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An Epidemiologically Significant Epitope of a 1998 Human Influenza Virus Neuraminidase Forms a Highly Hydrated Interface in the NA–Antibody Complex

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The crystal structure of the complex between neuraminidase (NA) of influenza virus A/Memphis/31/98 (H3N2) and Fab of monoclonal antibody Mem5 has been determined at 2.1 Å resolution and shows a novel pattern of interactions compared to other NA-Fab structures. The interface buries a large area of 2400 Å^2 and the surfaces have high complementarity. However, the interface is also highly hydrated. There are 33 water molecules in the interface ≥95% buried from bulk solvent, but only 13 of these are isolated from other water molecules. The rest are involved in an intricate network of water-mediated hydrogen bonds throughout the interface, stabilizing the complex. Glu199 on NA, the most critical side-chain to the interaction as previously determined by escape mutant analysis and site-directed mutation, is located in a non-aqueous island. Glu199 and three other residues that contribute the major part of the antigen buried surface of the complex have mutated in human influenza viruses isolated after 1998, confirming that Mem5 identifies an epidemiologically important antigenic site. We conclude that antibody selection of NA variants is a significant component of recent antigenic drift in human H3N2 influenza viruses, supporting the idea that influenza vaccines should contain NA in addition to hemagglutinin.

Keywords: neuraminidase–Fab structure; influenza virus; antigenic drift; water-mediated hydrogen bonds

Introduction

Influenza virus, an Orthomyxovirus, has a negative-stranded RNA segmented genome that contains coding information for about ten proteins. Influenza type A viruses are further classified into antigenic subtypes of HA (H1 to H16) and NA (N1 to N9). Epidemic human viruses have so far only included H1, H2, H3, N1 and N2 antigens, but all subtypes have been isolated from avian species. Humans have recently been lethally infected with viruses containing H5 or H7 HA and there is considerable concern that these or other avian subtypes could appear in human viruses and cause a major pandemic. The two major surface glycoproteins of the virus, neuraminidase (NA) and hemagglutinin (HA), are embedded in the lipid membrane of the virus and form spikes on the outer surface. Neutralizing antibodies raised by either vaccination or infection are directed to the native forms of these surface glycoproteins, and bound antibody inhibits their functions. The HA is a trimer, and it binds to sialic acid receptors on the cell surface. The NA is a tetramer of identical subunits of 50,000 M_r. NA cleaves terminal sialic acid from glycoconjugates such as those on the viral glycoproteins and the surface of target cells in the respiratory tract.[1,2] NA is thus a receptor-destroying enzyme, removing sialic acid from carbohydrate chains attached to HA and NA. If NA

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Abbreviations used: HA, haemagglutinin; NA, neuraminidase; CDR, complementarity-determining region.

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activity is inhibited, either by antibodies or by substrate analogue inhibitors, the progeny virus particles are trapped in aggregates at the cell surface and the infection is terminated.[3,4] Antibodies against NA are also effective in preventing disease.[5] Neutralizing antibodies against the HA block its binding to receptor, and crystal structures have shown that this steric blocking occurs even when the epitope is a considerable distance from the sialic acid binding site.[6] The HA is more abundant on the virus than NA and shows more variation, thus it is considered the major neutralizing antigen and it is the only antigen measured during vaccine production.

As a consequence of immune selection in the human population, influenza viruses progressively accumulate mutations that progressively change their antigenic properties, and this "antigenic drift" results in recurrent epidemics in the human population and an annual need to update the influenza vaccine. Molecular studies showed that monoclonal antibodies could be used to model antigenic drift in the early N2 NAs of human viruses A/RI/5$^+$/57 and A/Tokyo/3/67.[7–10] The crystal structure of Tokyo/67 NA is known[11] and antibody complexes of the avian N9 NA were determined,[12–14] but there is no crystal structure of N2 NA complexed with antibody.

To investigate antigenic drift of N2 NA in recent human H3N2 viruses, we raised monoclonal antibodies against the NA of A/Memphis/31/98, which is antigenically like A/Sydney/5/97.[15] One of these antibodies, Mem5, selected escape mutations at a site different from epitopes in other NA–Fab complexes, and a low-resolution structure confirmed that the antibody binds in the region where escape mutations were found.[15] We now report the crystal structure of the Memphis/98 NA–Mem5 Fab complex refined to 2.1 Å. This complex has a much more hydrated interface structure compared to the crystal structures of N9 NA–Fab complexes.[12,13] Sequence changes have occurred in this epitope in human viruses isolated subsequently, indicating that the Mem5 antibody has identified an antigenic region that is immunologically important in recent H3N2 virus evolution.

Results and Discussion

Data were collected at room temperature to 3.0 Å and from frozen crystals to 2.1 Å resolution. The data collection and refinement statistics are shown in Table 1. We have used the low temperature data in the analyses below except for comparison as noted. The final model includes amino acid residues 82–469 and 12 sugar residues of NA, amino acid residues 1–116, 148–176, and 186–190 of the Fab heavy chain, amino acid residues 1–113, 136–143, and 164–171 of the Fab light chain, and 506 water molecules. Two glucose molecules from the cryoprotectant are found in the high-resolution structure. One of these is in the interface, but comparison with the room temperature model, where there is no glucose, shows that it does not significantly influence the binding. The asymmetric unit contains one-fourth of the tetrameric N2 neuraminidase–Mem5 Fab complex and the structure is shown in Figure 1(a). The antibody binds to loops surrounding the active site cavity but on the opposite side to the region bound by N9 antibodies NC41 and NC10 (Figure 1(b) and (c)). The center of the Mem5 binding site is further from the neuraminidase active site than is the NC41 binding

Table 1. X-ray data collection and refinement statistics for crystals of A/Memphis/31/98 N2 NA complexed with Mem5 Fab

Crystal	N2–Mem5	N2–Mem5
Data collection temperature (°C)	20	−166
Space group	$P42_12$	$P42_12$
Unit cell parameters a, c (Å)	159.75, 104.11	155.06, 102.65
Total observations	48,044	458,140
Unique reflections	23,223	73,146
IP frames	36	170
R_{sym}[a] overall/outer shell (%)	11.4/33.9	10.4/32.1
Resolution (Å)	3.0	2.10
Completeness (%)	93.7	99.98
Mean $I/\sigma I$ overall/outer shell	10.0/3.28	17.6/2.06
V_M[b] (Å^3)	3.5	3.2
Refinement range (Å)	20.0–3.0	20.0–2.1
Overall B value (Å^2)	n/a	32.9
Number of non-hydrogen atoms	4913	5301
Number of solvent atoms	0	506
R_{cryst} (%)	26.7	18.8
R_{free} (%)	31.2	22.4
Ramachandran plot: % in most favorable regions (% non-Gly residues in disallowed)	82.7 (2.5)	83.4 (0.09)
rms deviation in bonds (Å), angles (deg.)	0.009, 1.6	0.025, 1.986[c]

[a] $R_{sym} = \frac{\Sigma|I-\langle I\rangle|}{\Sigma I}$ averaged over the number of frames.
[b] Matthews coefficient.[57]
[c] The rms deviation in bond lengths and angles is high, probably due to unattached segments in the C domain. When the C domains are omitted, the rmsds values drop to 0.018 Å and 1.58°.