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Antibodies That Engage the Hemagglutinin Receptor-Binding Site of Influenza B Viruses

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Abstract

We describe cross-reactive human antibodies recognizing influenza B viruses spanning nearly 80 years of antigenic drift. Structures show that they engage the receptor-binding site (RBS) of the viral hemagglutinin with strong similarities to their influenza A counterparts, despite structural differences between the RBS of influenza A and B. Our data show that these antibodies readily cross-react with both influenza B Victoria and Yamagata lineages. We also note that all antibodies are encoded by IGHV3-9/IGK1-33. Future research will provide insight into the prevalence of these antibodies in the human population.

Graphical Abstract

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Author Contributions

G.B. designed and performed the research and analyzed the data; G.B. and S.C.H. wrote the paper.

The authors declare no competing financial interest.

Supporting Information

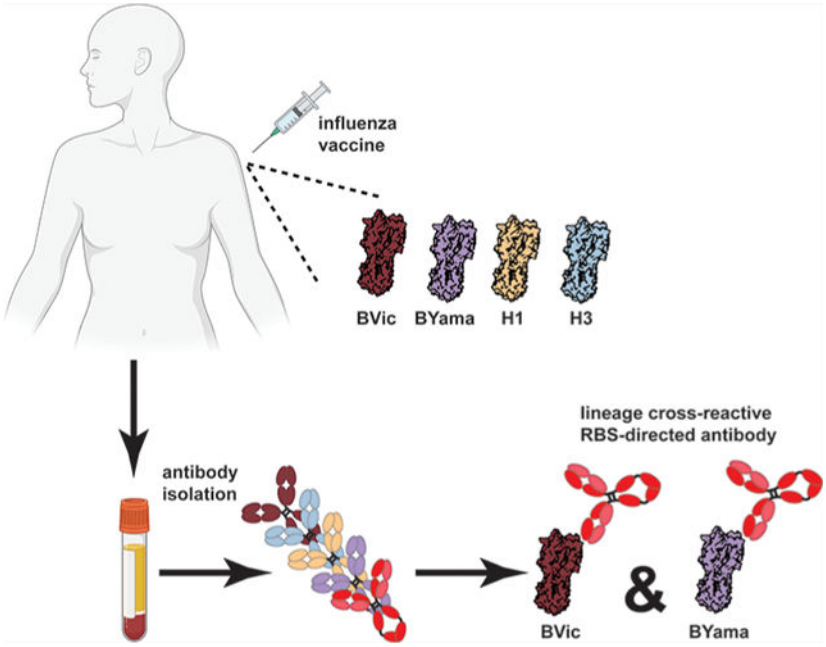
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsinfecdis.0c00726.

Antibody sequence alignments; details of the affinity measurements and molecular interactions; composite annealed omit maps of the Fab HCDR3; antigenic evolution of contemporary B Victoria HAs; data collection and refinement statistics (PDF)

Accession Codes

Coordinates and structure factors have been deposited in the Protein Data Bank under accession codes 7KQG, 7KQH, and 7KQI for the H1272, H2365, and H1209 complexes, respectively.

Complete contact information is available at: https://pubs.acs.org/10.1021/acsinfecdis.0c00726



Keywords

influenza vaccine; hemagglutinin; B-cell memory

The substantial morbidity and mortality from influenza viral infections have prompted intensive efforts to design more broadly effective vaccines.[1] Two influenza A subtypes, H1N1 and H3N2, and two influenza B lineages, Victoria and Yamagata, currently cocirculate in the human population.[2] Influenza B viruses derive from a common ancestral strain that evolved into two antigenically distinct lineages in the 1980s.[3] Influenza B infections have recently increased and now surpass those by H1N1 influenza A viruses, especially in infants.[4] Traditional vaccine approaches have historically centered on the circulating H1 and H3 influenza A strains, but influenza B viruses now elicit almost equal attention.

Influenza hemagglutinin (HA) is both the attachment protein recognizing sialic acid on host cells and the viral fusogen;[5,6] it is the more abundant of the two glycoproteins on the virion surface.[7] The characterization of B-cell responses to HAs of influenza A has identified conserved epitopes on the viral glycoprotein—the receptor-binding site (RBS), the head interface, and the membrane-proximal stem—and has yielded antibodies, the so-called broadly protective antibodies (bpAbs), that recognize a wide range of strains.[8–15] We, and others, have identified bpAbs that target the receptor-binding site (RBS)[12,13,15] or the head interface epitope on influenza A HA.[8,9,14] For the former class, we showed that these Abs mimic the HA receptor, sialic acid, by providing a critical dipeptide on the tip of their heavy-chain complementarity determining region 3 (HCDR3). For the latter class, we have found diverse ways to recognize a core epitope in the 220-loop of HA. Comparably detailed structural analyses of RBS-directed antibodies against influenza B virus HA have not yet been reported.

We examined paired heavy- and light-chain antibody sequences from plasmablasts of human donors administered trivalent, inactivated seasonal vaccines from 2007 to 2008 (H1 Solomon Islands/03/2006, H3 Wisconsin/67/2005, and B/Malaysia/06/2004) or 2008 to 2009 (H1 Brisbane/59/2007, H3 Uruguay/716/2007, and B/Florida/04/2006). We previously reported influenza A-reactive antibodies from donors in this cohort.[16,17] From these donors, we identified antibodies that bind HAs from both the Yamagata and Victoria influenza B lineages (Figures 1A and S1). Using vaccine HA components B/Malaysia/06/2004 and B/Florida/04/2006, we identified a three-membered antibody lineage "1261" comprising antibodies H1207, H1209, and H1235 (Figure 1C) as well as two "orphan" Abs H1272 and H2365. We selected one 1261 lineage member and the two orphan Abs for further biochemical characterization. We expressed and purified Fabs (to avoid any avidity effects) and measured affinities to monomeric HA1 "heads" using biolayer interferometry (BLI). All three Fabs cross-reacted with B/Yamagata and B/Victoria lineages and bound all HAs tested with variable affinities ranging from low nM to μM (Figures 1B and S2).

The isolated antibodies provide exceptional breadth by recognizing historical HAs from the 1940s to today. Both the lineage and orphan Abs have relatively long, 20-residue HCDR3s, with a central dipeptide motif of a hydrophobic and an acidic residue at its tip (Figure 1C). As in the case of influenza A RBS-directed Abs, this dipeptide would likely make RBS contacts that mimic those made by sialic acid. We therefore tested the impact on affinity to HA by replacing the hydrophobic (Met) or acidic (Asp) residues in H2365 with alanine (Figure 1D). Both substitutions lowered the affinity (i.e., increased overall K_D) by accelerating the dissociation rate, indicating that both the hydrophobic and the acidic moieties in the HCDR3 are necessary for strong binding to the HA RBS. The Asp substitution had a more pronounced effect than the hydrophobic change.

We determined the crystal structures of all three antibodies in complex with HA (Figure 2C,E,G, Table S1). The Abs engage the viral RBS, converging on a mode of molecular recognition similar to the modes of their influenza A counterparts (compare Figure 2C,E,G with Figure 2A). The central recognition element is a crucial dipeptide at the tip of HCDR3 that mimics many of the sialic acid contacts (Figures 2D,F,H and S4). The antigen-combining site includes additional contacts between HCDR2 and the HA 190-helix and between LCDR3 and the HA 150-loop (Figure S3). In the critical dipeptide, Asp supplies hydrogen bonds to the conserved Ser140 and to Gln239, thus mimicking the carboxylic acid moiety of sialic acid (Figure 2B), while the hydrophobic residue Met or Trp of the antibody contacts the conserved Trp158 at the base of the RBS, mimicking the –CH3 of the sialic acid acetamido group (Figure 2D,F,H). A noted difference in the influenza B HA RBS is a Phe at position 95 (Figure 2D,F,H) instead of the Tyr conserved in influenza A. B HAs thus contain one less hydrogen bond donor in their RBS. This evolutionarily conserved substitution reduces the affinity for the sialic acid receptor.[18] Our study suggests this has no effect on eliciting the RBS-directed antibodies.

It is worth noting that the contemporary B Victoria lineage viruses evolved during the 2016/2017 season to incorporate two (K162/N163) or three (K162/N163/D164) amino acid deletions. Recent studies showed that these viruses are antigenically distinct from each other and from the progenitor virus that lacks the deletions.[19] One representative virus of the

K162/N163 cluster is the B/Florida/84/2017 isolate (Figure S5A). All antibodies tested in this study preserve binding to that isolate (Figure 1B) indicating that individuals with such antibodies would most likely still be protected against the current B Victoria strains. Another feature of this strain is a putative N-linked glycosylation site at position 197 (Figure S5A) that could potentially interfere with antibody binding (Figure S5B). The historical HAs used in this study do not have a glycosite at that position, but the structures presented here indicate that, even if that particular site were glycosylated, it would most likely not impede the binding of our antibodies. For reference, we found that a previously characterized Crucell antibody CR8033 failed to bind the B/Florida/84/2017 HA (Figure S5C).

All 5 antibodies identified in this study derive from the V_H3-9 gene recombined with J_H6. We also note that, in all cases, the antibody heavy-chains pair with the same light kappa-chain IGK1-33. We speculate that the potential germline bias stems from two principal requirements: (1) engagement of the 190-helix through V_H3-9 encoded HCDR2 and (2) the length of the HCDR3 provided by the J_H6 gene segment. Further studies on much larger donor cohorts are necessary to corroborate these speculations.

Our study identifies the molecular signatures of human antibodies that engage influenza B HA by receptor mimicry and suggests a therapeutic potential for such RBS-directed antibodies. We also raise the possibility of a “universal” vaccine that elicits RBS-directed antibodies against both A and B influenza viruses.

METHODS

Expression and Purification of HA.

Influenza B HA1 “head” constructs were cloned into a pFastBac vector for insect cell expression (Hi5 cells) as previously described.[13,20] Of note, these insect cell produced HAs do not contain sialic acids on their N-linked glycans and are therefore better mimics of HAs on flu viruses than would be the case had they been expressed in mammalian cells. All constructs were confirmed by DNA sequencing at the DNA Sequencing Core Facility at the Dana Farber Cancer Institute. For biolayer interferometry (BLI) and crystallography, the HA1 head constructs contained an HRV 3C-cleavable C-terminal 6xHis tag. All constructs were purified from the supernatants by passage over Co^{2+}-NTA TALON resin (Takara) followed by gel filtration chromatography on a Superdex 200 Increase (GE Healthcare) in 10 mM Tris-HCl and 150 mM NaCl at pH 7.5. For BLI and crystallography, the tags were removed using HRV 3C protease (ThermoScientific) and the protein was repurified using the Co^{2+}-NTA TALON resin to remove the protease, tag, and noncleaved protein.

Fab Expression and Purification.

For Fab production, the genes for the heavy- and light-chain variable domains were synthesized and codon optimized by Integrated DNA Technologies and subcloned into pVRC protein expression vectors containing human heavy- and light-chain constant domains, as previously described.[13,20] Heavy-chain constructs for Fab production contained an HRV 3C-cleavable 6xHis tag. Constructs were confirmed by sequencing at the DNA Sequencing Core Facility at the Dana Farber Cancer Institute. Fabs were produced by

transient transfection in a suspension of 293F cells using polyethylenamine (PEI, Polysciences). Supernatants were harvested 4–5 days later and clarified by centrifugation. Fabs were purified using Co^{2+}-NTA TALON resin (Takara) followed by gel filtration chromatography on a Superdex 200 Increase (GE Healthcare) in 10 mM Tris-HCl and 150 mM NaCl at pH 7.5. For BLI and crystallography, the tags were removed using HRV 3C protease (ThermoScientific) and the protein was repurified using Co^{2+}-NTA TALON resin to remove the protease, tag, and noncleaved protein.

Interferometry Binding Experiments.

Interferometry experiments were performed using a BLItz instrument (fortéBIO Pall Corporation). Histidine-tagged HA heads or full-length HAs were immobilized on a Ni^{2+}-NTA biosensor, and cleaved Fabs were then applied to obtain binding affinities. Single-hit concentrations were tested at 20 μM for binding. All measurements were repeated in independent experiments. K_D was obtained through local fit of the curves by applying a 1:1 binding isotherm model using vendor-supplied software. All experiments were performed in 10 mM Tris-HCl and 150 mM NaCl at pH 7.5 and at room temperature.

Crystallization and Data Collection.

Influenza B HA1 heads and Fabs were incubated at a 1:1.5 molar ratio, respectively. The complex was isolated by size exclusion chromatography using a 24 mL Superdex Increase equilibrated in 10 mM Tris-HCl and 150 mM NaCl. Crystallization was achieved at 15–18 mg/mL of the complex by hanging drop vapor diffusion at 18 °C. Crystals were grown as follows: the H1209 complex in 200 mM sodium citrate, pH 7.0, with 20% (wt/vol) PEG 3350; the H2365 complex in 100 mM HEPES, pH 7.5, with 25% (wt/vol) PEG 3350; the H2365 complex in 100 mM bis-Tris, pH 6.5, with 20% PEG MME 5000. Crystals were cryoprotected in mother liquor supplemented with 25% (v/v) glycerol and flash-frozen in liquid nitrogen. Data were collected at 0.999 Å with a rotation of 1° per image on the 8.2.2 beamline, Advanced Light Source, at Berkley National Laboratory or on the beamline ID-24-C at the Advanced Photon Source (Argonne National Laboratory).

Structure Determination and Analysis.

X-ray diffraction data were processed using XDS.[21] While CC1/2 (at 0.1% significance level) was used to select the resolution cutoff for all data sets, unusually high R factors were noted for the H1209 antibody complex. Indeed, the data processing and structure refinement statistics become poor at resolutions better than 5 Å. The sequence and the structure similarity with the H2365 complex, for which the diffraction data were of better quality, however, make us confident about the structural analyses and the conclusions we draw from such analyses. We calculated the composite annealed omit maps for the H2365 and the H1209 complexes (Figure S2) to illustrate the confidence of tracing the Fab HCDR3 loops in the electron density. The structures were determined by molecular replacement using PHASER[22,23] with the B/Florida/4/2006 HA1 head (PDB ID: 4FQJ) and Fab CR8033 (PDB ID: 4FQL) as search models.[24] Refinement was performed using PHENIX.[25] Model building was done in COOT[26] and assessed with MolProbity.[27] N-Linked glycan stereochemistry was assessed with Privateer.[28] Figures were generated using the PyMOL Molecular Graphics System (v2.4.0; Schrödinger LLC).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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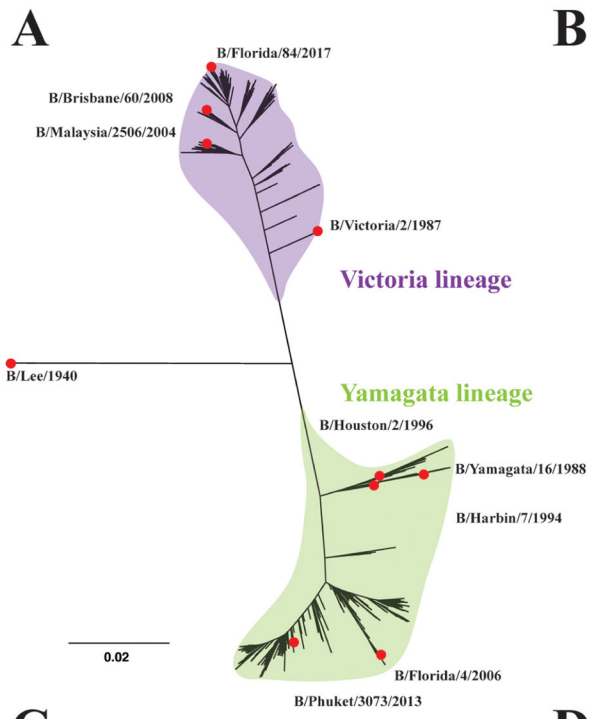

B

	Influenza strain	Antibody (Fab) affinity H2365	H1209	H1272
	B/Lee/1940	0.04	0.03	0.13
Victoria	B/Victoria/2/1987	0.01	0.01	0.01
Victoria	B/Malaysia/2506/2004	1.6	0.06	0.12
Victoria	B/Brisbane/60/2008	6.9	4.7	N.D.
Victoria	B/Florida/84/2017	7.9	0.9	0.9
Yamagata	B/Yamagata/16/1988	0.01	0.08	0.16
Yamagata	B/Harbin/7/1994	0.03	0.02	0.05
Yamagata	B/Houston/2/1996	0.01	0.75	0.13
Yamagata	B/Florida/2/2006	0.01	0.07	0.17
Yamagata	B/Phuket/3073/2013	0.05	0.53	0.03

C

Donor	mAb	HCDR3
TIV021	H2365	CAKDLRASSSMDYYYYSGMDVW
TIV01	H1207	CAKDFRSSSSMDYFYYYGMGVW
TIV01	H1209	CAKDFRSSSSMDYFYYYGMGVW
TIV01	H1235	CAKDFRSSSSMDYYYYYGMGVW
TIV01	H1272	CAKDRVDTTSWDYYFHKSMDVW

D

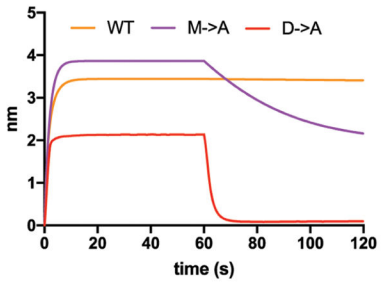

Figure 1.

Influenza B hemagglutinin phylogeny and cross-lineage binding antibodies. (A) Phylogenetic tree of influenza B viruses rooted on the ancestral B/Lee/1940 sequence. The divergent, cocirculating lineages Victoria and Yamagata are highlighted in purple and green, respectively. At the tips of the branches, highlighted with red circles, are the influenza B seasonal strains whose recombinant HA proteins were tested for binding with the antibodies. (B) Affinity measurements of the Fab to monomeric HA heads. The “heatmap” color scheme is an arbitrary visualization aid. Warm colors are high affinity and cool colors, low affinity. The calculated K_D values are reported in μM. (C) Sequence alignment of the antibody heavy complementarity determining region 3 (HCDR3) loops of the 5 antibodies isolated from 2 donors. The critical dipeptide motif is highlighted. (D) Biolayer interferometry binding isotherms for the H2365 wild-type (WT) and its mutants Met102Ala (M → A) and Asp103Ala (D → A) binding to the B/Phuket/3073/2013 HA head.

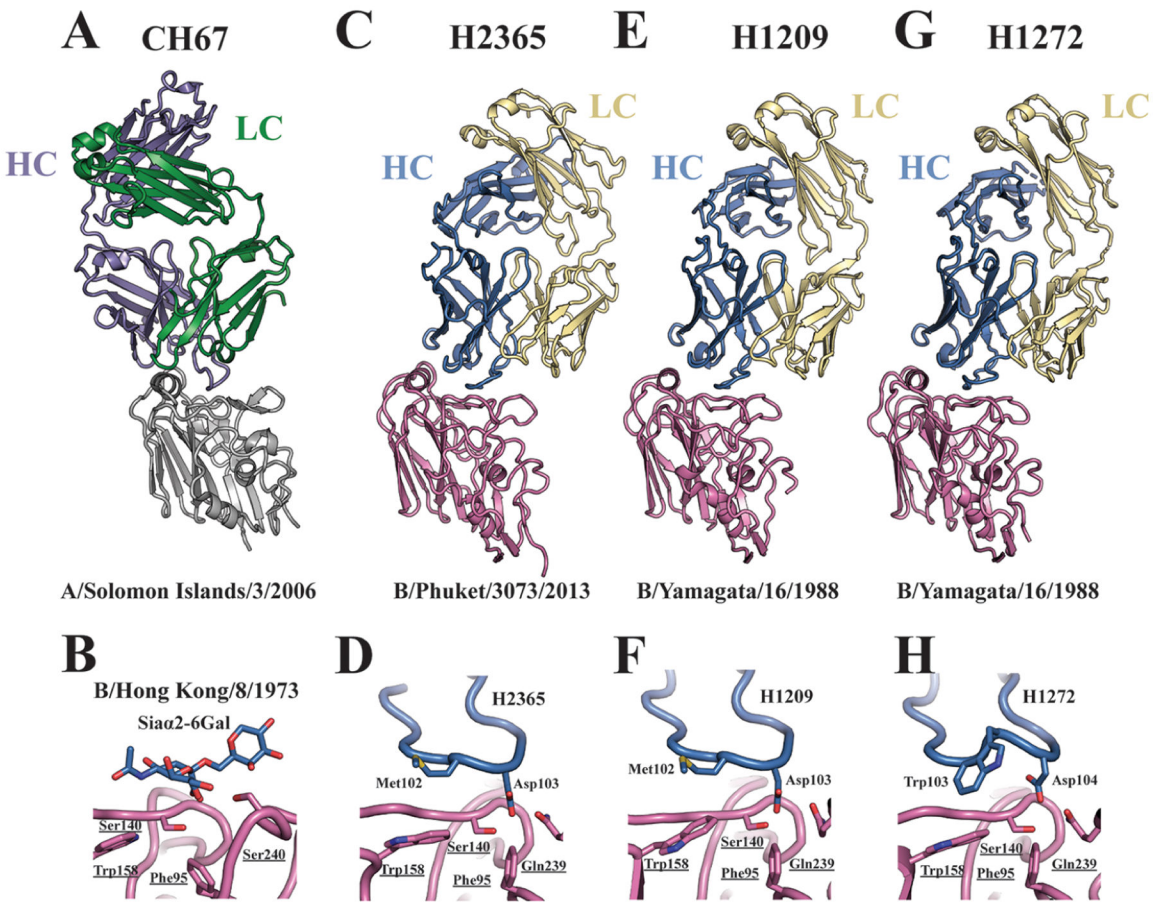

Figure 2.

Structural analyses of the antibody–hemagglutinin complexes. CH67 Fabs in complex with an influenza A HA head (PDB ID 4HKX) is shown for reference in (A). Antibody receptor mimicry through the critical dipeptide motif is made in comparison with a sialic acid bound HA structure of B/Hong Kong/8/1973 (PDB ID: 2RFU) shown in (B). Overall structures of the H2365 (C), H1209 (E), and H1272 (G) Fabs in complex with influenza B HA heads. Essential HCDR3 amino acid contacts are detailed in (D), (F), and (H). The orientation of the structures in (D), (F), and (H) is as looking from the left, i.e., from the 190-helix in (C), (E), and (G), respectively. Essential HA residues are underlined.