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# Preferential Selection of Receptor-Binding Variants of Influenza Virus Hemagglutinin by the Neutralizing Antibody Repertoire of Transgenic Mice Expressing a Human Immunoglobulin μ Minigene

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**An analysis was made of the neutralizing antibody repertoire, for influenza virus hemagglutinin (HA) of transgenic mice expressing a human immunoglobulin μ (IgH) minigene, by monoclonal antibody (MAb) selection and sequencing of the HA genes of X31 (H3N2 subtype) laboratory variants. Whereas previously reported laboratory variants, selected in ovo with high-affinity murine MAbs of the IgG class, differed from wild-type virus by a single amino acid residue change in one of the major antigenic sites, neutralizing MAbs from transgenic donors selected novel variant viruses with altered receptor-binding specificity and contained residue changes in both the receptor-binding pocket (HA1 225 or HA1 226) and an antigenic site (HA1 135, HA1 145, or HA1 158). Changes in receptor-binding specificities of the variant viruses were confirmed by their resistance to inhibition by horse serum glycoproteins and altered binding to neoglycoproteins. The residue changes in variant virus V-21.2 (HA1 135 G→R, 225 G→D) abrogated neutralization by each of the MAbs; nevertheless V-21.2 was recognized by its own selecting MAb in enzyme-linked immunosorbent assay and therefore qualified as an adsorptive mutant rather than an antigenic variant. We consider that a low-affinity neutralizing antibody response may preferentially select for receptor-binding variants of influenza virus HA.**

Recent studies on protective immunity to virus infection highlight two distinguishing features of the neutralizing antibody (Ab) response: first, studies by Roost et al., using vesicular stomatitis virus in a murine model system, report rapid onset of high-affinity serum immunoglobulin G (IgG) responses and on-rate kinetics, coincident with viral clearance (11). There was no further affinity maturation with time or reexposure to pathogen. This contrasts with a typical anamnestic response, following immunization with model hapten-protein conjugates, in which a progressive increase in Ab affinity (typically from $10^{-5}$ to $10^{-8}$ M) occurs over a period of several months (2, 6, 8). Second, studies from our laboratory have shown marked immunodominance in the neutralizing Ab response following influenza virus infection of major histocompatibility complex congenic mice (9, 13, 15).

Influenza virus hemagglutinin (HA) provides a paradigm for structural studies on the immunogenicity of a protective viral antigen: the three-dimensional structure is known for bromelain-cleaved HA of the H3 subtype (19, 20) and more recently for HA complexed to Fab fragments of a neutralizing monoclonal Ab (MAb) (1). Sequence analyses of natural and laboratory variants have identified amino acid residues in the membrane-distal ectodomain of the HA1 subunit that have featured in antigenic drift (19). Despite the diversity of available antigenic sites and the facility for antigenic change, we have found that a majority of neutralizing MAbs established from individual major histocompatibility complex congenic mice following viral infection select the same amino acid residue change in laboratory variants (HA1 198 A→E for the *H-2*$^d$ haplotype and HA1 158 G→E for the *H-2*$^k$ haplotype) (9, 13, 15). Immunodominance during viral infection, but with minor representation (<10%) of Ab specificities for other antigenic sites, was not accounted for by an oligoclonal B-memory-cell response. Sequence analyses of heavy- and light-chain gene usage for MAbs established from the same individual of the same isotype and which had selected the same single amino acid residue change in laboratory variants indicated that a minimum of three to six progenitor cells had contributed to the individual's immune repertoire (9).

The aim of this study was to extend these studies of immunodominance to a murine system in which an IgM-to-IgG class switch, and concomitant affinity maturation, was absent and in which $V_H$ gene usage was restricted. Advantage was taken of a transgenic model: mice bearing a human IgH gene minilocus and lacking a functional mouse IgH locus. The transgenic line used in this work has been described in detail (16, 17). It contains a human IgH minilocus with human $V_H$ ($V_H$ 26) and $D_H$ ($D_{Q52}$) elements and all of the $J_H$ and secretory Cμ elements in association with an additional $V_H$ element (mouse $V_H$ 186.2) with targeted disruption of the membrane exon of mouse Cμ. A panel of neutralizing MAbs was established following immunization with X31 virus (H3N2 subtype), and recognition specificity was deduced by in ovo selection and sequencing of laboratory variant viruses.

In contrast to the majority of previously reported laboratory variants, selected with high-affinity MAbs of the IgG class and differing from wild-type virus by a single amino acid change within one of the five major antigenic sites (19), we find that MAbs from transgenic mice select novel variant viruses with amino acid substitutions at both conserved positions, constituting part of the receptor-binding pocket (18) and residue changes within known antigenic regions proximal to the receptor-binding site. A low-affinity Ab response may, therefore, preferentially select for mutations in conserved regions that

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TABLE 1. HI specificities of MAbs for laboratory variants of X31

| MAb | HI titer[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X31 | Site E | | Site A | | | | | Site B | | | Site C |
| | | 62 (I→R[b]) | 63 (D→N) | 135 (G→R) | 140 (K→L) | 142 (G→R) | 145 (S→N) | 158 (G→E) | 189 (Q→H) | 196 (V→N) | 198 (A→E) | 273 (P→L) |
| 7.1 | 12,800 | 3,200 | 3,200 | 1,600 | 12,000 | 6,400 | 1,600 | 25,600 | 6,400 | 12,800 | 6,400 | 3,200 |
| 21.2 | 51,200 | 51,200 | 12,800 | 6,400 | 6,400 | 12,800 | 3,200 | 12,800 | 12,800 | 25,600 | 12,800 | 12,800 |
| 1.1 | 25,600 | 6,400 | 6,400 | <100 | 12,800 | 6,400 | 1,600 | 12,800 | 6,400 | 6,400 | 6,400 | 6,400 |
| 4.1 | 6,400 | 6,400 | 3,200 | <100 | 3,200 | 3,200 | 1,600 | 3,200 | 3,200 | 6,400 | 3,200 | 3,200 |

[a] HI titer for MAb-containing ascites fluid in the agglutination of turkey erythrocytes by X31 or its laboratory variants.
[b] Amino acid substitution in the HA1 subunit.

constitute part of the receptor-binding pocket, not commonly selected for by high-affinity B-memory-cell responses, and provide a further determinant of antigenic change.

## MATERIALS AND METHODS

**Human IgH transgenic mice.** Details of the derivation and characterization of the founder line have been reported elsewhere (3, 16, 17).

**Viruses.** Influenza viruses were grown in the allantoic cavity of 10-day-old embryonated chicken eggs. X31 virus is a recombinant expressing surface glycoproteins of the H3N2 subtype. Variant viruses were obtained by mixing equal volumes of allantoic fluid from X31-infected embryonated eggs and ascitic fluid containing an anti-HA MAb. Following incubation at room temperature for 30 min, the virus-MAb mixture was inoculated into embryonated eggs, and the variants were cloned by limiting dilution. Viruses were purified from allantoic fluid by precipitation with polyethylene glycol (5% [wt/vol]; molecular weight 6,000) followed by sucrose gradient (15 to 40% [wt/vol]) centrifugation.

**Production of MAbs.** Human IgH transgenic mice were immunized by intraperitoneal injection of UV-inactivated X31 virus (10,000 hemagglutination units [HAU]) and boosted 6 weeks later (1,000 HAU). Three days after boosting, splenic lymphocytes were used for the production of MAbs. Hybridomas were screened for anti-HA activity by hemagglutination inhibition (HI) assay using turkey erythrocytes and expanded as ascites in pristane-primed *nu/nu* mice.

**Nucleotide sequence analyses of HA genes.** RNA was phenol extracted from purified virus preparations followed by an ether wash. Nucleotide sequences were determined by using the dideoxynucleotide chain termination method, with primers as described elsewhere (4).

**ELISA for MAb specificity.** MAbs were assayed for their recognition specificity in an enzyme-linked immunosorbent assay (ELISA) using plates (Maxisorb; NUNC) coated with purified virus (1,000 HAU/ml), either X31 or variant viruses. Bound Abs were detected with alkaline phosphatase-conjugated goat Abs specific for mouse λ or κ light chain (Sigma, Poole, United Kingdom).

**Virus specificity for neoglycoproteins.** Substrates, *N*-acetyllactosamine-bovine serum albumin (BSA) (NGP0201), and 3′sialyl-*N*-acetyllactosamine-BSA (NGP0301) were from Dextra Laboratories (Reading, United Kingdom). We prepared the α-2,6 derivative by enzymatic modification of *N*-acetyllactosamine-BSA, using α-2-6-specific neuraminyltransferase and CMP-*N*-acetylneuraminidate, as recommended by the manufacturer (Boehringer Mannheim, East Sussex, United Kingdom). ELISA plates were coated overnight with neoglycoproteins and blocked by incubation with BSA (5 mg/ml). Bound virus was detected with biotin-conjugated goat anti-HA and alkaline phosphatase-conjugated streptavidin.

**Neuraminidase treatment of MAbs.** Hybridoma-containing ascitic fluid was treated with neuraminidase (1 U/ml) as directed by the manufacturer (Boehringer Mannheim) for 16 h at 37°C and inactivated by incubation at 56°C for 1 h.

TABLE 2. HI specificities of MAbs for natural isolates of the H1, H2, or H3 subtype

| MAb | HI titer[a] | | | | |
|---|---|---|---|---|---|
| | ENG/69 (H3) | HK/71 (H3) | PC/73 (H3) | VIC/75 (H3) | TEX/77 (H3) |
| 7.1 | 100 | 200 | 800 | 800 | 400 |
| 21.2 | 6,400 | 3,200 | 12,800 | 6,400 | 100 |
| 1.1 | 1,600 | 400 | 400 | 400 | 100 |
| 4.1 | 3,200 | 400 | <100 | 100 | <100 |

[a] HI titers for isolates JAP/57 (H2) and PR8/34 (H1) were below 100 in all cases.

## RESULTS

**MAbs cross-reactive for H3 subtype viruses.** MAbs were established from transgenic donors (carrying a human IgH minigene) after immunization with X31 virus and characterized initially in HI tests (an index of virus neutralization). Their recognition specificity for HA was studied by using a panel of laboratory variants differing from the wild type by single-residue changes in one of the major antigenic sites (Table 1) and also with a selection of natural isolates of the H1, H2, and H3 subtypes (Table 2).

Each of the laboratory variants was recognized in HI tests, with the exception of (135 G→R) and its lack of recognition by MAb 1.1 and 4.1. However, MAbs 21.2 and 1.1 were broadly cross-reactive for natural variants of the H3 subtype, isolated between 1969 and 1976, but failed to recognize an H1 (PR8/34) or H2 (JAP/57) subtype virus. This contrasts with the recognition specificity of murine MAbs, specific for X31 HA, which fail to recognize natural isolates such as PC/73 and VIC/75, which have altered significantly during antigenic drift (5).

**Variant virus selection.** For definitive assignment of Ab recognition specificity, X31 laboratory variants were selected with each MAb, and their HA genes were sequenced in parallel with the wild type. Table 3 summarizes the amino acid residue changes of the selected variant viruses. The first noteworthy point is that three of four variants contain two nucleotide substitutions and corresponding amino acid changes in the HA1 subunit. Second, variants V-7.1 and V-21.2 have residue changes within a conserved region (HA1 225 or 226) that is part of the receptor-binding pocket (18). Variant V-4.1 had a single residue change (135 G→R) at a known antigenic site (19) which accounts for the initial specificity of the selecting MAb in HI tests (Table 1).

**Variant virus selection by sialic acid-free MAbs.** Since MAbs are glycoproteins with terminal sialic acid on their *N*-glycan side chains, it was possible that changes within the

TABLE 3. Amino acid substitutions in laboratory variants of X31 virus, selected with MAbs from transgenic donors

| MAb | Laboratory variant | Amino acid change[a] |
|---|---|---|
| M7.1 | V-7.1 | 145 S→N |
| | | 226 L→P |
| M21.2 | V-21.2 | 135 G→R |
| | | 225 G→D |
| M1.1 | V-1.1 | 135 G→R |
| | | 158 G→E |
| M4.1 | V-4.1 | 135 G→R |

[a] Amino acid substitution in the HA1 subunit, as deduced by sequencing HA genes of cloned laboratory variant viruses.

TABLE 4. Amino acid substitutions in laboratory variants of X31, selected with neuraminidase-treated MAbs from transgenic donors

| MAb | Laboratory variant | Amino acid change in HA1 |
|---|---|---|
| M-7.1Na | V-7.1Na | 135 G→R<br>225 G→D |
| M-7.1Nb | V-7.1Nb | 145 S→N<br>226 L→P |
| M-21.2N | V-21.2N | 135 G→R<br>225 G→D |
| M-1.1N | V-1.1N | 145 S→R<br>158 G→E |

receptor-binding pocket had been selected for by carbohydrate rather than MAb-specific neutralization. Therefore, MAbs 1.1, 7.1, and 21.2 were treated with neuraminidase (to remove terminal sialic acid) before in ovo variant selection. The efficacy of enzymatic treatment was confirmed by a reciprocal change in reactivity of MAbs with peanut agglutinin, specific for terminal galactose, or elderberry lectin, specific for sialic acid, in ELISAs (data not presented).

Desialylated MAbs were still reactive with X31 by HI tests and selected further variant viruses. Table 4 shows the amino acid residue changes in their HA genes. MAb 7.1N, which had previously selected V-7.1 (145 S→N, 226 L→P), now generated the variant V-7.1Na (135 G→R, 225 G→D). This same mutant had been selected previously by MAb 21.2. Accordingly, we wished to establish whether the removal of sialic acid had affected the specificity of antibody selection. A further round of selection with MAb 7.1N produced variant V-7.1Nb, which had residue changes identical to those of V-7.1 (at positions 145 and 226) previously selected with the untreated MAb.

MAb 21.2N selected the same variant virus as the corresponding untreated MAb, and MAb 1.1N produced a variant, V-1.1N (145 S→R, 158 G→E), that was distinct from V-1.1 (135 G→R, 158 G→E). We conclude that the presence of terminal sialic acid in the MAbs is not required for HI activity or for the selection of such variant viruses.

**MAb fine specificity.** Table 5 is a summary of fine specificity for the MAbs in HI tests using the panel of selected variants, and includes X31 variants with single residue changes at equivalent positions in the HA1 subunit. Variation in HI titers for X31 (compare Table 1 with Table 5) is due to the use of different batches of MAb-containing ascites in assays performed at different times. Interestingly, V-21.2 (135 G→R, 225 G→D) was resistant to HI with the entire panel of MAbs,

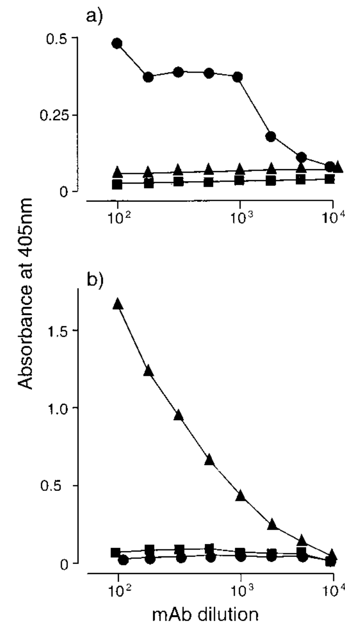


FIG. 1. MAb recognition of variant viruses by ELISA. (a) Reactivity of MAb 2.12 (●), MAb 7.1 (■), or MAb 4.1 (▲) with variant virus V-21.2. (b) Reactivity of MAb 1.1 with variant virus V-1.1 (●), V-4.1 (■), or V-7.1 (▲).

despite the observed differences between MAbs in their selection of escape mutants (Table 2) and in their differing sensitivities to the single residue change (135 G→R) in V-4.1 (but see results of ELISA tests, below). That two amino acid changes were required to abrogate recognition by the selecting MAb is suggested by the reactivity profiles of MAbs 7.1 and 21.2, which recognize laboratory variants containing single residue changes at HA1 135, 145, and 226.

**Novel phenotype of variant V-21.2.** Variant viruses with altered receptor-binding specificity, and which are HI negative for their selecting MAbs, are frequently recognized by ELISA (see Discussion). Variant V-21.2 exhibits such a phenotype and is recognized by its selecting MAb in ELISA (Fig. 1a) but not in HI assays (Table 5). Paradoxically, MAbs 7.1 and 4.1 failed to bind V-21.2 (or their own selected variants) in ELISA. Similarly, MAb 1.1 was sensitive to a single amino acid residue change at HA1 135 (Fig. 1b) and failed to recognize V-4.1 (135 G→R) or its selected variant V-1.1 (135 G→R, 158 G→E).

Residue changes in HA1 135 G→R, 225 G→D are therefore

TABLE 5. Fine specificity of MAbs for variant viruses in HI tests

| MAb | HI titer[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | V-7.1Nb (145 S→N, 226 L→P) | V-1.1 (135 G→R, 158 G→E) | V-4.1 (135 G→R) | V-3.41 (158 G→E) | V-9.51 (145 S→N) | X31-HS (226 L→Q)[b] | X31 |
| 7.1 | <100 | 3,200 | 6,400 | 12,800 | 6,400 | 6,400 | 12,800 |
| 7.1N | <100 | 800 | 1,600 | 6,400 | 3,200 | 3,200 | 6,400 |
| 21.2 | <100 | 6,400 | 12,800 | 12,800 | 6,400 | 6,400 | 12,800 |
| 21.2N | <100 | 1,600 | 3,200 | 12,800 | 3,200 | 3,200 | 12,800 |
| 1.1 | 12,800 | <100 | <100 | 6,400 | 1,600 | 1,600 | 6,400 |
| 1.1N | 1,600 | <100 | <100 | 6,400 | 1,600 | 1,600 | 3,200 |
| 4.1 | 12,800 | <100 | <100 | 6,400 | 3,200 | 3,200 | 6,400 |

[a] All titers for V-21.2 (135 G→R, 225 G→D) were less than 100.
[b] A variant of X31 selected for resistance to treatment with horse serum glycoproteins (5).

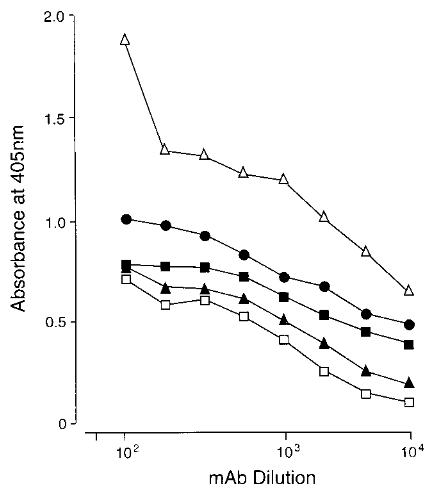


FIG. 2. MAb 21.2 recognition of variant viruses by ELISA. ●, X31; □, V-7.1 (145 S→N, 226 L→P); △, V-1.1 (135 G→R, 158 G→E); ▲, V-4.1; ■, X31-HS (226 L→Q).

sufficient to abrogate V-21.2 neutralization by the entire panel of MAbs.

**MAb 21.2 specificity in ELISA.** The contrasting specificities shown by MAb 21.2 for its selected variant in HI tests and ELISA was mirrored in its reactivity in ELISA with the other variants (Fig. 2). Although variant virus V-7.1 (145 S→N, 226 L→P) was found to be HI negative for MAb 21.2 (Table 5), it was recognized in ELISA by the same MAb and therefore qualified as a receptor-binding variant. Interestingly, MAb 21.2 showed heteroclytic reactivity for V-1.1 (135 G→R, 158 G→E).

**Changes in receptor-binding specificity.** Receptor-binding variants of X31 can be selected for their resistance to horse serum inhibition and show preferential specificity for the α-2,3 sialyl anomeric linkage, compared to wild-type preference for α-2,6 sialyl glycoconjugates (10). Table 6 shows the susceptibility to horse serum inhibition of hemagglutination by X31, horse serum-resistant variant X31-HS, or the variant viruses reported here. Each variant virus exhibited increased resistance to HI, consistent with their altered receptor specificities.

**Variant virus specificity for α-2,3 or α-2,6 sialyl glycoconjugates.** The receptor specificities of X31 and its variants were investigated by ELISA using α2,3 SLB or α2,6 SLB neoglycoproteins as substrates.

First, consider the binding specificity of X31 or X31-HS (226 L→Q), its receptor-binding variant: a single residue change abrogates virus binding to the α-2,6 SLB substrate but does not affect recognition of α-2,3 SLB (Fig. 3). All of the variant viruses selected herein exhibit reduced binding for α-2,6 SLB,

TABLE 6. Variant virus resistance to horse serum inhibition of hemagglutination

| Virus | Horse serum inhibition titer[a] |
|---|---|
| X31 | 1,280 |
| X31-HS (226 L→Q) | <100 |
| V-7.1 (145 S→N, 226 L→P) | <100 |
| V-21.2 (135 G→R, 225 G→D) | <100 |
| V-1.1 (135 G→R, 158 G→E) | <100 |
| V-1.1N (145 S→R, 158 G→E) | <100 |

[a] Titer for hemagglutinin inhibition by serial dilution of horse serum.

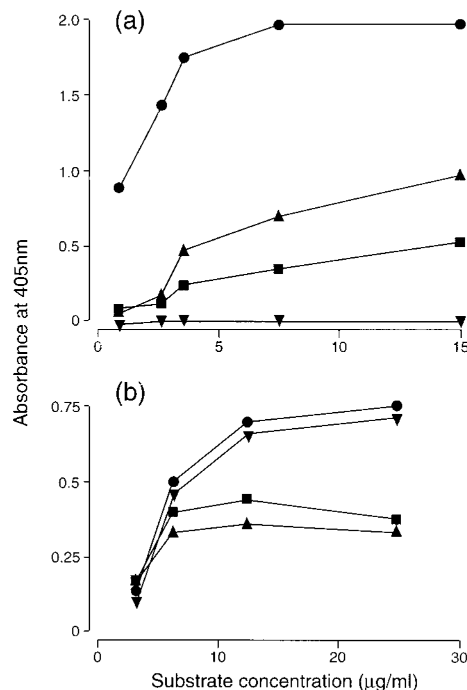


FIG. 3. Receptor-binding specificities of X31 and its variants for sialyl α-2,6 (a) or sialyl α-2,3 (b) *N*-acetyllactosaminyl-BSA in ELISA. ●, X31; ■,V-7.1 (145 S→N, 226 L→P); ▲, V-21.2 (135 G→R, 225 G→D); ▼, X31-HS (226 L→Q).

particularly V-7.1 (145, 226), and this was consistent with a critical role for HA1 226 in receptor specificity. Moreover, whereas X31-HS exhibits a reciprocal change in receptor specificity, binding to the α-2,3 substrate as effectively as X31, there was a significant reduction in binding of V-7.1 or V-21.2 to either α-2,3 or α-2,6 substrates.

## DISCUSSION

MAb selection of influenza virus laboratory variants, and sequencing of their HA genes, provides a definitive assignment of Ab recognition specificity to the three-dimensional structure of a protective antigen. In most instances, laboratory variants differ from the wild type by a single amino acid residue change in a surface-exposed region on the membrane-distal ectodomain of the HA1 subunit, proximal to the receptor-binding pocket (19, 20). In contrast, residues that constitute part of the receptor-binding pocket are conserved within a subtype and confer specificity for the anomeric linkage of host sialyl glycoconjugate receptors. Viruses of the H3 subtype exhibit preference for the sialyl α-2,6 linkage, while receptor-binding variants, selected in vitro with glycoconjugate inhibitors such as equine $\alpha_2$-macroglobulin, exhibit preference for the sialyl α-2,3 linkage. Such variants contain a single residue change, HA1 226 L→Q, that is within the receptor-binding pocket (18).

A novel finding of this study is that neutralizing MAbs, established from human IgH transgenic mice, are broadly cross-reactive and select laboratory variants of X31 with altered receptor-binding specificity.

A distinguishing feature of the laboratory variants that have been characterized is the occurrence of two distinct amino acid residue changes, the first at a position corresponding to a known antigenic site (HA1 135 or HA1 145) and the second at a conserved residue (HA1 225 or HA1 226) that constitutes part of the receptor pocket and/or affects binding specificity

(HA1 158). Since the MAbs used for variant selection were N-linked glycoconjugates, it was possible that the terminal sialic acid residues of their carbohydrate side chains, rather than Ab recognition specificity, was a determinant of variant selection. However, following neuraminidase treatment, sialic acid-free MAbs were able to select variant viruses of similar phenotypes. For instance, MAb 7.1 (specific for variant 145 S→N, 226 L→P), following neuraminidase treatment, selected either the same variant virus or variant V-7.1N (135 G→R, 225 G→D), and MAb 21.2 selected the same variant virus (135 G→R 225 G→D) before or after treatment with neuraminidase.

It should be emphasized that we have never selected laboratory variants of this phenotype by using murine MAbs of the IgG class and the same X31 seed stock employed in the current study (9). However, receptor-binding or adsorptive mutants of influenza virus have been reported previously following selection with subneutralizing levels of either MAbs (5, 15, 21) or polyclonal Abs (7). In most instances, the variant virus was still recognized by the selecting Ab in ELISA, although there was resistance to Ab neutralization or HI. Daniels et al. (5) documented a particularly novel IgG1 MAb that was broadly cross-reactive for natural isolates and selected a receptor-binding variant of X31 expressing HA1 193 S→N, 226 L→P or alternatively a variant with deletion of residues HA1 224 to 230 (RGLSSRI). Similarly, there were changes at both a major antigenic site (HA1 193) and a conserved residue within the receptor pocket (HA1 226).

In previous reports, receptor-binding variants of influenza virus were still recognized by the selecting MAb in ELISA, and the variants V-21.2 (135 G→R, 225 G→D) and V-7.1 (145 S→N, 226 L→P) exhibit similar phenotypes. In contrast, MAb 1.1-selected variants (135 G→R, 158 G→E or 145 S→R, 158 G→E) were not recognized in ELISA and therefore qualify as antigenic variants. Nevertheless, all of the MAb-selected variants showed altered receptor-binding specificity, as defined by resistance to horse serum inhibition or reduced binding to sialyl glycoconjugates. What should be further emphasized is the critical effect of the residue changes in V-21.2 (135 G→R, 225 G→D) on HA antigenicity, in so much as the other MAbs failed to neutralize this virus.

Figure 4 indicates schematically the HA receptor-binding site occupied by sialic acid (as shown in reference 18) and the possible hydrogen-bonded contacts to its receptor. The floor of the pocket contains 98Y and 153W; 190E and 194L project from a short α-helix and together with 183H and 155T define the rear of the cleft. Residues 134 to 138 are on the right, and residues 224 to 228 are on the left. The molecular locations of the residues that have featured in the variant viruses, reported herein, are evident (with the exception of HA1 145 or HA1 158, which constitutes part of the "second shell" [18]) and are consistent with their altered receptor-binding specificities.

It should be emphasized that the host IgM antibody response to influenza virus infection is unlikely to be a major determinant of immune protection and/or antigenic variation, because of the nature of the virus replicative cycle and rapid Ab class switching. Influenza virus does not establish systemic infection and is restricted to the respiratory tract, due to a requirement for apical budding from epithelial cells into the bronchial lumen (12, 14). Consequently, virus neutralization or clearance is mediated for the most part by secretory IgA antibodies and/or transudation of serum IgG, both of which would be of moderate affinity.

We speculate that low-affinity Ab responses during influenza virus infection might bias the repertoire to the selection of receptor-binding variant viruses, whereas high-affinity B-memory-cell responses would be, for the most part, focused on surface-exposed loop regions that are major antigenic sites (19, 20) and for which the virus has evolved an efficient strategy of immune evasion. It is of some interest that recent natural isolates (e.g., A/Beijing/92) obtained between 1991 and 1993 contain the substitution HA1 226 L→Q, within the receptor-binding pocket (7a).

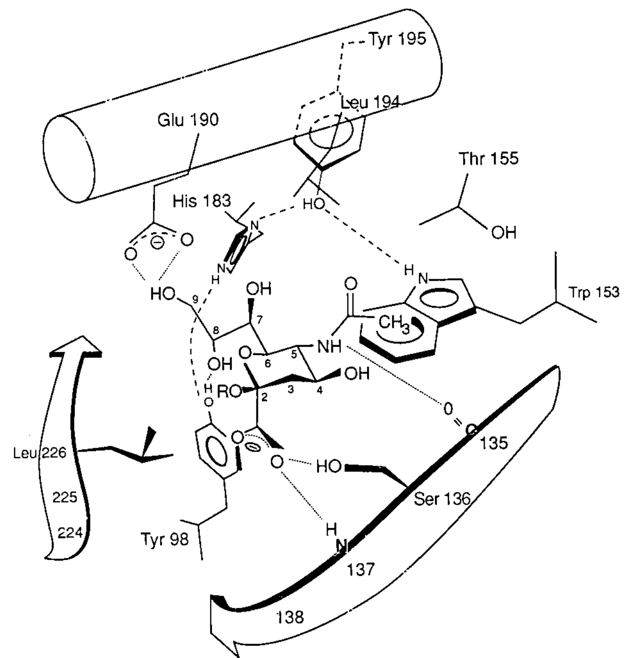


FIG. 4. Schematic representation of the influenza virus HA receptor-binding site, occupied by sialic acid as illustrated in reference 18.

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