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Mapping of herpes simplex virus 1 genes with mutations which overcome host restrictions to infection

(glycoprotein D/homologous interference/virus entry)

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ABSTRACT **Earlier studies have shown that the thymidine kinase-negative baby hamster kidney (BHKTK$^-$) cell lines expressing constitutively the herpes simplex virus 1 (HSV-1) glycoprotein D (gD), designated BJ, restrict infection by HSV-1 at the level of virus entry. U10, a HSV-1 mutant not restricted by the BJ cells, carried the substitution of proline for Leu^{25} in the gD gene, suggesting that gD encodes a specialized domain which precludes virus entry into cells expressing gD. Analyses of a new series of 36 unrestricted viral mutants showed the following. (*i*) Only two mutants contained mutations at a site which did not overlap with the previously reported mutation. A representative of a previously mapped mutant and one of the two new mutants were examined in detail. Thus, in the gD of mutant U30 Ala^{185} was replaced by threonine, whereas in gD of U21, Ala^{185} and Leu^{25} were replaced with threonine and proline, respectively. U30 and U21 multiplied better than the wild-type parent virus in the parental BHKTK$^-$ cells. (*ii*) Transfer of the gD gene from U21 or U30 to wild-type parent virus or to the gD$^-$ virus FgDβ yielded recombinants which, while capable of infecting BJ cells, were considerably less efficient than the parent unrestricted mutants, suggesting that the latter contained additional mutations which were responsible in part for the unrestricted phenotype. Conversely, marker rescue of mutant viruses with wild-type gD reduced but did not abrogate entirely the unrestricted phenotype. (*iii*) Mutations in gD which conferred the unrestricted phenotype were not random. (*iv*) gD plays a role in the restriction, inasmuch as preincubation of cells expressing gD with antibodies to gD abolished restriction. (*v*) In mutant R5000, the gD substitution Ser^{140} to Asn was capable of overcoming a restriction of a BHKTK$^-$ clonal line which does not express gD but conferred very low ability to replicate on BJ cells. We conclude that (*a*) uncloned stocks of BHKTK$^-$ cells exhibit a low level restriction to infection with wild-type virus, (*b*) clonal lines of BHKTK$^-$ cells which vary with respect to the stringency of restriction express either allelic genes differing in the properties of their products or products of different genes, and (*c*) both the restricted and unrestricted phenotypes reflect the interactions of gD with these cellular products. The implications of these conclusions with respect to the restriction imposed on BHK cells by the expression of gD are discussed.**

The notion that cells can be rendered resistant to infection by virtue of a viral product has sustained scientific efforts for many decades. It led to the discovery of both viral interference and interferon, it feeds the dream of as yet undiscovered modalities for prevention of infection, and, in a more practical sense, it is a key to the elucidation of the early stages of viral entry into cells at a molecular level. Indeed, the mechanisms exhibited by viral products to prevent infection are both instructive and varied. For example, neuraminidase encoded by influenza virus removes terminal sialic acid residues, which act as receptors, and precludes the initial attachment of virus to cells (1). The *env* gene product encoded by some retroviruses sequesters the corresponding cellular receptor, which becomes unavailable for infection of the cells with a retrovirus that uses the same receptor (2, 3). A totally different mechanism is exhibited by human immunodeficiency virus type 1, which has evolved several strategies to down-regulate the synthesis of the cellular receptor CD4 in infected lymphocytes (see ref. 4). Restrictions have been reported also in plant virus systems (5). Previously we reported that wild-type herpes simplex virus 1 (HSV-1) attached to baby hamster kidney cells lacking thymidine kinase (BHKTK$^-$) but expressing the wild-type glycoprotein D (gD) gene of HSV-1 (BJ cells) was endocytosed and degraded, and infection did not ensue (6, 7). An unrestricted mutant selected on the basis of its ability to infect BJ cells and designated as U10 exhibited a mutated gD gene in which Leu^{25} was replaced with proline (8). That this substitution conferred the altered phenotype became evident from the observation that a mutant selected for its resistance to the monoclonal antibody (mAb) AP7 (9) but exhibiting the same amino acid substitution was able to infect BJ cells (8).

gD is an essential glycoprotein necessary for post-attachment entry of HSV-1 into cells (10). It interacts with a relatively low-affinity receptor in the plasma membrane (10–12). A fusogenic activity of gD is supported by the observations that cell lines expressing low levels of gD undergo spontaneous polykaryocytosis (13) and antibodies to gD inhibit cell–cell fusion by syncytial mutants (14).

In this report we show that whereas gD indeed imposes a restriction to infection, additional host factors also play a role, and that mutations elsewhere in the viral genome enhance the capacity of mutant gD molecules to overcome restrictions due to either gD or cellular factors. These conclusions are based on studies of additional mutants unrestricted for replication in BJ cells and on a viral mutant shown to overcome the restriction expressed by a cell line carrying the U_S11 gene (15, 16).

MATERIALS AND METHODS

Cells and Viruses. BHKTK$^-$ and rabbit skin cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum. Vero cells and the clonal cell lines were grown in DMEM supplemented with 5% fetal bovine serum. The BJ10 clonal line (hereafter referred to as BJ) and the $U_S11c119$ clonal cell line (hereafter referred to as U_S11) have been described (6, 15, 16). Table 1 lists the derivation and properties of the viruses used in this study.

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Abbreviations: HSV, herpes simplex virus; mAb, monoclonal antibody; gD, glycoprotein D; BHKTK$^-$ cells, thymidine kinase-negative baby hamster kidney cells.

Table 1. Summary of HSV-1 mutant, recombinant, and repaired viruses

Virus	Characteristics	gD mutation (ref.)
HSV-1(F)	Wild type	None (17)
FgDβ	No gD	— (10)
	Mutants selected for growth in restricted cells	
U10	Selected in BJ cells	Leu^{25} → Pro (8)
U21*	Selected in BJ cells	Leu^{25} → Pro, Ala^{185} → Thr
U30*	Selected in BJ cells	Ala^{185} → Thr
R5000	Selected in U_S11 cells	Ser^{140} → Asn (16)
	Mutants resistant to neutralization by anti-gD mAbs	
$AP7^R$	AP7-resistant	Leu^{25} → Pro (9)
$LP2^R$	AP2-resistant	Ser^{216} → Asn (9)
$LP14^R$	AP14-resistant	Arg^{16} → His (9)
$AP12^R$	AP12-resistant	Ile^{129} → Thr (9)
	Marker transfer of mutant gD gene to HSV-1(F)	
RFU10†		Leu^{25} → Pro
RFU21†		Leu^{25} → Pro, Ala^{185} → Thr
R5001		Ser^{140} → Asn
	Marker transfer of mutant gD to gD^- FgDβ virus	
RβU21		Leu^{25} → Pro, Ala^{185} → Thr
RβU30‡		Ala^{185} → Thr
	Marker rescue of mutants with wild-type gD gene	
R_SU10¶		Wild-type gD
R_SU21¶		Wild-type gD

*U21 and U30 were derived by four serial passages of HSV-1(F) in the BJ10 clonal line exposed to 10 plaque-forming units per cell. Viral progeny were plaque purified five times in Vero cells. The *Bam*HI J DNA fragment derived from mutant DNAs was cloned in pUC19 and the nucleotide sequence of gD was determined by dideoxy sequencing with Sequenase version 2 (United States Biochemical).

†The *Sac* I–*Mlu* I viral DNA fragments cleaved from plasmids carrying the *Bam*HI J fragments were cotransfected with HSV-1(F) DNA onto rabbit skin cells. The progeny of the transfections were passaged once in BJ cells, plaque purified five times on Vero cells, and selected for lack of reactivity with mAb AP7.

‡The donor fragment, the *Sac* I–*Mlu* I fragment from the *Bam*HI J fragment of U21 or U30 viral DNA, was cotransfected with FgDβ DNA. Progeny were selected for ability to form plaques in Vero cells.

¶The donor fragment, the *Sac* I fragment from the *Bam*HI J fragment of HSV-1(F), was cotransfected with U10 or U21 DNA. Progeny were selected for reactivity with mAb AP7.

Antibodies. mAbs HD1 (18) and AP7 (9) have been described. mAbs 30 and 2G9 to HSV-1 gD and gC, respectively, were derived by L. Foà-Tomasi and G.C.-F. (unpublished work).

RESULTS

Mutants U21, U30, and R5000 Are Unrestricted in BJ Cells. U21 and U30 belong to a panel of 36 mutants selected, as described in the legend to Table 1, for unrestricted growth in BJ cells. U21 and U30 overcome the restriction in BJ cells, as inferred by their ability to direct viral protein synthesis (Fig. 1, lanes 2 and 7) and to replicate and to form plaques (Table 2) in these cells relative to the wild-type virus. Surprisingly, the mutant R5000, selected for unrestricted growth in U_S11 cells, also induced viral protein synthesis (Fig. 1, lane 14) and replicated and formed plaques in BJ cells (Table 2). Overall, U21 and U30 were more efficient than the previously described U10 mutant (8) in overcoming the BJ cell restriction. U10, in turn, was very similar if not slightly more efficient than R5000. The gD gene of both U21 and U30

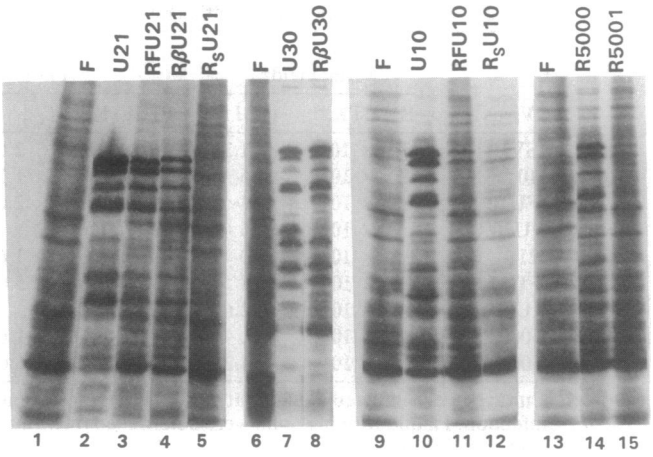

FIG. 1. Fluorographic image of proteins synthesized in BJ cells infected with the wild-type virus HSV-1(F) (lanes 1, 6, 9, and 13), the mutants U21 (lane 2), U10 (lane 10), U30 (lane 7), and R5000 (lane 14), the recombinants RFU21 (lane 3), RβU21 (lane 4), RFU10 (lane 11), RβU30 (lane 8), and R5001 (lane 15), or the rescued viruses R_SU21 (lane 5) and R_SU10 (lane 12). Cells were infected with virus at 10 plaque-forming units per cell. Fifteen hours after infection, cells were metabolically labeled with a mixture of [^{35}S]methionine and [^{35}S]cysteine (Tran^{35}S-label, ICN), 45 μCi/ml of medium containing no methionine, for 1 hr and harvested. Proteins were separated by electrophoresis in sodium dodecyl sulfate/8.5% polyacrylamide gels crosslinked with *N,N'*-diallyltartadiamide. Fixed gels were soaked in Amplify (Amersham) and exposed to Kodak X-Omat films for fluorography.

was found to encode a threonine in place of the Ala^{185}. The gD gene of U21 carried an additional substitution, that of proline in place of Leu^{25}. This is the same substitution found in U10 and in the mAb AP7-resistant mutant (8, 9). As might be expected, the U21 mutant also fails to react with mAb AP7. Indeed, the vast majority of the mutants derived in this study (34 of 36) did not react with mAb AP7.

Mutations Which Overcome the Restriction Imposed by BJ Cells Map in the gD Gene and in Genes Other than gD. This conclusion was inferred from analysis of two series of viral constructs. The first comprised recombinant viruses RFU21, RFU10, RβU21, and RβU30, made by marker transfer of a mutant gD gene from U21 or U10 into HSV-1(F), the wild-type parent virus, or of gD from U21 or U30 into the gD^- virus FgDβ. RFU21, RFU10, RβU21, and RβU30 were found to be able to direct viral protein synthesis in BJ cells (Fig. 1, lanes 3, 4, 11, and 8), and RFU21 and RFU10 were able to replicate and to form plaques in BJ cells (Table 2). The recombinants RβU21 and RβU30 were not characterized further, as the parental virus FgDβ is syncytial—i.e., contains mutations not yet identified which might have affected our results. A key conclusion from these studies is that the capacity of the recombinant viruses to infect and spread from cell to cell in BJ cells was not as efficient as that of their respective mutants.

The second series of recombinant viruses, R_SU21 and R_SU10, was made by rescue of U21 or U10 mutants with wild-type gD from HSV-1(F). The phenotype of R_SU21 was similar to that of the wild-type virus, whereas R_SU10 maintained the ability to replicate in BJ cells and, to a very low level, also to direct viral protein synthesis (Fig. 1, lanes 5 and 12). We conclude that the mutations in the gD gene of U21, U10, and U30 are sufficient to confer the ability to infect BJ cells and that additional mutations mapping outside gD contribute to the unrestricted phenotype of the mutants in BJ cells.

Uncloned BHKTK$^-$ Cells Show a Low-Level Restriction to HSV-1(F). U21 (Table 2) and U30 (data not shown) carry

Table 2. Virus yield and plating efficiency

Virus	Virus yield*		Plating efficiency†			Ratio of plating efficiencies, no. × 10^4	
	BHKTK⁻	BJ	BHKTK⁻	BJ	Us11	BJ/BHKTK⁻	Us11/BHKTK⁻
HSV-1(F)	3.9×10^3	1.3	2.0×10^9	2.0×10^4	1.2×10^5	0.1	1
U10	5.5×10^3	16	7.5×10^8	1.2×10^6	4.0×10^6	16	53
RFU10	15×10^3	14	6.0×10^8	2.0×10^5	8.0×10^4	3	1
RsU10	11×10^3	16	ND	ND	ND	—	—
U21	53×10^3	180	6.6×10^8	1.6×10^6	2.4×10^6	24	36
RFU21	20×10^3	8	1.4×10^8	5.0×10^4	2.5×10^5	4	18
RsU21	58×10^3	2.6	ND	ND	ND	—	—
R5000	4.7×10^3	11	1.0×10^8	1.0×10^5	7.0×10^5	10	70
R5001	2.7×10^3	4.2	5.6×10^8	6.0×10^4	2.8×10^6	1	50

*Cultures were infected at an input multiplicity of infection of 0.5 plaque-forming unit per cell and frozen at 3 or 24 hr after infection. Plaque-forming units were determined by titration on Vero cells. The number of plaque-forming units at 3 hr was subtracted from the number at 24 hr.

†Triplicate cultures for each cell line were infected with 10-fold dilutions of the viruses. Plaques were scored 48 hr later by immunoperoxidase staining with mAb 30 to gD and the avidin-amplified kit from Vector Laboratories.

mutations which enabled the viruses to replicate to at least 10-fold higher titers in BHKTK⁻ cells than the parent HSV-1(F). For U21, the genetic location of this mutation is unclear, inasmuch as the recombinants produced by marker transfer and those produced by rescue of gD with the wild-type gene showed a similar phenotype.

Differentiation of the Restriction in BJ Cells and Us11 Cells. Inasmuch as the mutant R5000, which was selected for unrestricted growth in Us11 cells, was able to infect BJ cells, the questions arose whether (*i*) the mutants selected for unrestricted growth in BJ cells were also unrestricted in Us11 cells, (*ii*) the mutations in the gD gene of the BJ cell-unrestricted mutants could confer the ability to infect the Us11 cells, and, vice-versa, (*iii*) whether the mutation in gD of R5000, which fully accounted for its ability to infect Us11 cells (ref. 16, Table 2), was sufficient to confer the ability to infect BJ cells. The results and conclusions of a comparative plating efficiency experiment done in BJ, Us11, and BHKTK⁻ cells (Table 2) were as follows. The BJ cells were more restrictive than the Us11 cells, inasmuch as the plating efficiency of the mutants, with possible exception of U21, was higher in Us11 cells than in BJ cells. In addition, the efficiency of marker transfer by recombinants carrying the mutated gD gene was 5- to 10-fold lower in BJ cells than that by the parental mutant. With the exception of RFU10, the efficacy of the recombinants and parent viruses to form plaques in Us11 cells differed by a much smaller ratio (≤2-fold). More important, the U21 and U10 viruses, unrestricted in BJ cells, were also unrestricted in Us11 cells. However, the unrestricted phenotype for replication in Us11 cells cannot be attributed solely to the mutations in gD. For example, the phenotype for plaque formation by U21 and R5000 was fully transferred by the gD gene to recombinants RFU21 and R5001. The gD gene of U10, however, failed to transfer this phenotype to its recombinant RFU10. Neither mutated gD of these viruses fully transferred the ability of the mutant viruses to form plaques in BJ cells. We conclude that the various mutations in gD differ on their ability to overcome different types of restrictions.

gD Substitutions Present in the BJ Cell-Unrestricted Mutants Are Nonrandom. The mutations in gD which overcome restrictions to infection of BJ cells were shown to map at Leu^{25}, Ser^{140}, and Ala^{185}. To test the specificity of these domains further, we examined the restriction phenotype of three mutants with substitutions in gD at amino acids 16, 129, and 216 selected for resistance to mAbs LP14, AP12, and LP2, respectively (Table 1). Viral protein synthesis was not detectable in BJ cells exposed to any of these mutants (Fig. 2, lanes 8–10). We conclude that the gD mutations which conferred an unrestricted BJ cell phenotype were not random and that the amino acid substitutions at positions 16, 129, and 216 did not disrupt the gD-domain target of BJ cell restriction.

gD Present on the Surface of BJ Cells Confers at Least Partial Restriction to Infection by Wild-Type and Recombinant Viruses. The conclusion that cell surface expression of gD in BJ cells correlates with restriction to infection stems from the following experiment. BJ cells were preincubated for 1 hr prior to exposure to HSV-1(F) with increasing concentrations of mAb HD1 or AP7 or a single concentration of mAb 30 and labeled with [^{35}S]methionine at 15 hr after infection. Synthesis of labeled viral proteins was detectable in BJ cells exposed to all three mAbs (Fig. 3 *A*, lanes 3–10, and *B* lanes 2, 3, and 5) and increased in a dose-dependent fashion. Viral protein synthesis was not detected in cells exposed to mAb 2G9, which is directed to gC (Fig. 3*A*, lanes 11–14). The observation that all of the mAbs to gD tested were effective is at variance with previous observations showing that mAb AP7 but not mAb HD1 rendered BJ cells susceptible to infection by HSV-1(F) (8). Conceivably, the variance reflects the fact that in the present experiments the cells were treated with purified IgG, whereas in our earlier studies the cells were treated with crude ascites, which may have varied with respect to the concentration of immunoglobulins and may have contained impurities, particularly proteases. The results imply that the restriction to HSV-1 superinfection in BJ cells is a reversible effect mediated by the presence of gD on the

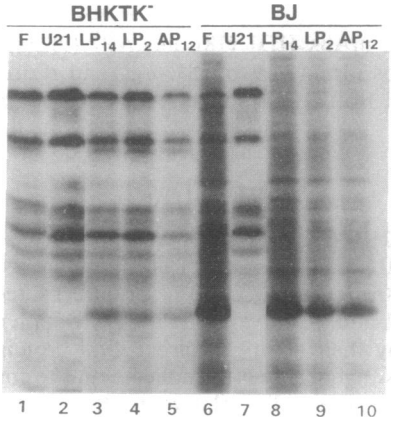

FIG. 2. Fluorographic image of proteins synthesized in BHKTK⁻ and BJ cells infected with the wild-type virus HSV-1(F) (lanes 1 and 6), the unrestricted mutant U21 (lanes 2 and 7), or the mutants resistant to neutralization by anti-gD mAbs LP14 (lanes 3 and 8), LP2 (lanes 4 and 9), and AP12 (lanes 5 and 10). Cells were infected at 10 plaque-forming units per cell and processed as described in the legend to Fig. 1.

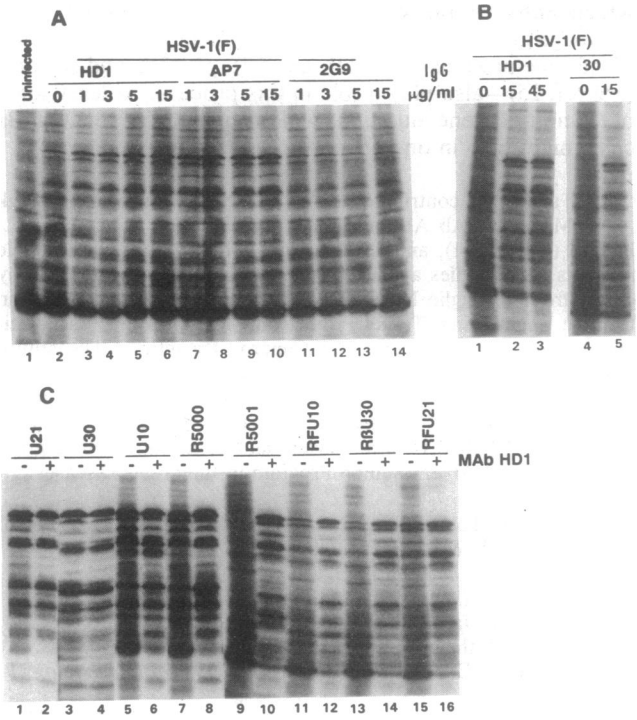

FIG. 3. Fluorographic image of proteins synthesized in BJ cells uninfected, infected with HSV-1(F) (*A* and *B*), or infected with unrestricted mutants and recombinants (*C*). Cells in 24-well dishes were exposed for 1 hr to the indicated amounts (purified IgG, μg/ml) of the mAbs HD1 (*A*, lanes 3–6; *B*, lanes 2 and 3), AP7 (*A*, lanes 7–10), and 30 (*B*, lane 5) to gD and 2G9 to HSV gC (*A*, lanes 11–14). For *C*, cells were exposed to 40 μg of HDI per ml of medium (even-numbered lanes). After removal of the antibodies, cells were infected with the indicated viruses at 10 plaque-forming units per cell. Cells were labeled and processed as described in the legend to Fig. 1.

plasma membranes of BJ cells and not the result of the selection of a mutant cell line resistant to HSV-1 infection. Preincubation of BJ cells with mAb HD1 enhanced the ability of BJ cells to become infected and sustain viral protein synthesis of marker-transfer recombinants carrying the mutant gD (Fig. 3*C*, lanes 8, 10, 12, 14, and 16).

DISCUSSION

In this report we show that (*i*) BHK clonal cell lines exhibit varying degrees of resistance to infection and replication by HSV, (*ii*) mutations which enable HSV to infect cells expressing gD map in part to the gD gene and in part elsewhere in the viral genome, and (*iii*) the mutations in gD which allow infection are nonrandom. Three key issues emerge from this study.

Number and Nature of the Restrictions to Infection by HSV in BHK Cell Lines Tested to Date. The cell lines tested define two types of restriction. (*i*) BHKTK$^-$ cells exhibit a low-level restriction to infection with wild-type virus, and mutants capable of overcoming this restriction can be selected. Evidence rests on two series of experiments. First, it could be expected that both wild-type HSV-1(F) and mutant viruses selected by passage in gD-expressing restricted cells would replicate equally well in parental BHKTK$^-$ cells. This is in fact not the case: U21 and U30 (data not shown) grew better on uncloned unrestricted BHKTK$^-$ cell lines than the wild-type parent HSV-1(F), notwithstanding the fact that all viruses grow equally well in BHK cells (data not shown). Second, restrictive clonal cell lines were readily derived from BHKTK$^-$ cells without expression of viral genes (16). These results suggest that BHKTK$^-$ cells carry an inherent host restriction and that passage of a human cell-derived virus through these cells results in the selection of a "BHKTK$^-$ cell-adapted" virus. The fundamental question is whether the restricted cell lines express different alleles of the same or different genes. Notwithstanding the surprising finding that the low-level restriction seen in BHKTK$^-$ and U_S11 cells is overcome to various degrees by mutations in gD, suggestive of a block at the virus entry level, we cannot exclude the possibility that the clonal cell lines also differ with respect to expression of genes which operate at a post-entry level.

(*ii*) The expression of gD in BHKTK$^-$ cells leads to a high-level restriction to infection by wild-type virus. The observation that preexposure of BJ cells with antibodies to gD enables infection by wild-type virus supports the confusion that expression of gD leads to a high-level restriction to infection which operates at the level of virus entry into cells. Other laboratories have reported a variety of cell lines expressing HSV gD or gD homologues which are resistant to infection by herpesviruses (19–21), and therefore the restriction to infection is independent of the nature of the cell line in which the glycoprotein is expressed. Inasmuch as the major block in BJ cells operates at the level of virus entry into the cells, it seems likely that the mutations described in this report affect this function.

Nature of the Mutations in HSV-1 Which Allow Infection of Restrictive Cells. Some nonrandom mutations in gD appear to be sufficient but not essential to enable HSV-1 to express viral proteins, replicate, and form plaques in BJ cells which express wild-type gD and in restrictive clonal lines (e.g., U_S11) which do not. The available data bear on three issues.

(*i*) At least three amino acid substitutions in gD—Leu^{25} to proline, Ala^{185} to threonine, and Ser^{140} to asparagine—have been noted in unrestricted mutants. Although the substitutions range over a span of 160 amino acids, these mutations are not random since mutants with substitutions in Arg^{16}, Ile^{129}, and Ser^{216} did not overcome the restriction of BJ cells.

(*ii*) Sufficiency of the gD mutations to overcome restrictions is sustained by the cumulative evidence that recombinants carrying gD of U10 and U21 have an overall ability to direct viral protein synthesis, to replicate, and to form plaques in BJ cells, although the degree to which the mutants perform these functions varies. With respect to necessity, 36 of 38 unrestricted mutants carry at least one mutation in the gD gene determined either by sequencing of the gene or by the observation that the selected mutant failed to react with mAb AP7. However, one of the unrestricted mutants derived in an earlier study (U5) did not exhibit a mutation in the gD gene (G.C.-F. and S. Qi, unpublished work). Notwithstanding the sample of one, we cannot conclude that mutations in gD are necessary to overcome the gamut of the host-range restrictions examined to date.

(*iii*) Of the various amino acid substitutions in gD, those at residues 25 and 185, present singly or in combination in the recombinants RFU10 and RFU21, enabled viral replication in the most stringently restrictive clonal lines derived from BJ cells. Conversely, the substitution of Ser^{140} enabled R5001 recombinant to productively infect the less restrictive clonal lines of BHKTK$^-$ cells lacking the gD gene. In each of these instances the most apparent restriction is at the level of entry of the virus into cells (7, 8, 16). One hypothesis to account for these findings is that the interaction of gD with the BHKTK$^-$ receptor may involve several sites on the gD molecule. Each of these mutations may alter the "fit" of gD to the receptor, but in varying degrees. Alternatively, different mutations might favor the interaction of gD with different host factors for which wild-type gD has a lower affinity.

Number and Mechanisms of Host-Range Restrictions Operating in BHK Cells. In earlier studies, the observation that wild-type gD restricted BHK cells from being infected with

wild-type virus, whereas mutated gD did not, led us to postulate that this restriction is mediated by the direct interaction of gD molecules on the surface of the virion and plasma membrane (8). This hypothesis is no longer tenable for two reasons. First, U_S11 cells lacking the gD gene restrict entry of wild-type virus and this phenotype is alleviated by a mutation in gD (16). Second, the possibility that anti-gD antibody sequesters the gD in the plasma membranes and renders it unavailable for direct interaction with virion gD is unlikely, since mutants which overcome restriction in BJ cells also overcome the restriction in U_S11 cells which do not express gD.

An alternative hypothesis is that the gD must interact directly and sequester cellular factors—i.e., either products of diverse cellular genes or those of allelic genes—and that preincubation of these cells with anti-gD antibody results in a release of the receptor which becomes available for interaction with the gD in virions. The finding that restriction in U_S11 cells may be overcome by mutations in gD, coupled with the evidence that viral mutants selected for ability to infect BJ cells could infect U_S11 cells, suggests that the two sets of clonal lines share a common restrictive mechanism. Of particular interest is the observation that the recombinant R5001, which carries the mutated gD from R5000, infects U_S11 cells efficiently but infects BJ cells very poorly; conversely, the recombinant RFU10, which carries the mutated gD from U10 virus, forms plaques in BJ but not in U_S11 cells, and the recombinant RFU21 which carries the mutated gD from U21 virus can form plaques in both BJ and US11 cells. Together these observations lead to the hypothesis of multiple receptors for gD.

A model consistent with the available data is that $BHKTK^-$ cells express at least two surface receptors of which one, designated as primary, has an affinity for gD and is sequestered by wild-type gD expressed in BJ cells. This receptor is released and becomes available to incoming wild-type virus by pretreatment of BJ cells with antibody to gD. The other receptor, which we shall designate as secondary, does not interact with wild-type gD but has a low affinity for a specific set of mutated gD molecules. The secondary receptor can be sequestered by mutated gD. However, the affinity of the secondary receptor for the mutant gD molecules may be lower than that of wild-type gD for the primary receptor. This may explain why cell lines expressing mutant gD are infectable by both wild-type virus (primary receptor is present) and viruses carrying mutated gD (low-affinity favors reequilibration of the mutant gD in the presence of viral particles attached to the surface of the cells). The mutant gD can interact also with the primary receptor, inasmuch as the infectivity of recombinants carrying mutant gDs was enhanced in BJ cells exposed to antibody to gD. The model predicts that in the restrictive clonal cell line U_S11, which does not express gD, the primary receptor is either absent or mutated.

Two aspects of this model should be noted. First, the model does not exclude the possibility that the primary and secondary receptors are products of nonallelic genes, and this point remains to be resolved. The second aspect of the model concerns the additional viral mutations outside the gD gene which enhance the capacity of HSV to infect BJ cells. Although gD plays a role in post-attachment entry, other viral membrane proteins—gB (22), gH (23), gL (24), and so forth (see ref. 25)—also play a role in this process. At least some of the non-gD gene mutations which enable HSV to infect BJ cells might map in one or more of these genes.

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