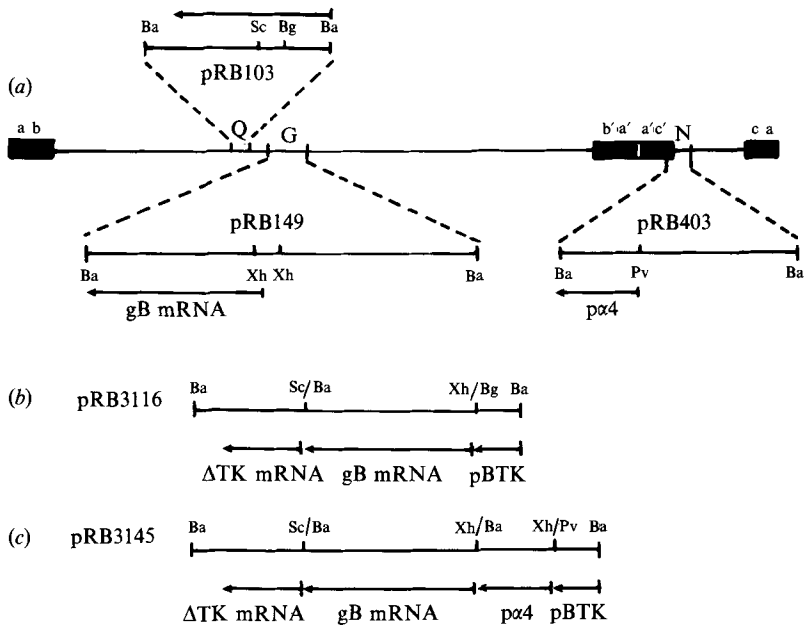

Fig. 1. Schematic diagram of the HSV-1 genome and location of the DNA fragments used in this study. The thin lines represent the unique sequences of the long and short components, whereas the filled rectangles represent the terminal sequences ab and ca internally repeated as the inverted sequences b'a'/a'c' (Roizman, 1979). Panel (*a*) also shows the expanded scales of the *Bam*HI restriction fragments Q, G and N which contain promoter–regulatory regions and coding sequences for the β_1 TK, γ_1 gB and α4 genes, respectively. The arrows indicate the direction of transcription. Plasmids pRB103, pRB149 and pRB403, which contain the cloned DNA sequences for these genes, have been described (Post *et al.*, 1980). (*b*) The construction of plasmid pRB3116 by subcloning the *Xho*I–*Bam*HI fragment from pRB149 into the *Bgl*II–*Sac*I sites of pRB103 such that the *Xho*I site was fused to the *Bgl*II site after T4 polymerase treatment, thus regenerating the *Xho*I site. ΔTK indicates the interruption of the TK gene. (*c*) The construction of plasmid pRB3145 by subcloning the *Bam*HI–*Pvu*II fragment from pRB403 which contains the α4 promoter–regulatory sequences into the *Xho*I site of pRB3116 in the proper transcriptional orientation. Restriction enzymes are abbreviated as follows: Ba, *Bam*HI; Sc, *Sac*I; Bg, *Bgl*II; Xh, *Xho*I; Pv, *Pvu*II.

αgB gene would interrupt the TK gene, TK$^-$ virus progeny were screened for the expression of HSV-1 gB using a biotin–avidin-enhanced surface immunoassay with HSV-1-specific antibody H1397 (Kousoulas *et al.*, 1984). Recombinant RH1G44, which specifies HSV-2 gB, reacts with HSV-2-specific H1360 antibody but not with HSV-1-specific H1397, whereas the HSV-1 αgB, which recombined into the RH1G44 genome, reacts with H1397 but not with H1360. A plaque-purified stock of the R3145 recombinant was prepared; this reacted with both H1397 and H1360 antibodies in the enhanced surface immunoassay. Restriction endonuclease digests verified the presence of the expected insert in the domain of the TK gene (data not shown). The initial stock of R3145 was expanded into a high-titre working stock for subsequent analysis of the chimeric gB gene product.

We next tested the effect of cycloheximide treatment of infected cells on the expression of the αgB chimeric gene in R3145. Cycloheximide (50 μg/ml) was added to cells 0·5 h before infection with R3145 and maintained in the medium for 6 h post-infection. The cells were then rinsed and replenished with medium containing [^{35}S]methionine (sp. act. > 400 Ci/mmol) and actinomycin D (10 μg/ml). Under these conditions the parental viruses RH1G44 and HSV-1 (F) failed to produce gB, which is the product of a γ_1 regulated gene. In contrast, R3145 virus expressed gB after cycloheximide reversal in the presence of actinomycin D, as would be predicted for a gene driven by an α gene promoter; however, the gB reacted with HSV-2-specific H1360 rather than HSV-1-specific H1397 antibody in immunoprecipitation tests, as we had expected (data not

Table 1. *Neutralization* by monoclonal antibodies in plaque reduction assays with parental and recombinant HSV strains*

	Monoclonal antibody			
	Type-common			
Virus	H126	H233	HSV-1-specific H1397	HSV-2-specific H1360
HSV-1 (F)	99†	100	100	0
HSV-2 (G)	99	97	0	100
RH1G44‡	100	99	0	100
R3145	100	100	0	100

* For neutralization tests, 200 to 300 p.f.u. of virus was reacted with 10 μl of mouse ascites fluid containing monoclonal antibodies for 2 h at room temperature, then plated on Vero cells (Kousoulas *et al.*, 1984).

† Numbers denote the percentage neutralization relative to antibody-negative control ascites. The data shown are the means of three separate tests.

‡ The properties of RH1G44 have been published (Tognon *et al.*, 1981).

shown). Analysis of the phenotype of R3145 by neutralization assays with these antibodies supported the cycloheximide reversal studies (Table 1). Like the RH1G44 parent virus, R3145 was neutralized only by HSV-2-specific H1360 antibody and not by H1397, indicating that both copies of gB expressed the type 2- but not the type 1-specific epitope. Subsequent analysis of the high-titre stock of recombinant R3145 revealed that it was uniformly negative in the enhanced surface immunoassay with type 1-specific H1397 antibody. One explanation for the observed reactivity of the product of the αgB gene is that it had acquired at least a portion of the coding domains of the HSV-2 gB gene by gene conversion.

In gene conversion, a portion or all of the sequences of a gene are replaced by a homologue of the gene. To determine whether HSV-1 gB DNA sequences had been replaced with HSV-2 DNA sequences, we used a panel of type-specific antibodies to gB to test the reactivity of the product of the αgB chimeric gene in R3145 after cycloheximide reversal. Representative immunoprecipitates formed by antibodies to gB in reactions with R3145 are shown in Fig. 2. Under the conditions of cycloheximide reversal (described above), we did not detect any leakage of the native γ_1-regulated gB and only the chimeric αgB was expressed. Results of these experiments, summarized in Table 2, indicate that the chimeric protein specified by the converted αgB gene lost reactivity with four HSV-1-specific antibodies (H1397, H1392, H1396 and H1839) and gained reactivity with three HSV-2-specific antibodies (H368, H357 and H1360). The observation that the chimeric αgB retained reactivity with a subset of HSV-1-specific antibodies indicated that only a portion of the DNA sequences were replaced.

The next step was to fine map the crossover sites by nucleotide sequence analysis of the R3145 αgB chimeric gene. Fig. 3 summarizes these results and shows the 5′ and 3′ crossover sites in the chimeric gB gene. In the 5′ domain of the αgB gene, the nucleotide sequence corresponds to that of HSV-1 gB until the sequence CGGTCGTGGT, which is shared by both HSV-1 and HSV-2 gB and shown by large bold letters in row R. The recombinant nucleotide sequence 3′ to this sequence corresponds to that of the HSV-2 gB. The crossover site is therefore in that sequence, i.e. within the 5′ transcribed, non-coding sequence, between nucleotides 107 and 117 5′ to the translation initiation site of the gB gene. It is noteworthy that the HSV-2 gB lacks several of the amino acids in the signal sequence, an observation also reported by others (Bzik *et al.*, 1986; Stuve *et al.*, 1987). The 3′ crossover site was mapped to the sequence ACGCACATCAAGGTGGGCCAGCCGCAGTACTAC, shown by large bold letters in row R and shared by both HSV-1 and HSV-2 gB. The recombinant virus sequences upstream from that sequence correspond to HSV-2 gB, whereas the sequences downstream correspond to HSV-1 gB. The crossover site is therefore between amino acids 402 and 412 of the HSV-1 gB. From comparisons of the amino acid sequence of HSV-1 (Bzik *et al.*, 1984; Pellett *et al.*, 1985) and HSV-2 gB (Bzik *et al.*, 1986), we may deduce that the αgB chimeric gene in R3145 consists of an amino-terminal portion of 405 to 415 amino acids encoded by HSV-2 gB and a carboxy-

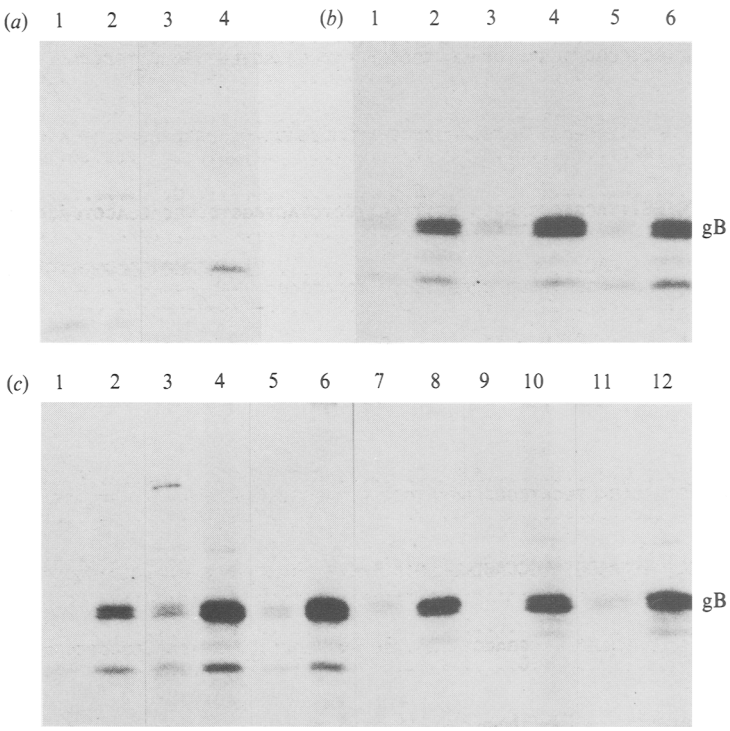

Fig. 2. Autoradiographs of electrophoretically separated polypeptides in precipitates obtained with monoclonal antibodies reacted with recombinant R3145. Extracts of R3145-infected HEp-2 cells were untreated (odd-numbered lanes) or treated (even-numbered lanes) with 50 μg/ml of cycloheximide, then labelled with [^{35}S]methionine in medium containing 1/10 the normal concentration of methionine and actinomycin D (10 μg/ml). Cells were extracted in phosphate-buffered saline lacking Mg^{2+} and Ca^{2+} and containing 1% Nonidet P40, 1% sodium deoxycholate and 0·01 mM each of TLCK and TPCK. Immune complexes were formed by mixing clarified extracts with Protein A–Sepharose beads coated with anti-mouse antisera followed by 10 μl of monoclonal antibody in the form of mouse ascites. The bound complexes were washed extensively with extraction buffer and electrophoresed in denaturing 9·25% polyacrylamide gels cross-linked with *NN'*-diallyltartardiamide. (*a*) Selected HSV-1-specific antibodies non-reactive with R3145 αgB (lanes 1 and 2, H1839; lanes 3 and 4, H1397); (*b*, *c*) HSV-2-specific (lanes 1 and 2, H1360; lanes 3 and 4, H368; lanes 5 and 6, H357) and HSV-1-specific (lanes 1 and 2, H1382; lanes 3 and 4, H1393; lanes 5 and 6, H1399; lanes 7 and 8, H1757; lanes 9 and 10, H1830; lanes 11 and 12, H1828) antibodies reactive with R3145 αgB, respectively. Relevant properties of most of these antibodies are listed in Table 2 and were reported previously (Chapsal & Pereira, 1988). The position of gB is indicated on the right.

terminal domain of 462 to 472 amino acids encoded by HSV-1 gB. In all of these calculations, the signal sequences (−29 to −1 for HSV-1 gB and −22 to −1 for HSV-2 gB) are not included in the numbering of HSV-1 and HSV-2 domains.

Of special interest is the observation that even though the recombinant virus was initially selected and plaque-purified on the basis of its reactivity with an HSV-1-specific antibody, the working virus stocks consisted of a recombinant virus population carrying an αgB chimeric gene that had undergone gene conversion. Gene conversion has been recorded in at least one other instance in which viruses carrying duplications of genes of different serotypes have been constructed (Pogue-Geile & Spear, 1986), and it may be assumed that this phenomenon is a possibility in all instances in which related but non-identical homologues are introduced into the same genome. Since it is expected that all populations of viruses containing non-identical homologues contain recombinant or converted genes, the question arises as to why the R3145 virus, carrying the αgB chimeric gene, underwent gene conversion. One possible explanation

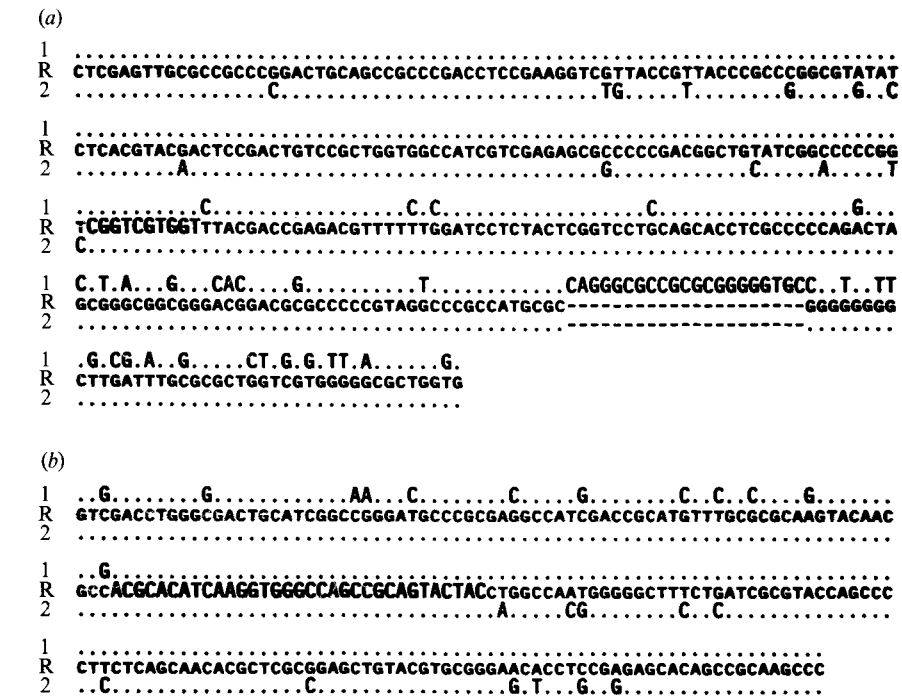

Fig. 3. Summary of the results of the nucleotide sequence analyses of the αgB gene showing the regions that contain the 5′ (*a*) and 3′ (*b*) crossover sites in recombinant R3145. Nucleotide sequences shown in the middle row (R) belong to R3145, upper row (1) to HSV-1, and lower row (2) to HSV-2. Dots indicate identity of nucleotide sequences. Dashes indicate the absence of corresponding nucleotide sequences of the HSV-1 gB in either the recombinant or the HSV-2 gB sequence. Letters in rows 1 or 2 indicate differences in the nucleotide sequence of HSV-1 or HSV-2, respectively, from that of the recombinant sequence. The crossover sites in the sequence shared by both HSV-1 and HSV-2 gB are shown by bold, enlarged letters. The 5′ crossover site is between nucleotides 107 and 117 relative to the translation initiation site, and the 3′ crossover site is between nucleotides 1206 and 1236 of the HSV-1 gB coding sequence.

Table 2. *Immunoprecipitation of type-specific monoclonal antibodies to gB with parental and recombinant HSV strains*

Monoclonal antibody	Virus tested			
	HSV-1 (F)	HSV-2 (G)	RH1G44	R3145
HSV-2-specific				
H368	−	+	+	+
H357	−	+	+	+
H1360	−	+	+	+
HSV-1-specific				
H1397	+	−	−	−
H1396	+	−	−	−
H1392	+	−	−	−
H1839	+	−	−	−
H1382	+	−	−	+
H1393	+	−	−	+
H1757	+	−	−	+
H1830	+	−	−	+

involves the relative functionality of the gene product. Recombinant viruses with heterologous gB genes are viable and replicate well, as reflected by the parental RH1G44 virus. It is conceivable that because gB molecules form dimers (Sarmiento & Spear, 1979), identical extracellular domains would yield a more stable structure or allow the virus to spread more efficiently from cell to cell. This hypothesis remains to be tested.

It is obvious that no single approach to mapping epitopes is likely to yield a complete map of all of the antigenic sites in the native conformation of a given protein. Epitopes dependent on primary sequence can be readily mapped with truncated proteins or with synthetic peptides; discontinuous epitopes dependent on secondary structure are more difficult to map, because mutations at distal sites may influence the folding and hence the ability of the protein to react with a specific antibody. The recombinant αgB protein consists of quasi-equal domains of HSV-1- and HSV-2-specific sequences and therefore is likely to conserve epitopes dependent on primary sequence. Because these domains are quite large, it is likely that at least some epitopes dependent on secondary structure would also be conserved. Indeed, results of studies that mapped epitopes by analyses of antibody-resistant mutants (Kousoulas *et al.*, 1984, 1988; Pellett *et al.*, 1985) and truncated derivatives of gB (L. Pereira, K. Kousoulas, M. Ali, B. Huo & T. Banks, unpublished data) are generally in agreement with those obtained in this study. Results of these experimental approaches indicate that epitopes recognized by antibodies H1392, H1396, H1397 and H1839 map in the N-terminal domain of the HSV-1 gB. This is consistent with the loss of reactivity of these antibodies with the recombinant αgB, the N-terminal portion of which was encoded by HSV-2 gB and the C-terminal portion by HSV-1 gB. Three of the antibodies, H1382, H1393 and H1757, mapped in these studies to the C-terminal half of gB, did not react with truncated forms of HSV-1 gB representative of the N-terminal half of the protein, a result also in accord with the results of this study. However, a difference emerged in the case of H1830. Studies with truncated HSV-1 gB proteins indicated that H1380 reacts with the N-terminal 190 amino acids of gB. In the present study, this antibody reacted with the αgB recombinant protein, which suggests that the epitope maps in the C-terminal half of the protein. It is conceivable, however, that epitope H1830 maps in the N-terminal portion of the glycoprotein but is masked or obstructed in the HSV-2 protein although not in the recombinant αgB protein. Intact recombinant gB molecules obtained by specifically directed recombination events can both confirm and extend the mapping of epitopes dependent on secondary structure.

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