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Structural Evidence for Recognition of a Single Epitope by Two Distinct Antibodies

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ABSTRACT **The structure of a complex between the hemagglutinin of influenza virus and the Fab of a neutralizing antibody was determined by X-ray crystallography at 2.8 Å resolution. This antibody and another which has only 56% sequence identity bind to the same epitope with very similar affinities and in the same orientation. One third of the interactions is conserved in the two complexes; a significant proportion of the interactions that differ are established by residues of the H3 complementarity-determining regions (CDR) which adopt distinct conformations in the two antibodies. This demonstrates that there is a definite flexibility in the selection of antibodies that bind to a given epitope, despite the high affinity of their complexes. This flexibility allows the humoral immune response to be redundant, a feature that may be useful in achieving longer lasting protection against evolving viral pathogens. Proteins 2000;40:572–578.**

Key words: influenza; hemagglutinin; antibody; structure; epitope; redundancy

INTRODUCTION

The ability to form noncovalent complexes of high affinity and specificity is a fundamental property of biological macromolecules; antibody-antigen complexes provide well-studied examples of such interactions. In addition to providing specific, high affinity ligands to foreign substances, another remarkable property of the humoral immune response is its redundancy, a property whereby several antibodies bind to a single antigen. There are several levels to this redundancy: antibodies binding to the same antigen may recognize distinct or overlapping epitopes. X-ray crystallography, which has allowed the structure of many antibody-antigen complexes to be determined, has provided examples of the recognition of overlapping regions by different proteins on a common target molecule[1–4] and is the best suited method to evaluate the degree of overlap between these regions. Even when two proteins recognize a common region on a target molecule, the degree of similarity of their interactions with this region may be different. In one case, the interactions of two antibodies with the common part of their epitopes have been found to differ completely;[3] here we report how a single epitope is recognized by two different antibodies through partially conserved interactions.

We describe the structure of the complex formed between the hemagglutinin of influenza virus strain X31 (X31 HA) and the Fab fragment of antibody BH151. The hemagglutinin is a trimer of identical subunits, each of which consists of two disulfide-linked polypeptides, HA_1 and HA_2. Structurally, each subunit consists of a membrane-proximal helix-rich stem structure and a membrane-distal globular domain where infectivity neutralizing antibodies bind.[5] We have previously described the structure of a complex formed between the HC45 antibody Fab fragment and X31 HA[6]. Both BH151 and HC45 antibodies neutralize viral infectivity. We show that, although the variable domains of these two antibodies display only 56% sequence identity (Table I), they bind to very closely related epitopes on the hemagglutinin with nearly identical dissociation constants (Kd = 8.9 ± 2 nM for BH151 and 3.4 ± 1.5 nM for HC45). The two complexes allow us to compare the interactions of the two antibodies with HA and illustrate one of the ways the humoral immune response achieves redundancy.

MATERIALS AND METHODS

Virus and Proteins

X31 virus, BHA and the membrane-distal domain of HA were purified as described.[7,8] BH151 antibody and Fab fragments were produced and purified as described.[9,10]

Structure Determination and Refinement

The crystallization of both the HC45 Fab and BH151 Fab complexes with X31 BHA and collection of some of the diffraction data have been described previously.[10] For the BH151 Fab–X31 BHA complex, we recorded a dataset on a MAR Image plate system at the ESRF beamline ID2 from a frozen single crystal (110°K) to a higher resolution than previously available. The data were processed with the HKL suite.[11] The structure of the BH151 Fab · X31 BHA

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TABLE I. Primary Sequences of the Variable Domains of Antibodies HC45 and BH151[†]

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Light Chain
bh151:                    QIILTQSPAIMSASPGEKVTMTCSAS--SDISY----MHWYQQKS
hc45:                     DVVM  T LSLPV L DQASIS RS QTLVH NGNTYL   L  P
                                                  CDR L1

bh151:                    DTSPKIWIYDTSKLASGVPARFSGSGSGTSYSLTISTMEAEDAATY
hc45:                     GQ   LL  KV NRF    D         DFT  I RV    LGV
                                CDR L2

bh151:                    YCHQRSSYP-TFGGGTKL
hc45:                     F S NTHV Y
                               CDR L3

Heavy Chain
bh151:                    QVQLQQSGAELMKPGPSVKISCKATGYSFSTYFIEWIRQRPGHGLEW
hc45:                           P    VR  A   L    S  TLT  WMN FK   DQ
                                                  CDR H1

bh151:                    IGEILPGSDNTNFNEKFKDRATFTADTPSNTAYMQLSSLTSEDSAVY
hc45:                       R D YDSE HY Q  K K IL V RS S
                               CDR H2

bh151:                    YCARP---TGRLWFS-YWGQGTL
hc45:                       T FLOITTIIYGMD      S
                                    CDR H3
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[†]The amino acids are represented by the one-letter code. When they are not indicated, HC45 Fab residues are identical to BH151 Fab ones. The residues belonging to the Complementarity Determining Regions (CDRs) are underlined.

complex was solved by molecular replacement using the AMoRe package.[12] The atomic coordinates of uncomplexed X31 BHA (PDB entry 1HGF) and of Hyhel5 Fab (PDB entry 2IFF) were used as starting models; Hyhel5 was chosen from the Fabs of known structure in the Protein Data Bank[13] because its light and heavy chains belong to the same sub-classes as BH151 (both IgG1,k) and display the greatest sequence identity with those of BH151.

The molecular replacement search was performed using data from 10 Å to 4 Å and three independent bodies: BHA, the constant domain dimer and the variable domain dimer of the Fab (excluding the CDR regions); splitting the Fab models avoids possible inconsistencies between the structures of the model and of the Fab in the crystal that are due to the known variability of the Fab elbow angle.[14] A clear solution for all three bodies came out of molecular replacement calculations: the correlation coefficient between calculated and observed structure factors after rigid body refinement was 0.55. 2Fo-Fc and Fo-Fc maps were calculated with the molecular replacement model and allowed us to place the residues corresponding to the BH151 sequence (except in the CDR regions) in the electron density. After a few cycles of positional refinement using XPLOR, the CDR regions were built into the electron density, using canonical structures as a guide, except for the CDR3 of the heavy chain which was built de novo. The final model was determined after alternate cycles of positional/temperature factor refinement using X-PLOR[15] and manual rebuilding using O.[16] The final model contains 7,252 non-hydrogen atoms and 108 water molecules, its geometry was checked with Procheck;[17] data processing and refinement statistics are shown in Table II.

TABLE II. Data Processing and Refinement Statistics

Data collection statistics	
Space group	P321
Unit cell	a = b = 138.15 Å; c = 129.5 Å α = β = 90°; γ = 120°
Resolution limit	2.8 Å
Observations	152,633
Unique reflections	49,210
Completeness[a]	99% (99%)[a]
Redundancy[a]	3.1 (3.0)[a]
Rmerge[a]	6.8% (33.6%)[a]
Refinement statistics	
Resolution range	7.0 Å–2.8 Å
Rfactor	19.6%
Rfree	29.8%
r.m.s.d. from ideal stereochemistry	
Bond lengths (Å)	0.013
Bond angles (°)	1.9

[a]Values in parentheses apply to the last resolution shell (2.85 Å–2.8 Å)

Primary Sequence Determination of the BH151 Antibody

The BH151 hybridoma resulted from fusion of spleen cells with NS1/1-Ag4-1 myeloma cells.[18] Cytoplasmic RNA was extracted from 10^7 hybridoma cells by standard procedures.[19] cDNAs were prepared in 50 μl reaction mixes using M-MLV reverse transcriptase with oligo $(dT)_{12-18}$ (all supplied by Gibco BRL, Gaithersburg, MD). The heavy chain fragment (~750 bp) was amplified using a mixture of 12 primers[20] in combination with Cγ2 (TATG-

CAAGGCTTACAACCACA). Kappa light chains (~450 bp) were amplified using a set of 11 primers each in combination with MKC.[20] PCR was performed in 100 μl mixes containing 10 mM Tris pH 8.8, 50 mM KCl, 2 mM $MgCl_2$, 250 μM each dNTP, 50 μg/ml Carageenan (Sigma Chemical Company, St. Louis, MO), 0.1 μM each primer, 2 μl cDNA product, 2 units Amplitaq (Cetus, Norwalk, CT) and 0.125 unit Pfu polymerase (Stratagene, La Jolla, CA). Mixes were incubated in a Stratagene Robocycler programmed for: 96°C/2 min, 55°C/1.5 min, 72°C/2 min × 1; 96°C/1.5 min, 55°C/1.5 min, 72°C/2 min × 30; 55°C/1.5 min, 72°C/5 min × 1. Products were purified on 0.8% SeaPlaque agarose gels (FMC) and bands excised for extraction with Millipore 0.45 μm filter units (Cat no: UFC3 OHV00) according to manufacturers instructions. After resuspension in 50 μl water, 2 μl aliquots were sequenced using PCR- and internal-primers with Amplitaq cycle-sequencing kits (ABI) and analyzed on an ABI 373A. Resulting sequences were assembled and translated using Staden software.[21] Two kappa chain fragments were generated using primers MKV-5 and MKV-2 which hybridize with the leader sequences.[20] Both products were sequenced and that generated with MKV-2 was found to contain a nucleotide deletion in its CDR3 coding region which results in a premature termination signal some 30 bases downstream. The alternate chain (generated with MKV-5) was assumed to be present in the functional antibody. Sequencing revealed that the heavy chain fragment was generated by pairing of primers MHV-7[20] and Cγ2. Sequences have been deposited with EMBL (accession numbers AJ251890 (γ) and AJ251891 (κ)).

Measurements of the Affinities of IgGs for the Ectodomain of HA

The measurements were performed with a BIAcore™ (Pharmacia Biosensor AB, Uppsala, Sweden), at 22°C, using an HBS buffer (150 mM NaCl, 3.4 mM EDTA, 10 mM Hepes pH 7.6) and flow-rates of 10 μl/min. Rabbit antibodies specific for the Fc fragment of mouse immunoglobulins (RamFc, Pharmacia) were coupled to CM5 sensor chips with the amine coupling kit (Pharmacia) as described.[22] These molecules were then used as a capturing ligand for BH151 and HC45 antibodies. The antibodies (at concentrations 10–20 μg/ml) were continuously injected until the spectrogram baseline was stabilized. The membrane-distal domain of HA (ca. 40 μl at concentrations ranging from 5 to 50 μg/ml) was injected and monitored for association and dissociation rates. The kinetic data were analyzed with the BIAevaluation 2.1 software and curves were corrected for the effects of non specific binding and difference of refractive index of the antigen molecules (corresponding to the signal generated in the absence of anti-HA antibodies). The relative affinities of whole antibodies for the monovalent ectodomain of HA have been evaluated as the ratio of the association and dissociation rates.[22] Regeneration was performed using 100 mM HCl, 0.05% Tween, leaving the RamFc molecules intact and allowing another cycle of measurements.

Structural Comparisons and Superpositions

The epitopes of the two antibodies on the BHA molecule were defined as follows: a BHA residue was considered to belong to the epitope if it is significantly buried in the complex (i.e., the difference between its solvent-accessible surface area in the free BHA molecule and in the complex is larger than 7 $Å^2$), and if at least one atom of the residue makes an atomic contact with an atom of the Fab fragment, at a distance compatible with either a hydrogen bond, a salt bridge or a Van der Waals interaction.[1] Accessible surface calculations were made according to the algorithm of Shrake and Rupley,[23] using a 1.4 Å radius probe. Superposition of atomic models was done using the algorithm of Kabsch.[24]

Coordinates

Coordinates have been deposited in the Protein Data Bank (accession code 1EO8).

RESULTS

Overview of the BH151 Fab–X31 HA Complex

The structure of the BH151 Fab–X31 HA complex was determined at 2.8-Å resolution. The BH151 epitope comprises 14 residues; all of these but one are located at the base of the eight-stranded antiparallel β-sheet structure that constitutes the membrane-distal domain of HA (Fig. 1a). 1,520$Å^2$ accessible surface area is buried upon complex formation. There are eight hydrogen bonds in the interface involving Fab and HA atoms and two additional hydrogen bonds are mediated by water molecules buried in the interface. These figures are in the range observed in antibody-antigen complexes.[25]

There is no change in the overall X31 HA structure upon complex formation as compared either to the uncomplexed molecule or to X31 HA in the complex with the Hc45 Fab (the root mean square deviation (r.m.s.d.) of the atomic positions of the peptide backbone atoms is 0.49 Å and 0.56 Å, respectively). Two significant local changes occur in HA upon X31 HA–Fab BH151 complex formation. The first one is a 4-Å movement of one of the Asp 63 carboxylate oxygens involving a 110° rotation of the χ_1 torsion angle. This is due to a change in the environment of this residue which establishes a salt bridge with the Fab residue H94 Arg in the complex. The second change is a movement of residue HA_1 92 Lys which prevents steric hindrance with the Fab. The lysine side chain changes its orientation and the Cα atom moves by 1.1 Å from its position in uncomplexed X31 HA; this movement of the main chain is completely dampened two residues away from HA_1 92 Lys. The local changes of HA structure upon X31 HA–BH151 Fab complex formation may account, at least in part, for the difficulties encountered in the prediction of the complex structure from those of uncomplexed X31 HA and BH151 Fab.[26]

All these features of the BH151 Fab–X31 HA complex are shared with the HC45 Fab–X31 HA complex. In particular, very similar structural changes of the same side chains were observed upon HC45 Fab–X31 HA complex formation[6] and six of the eight hydrogen bonds made

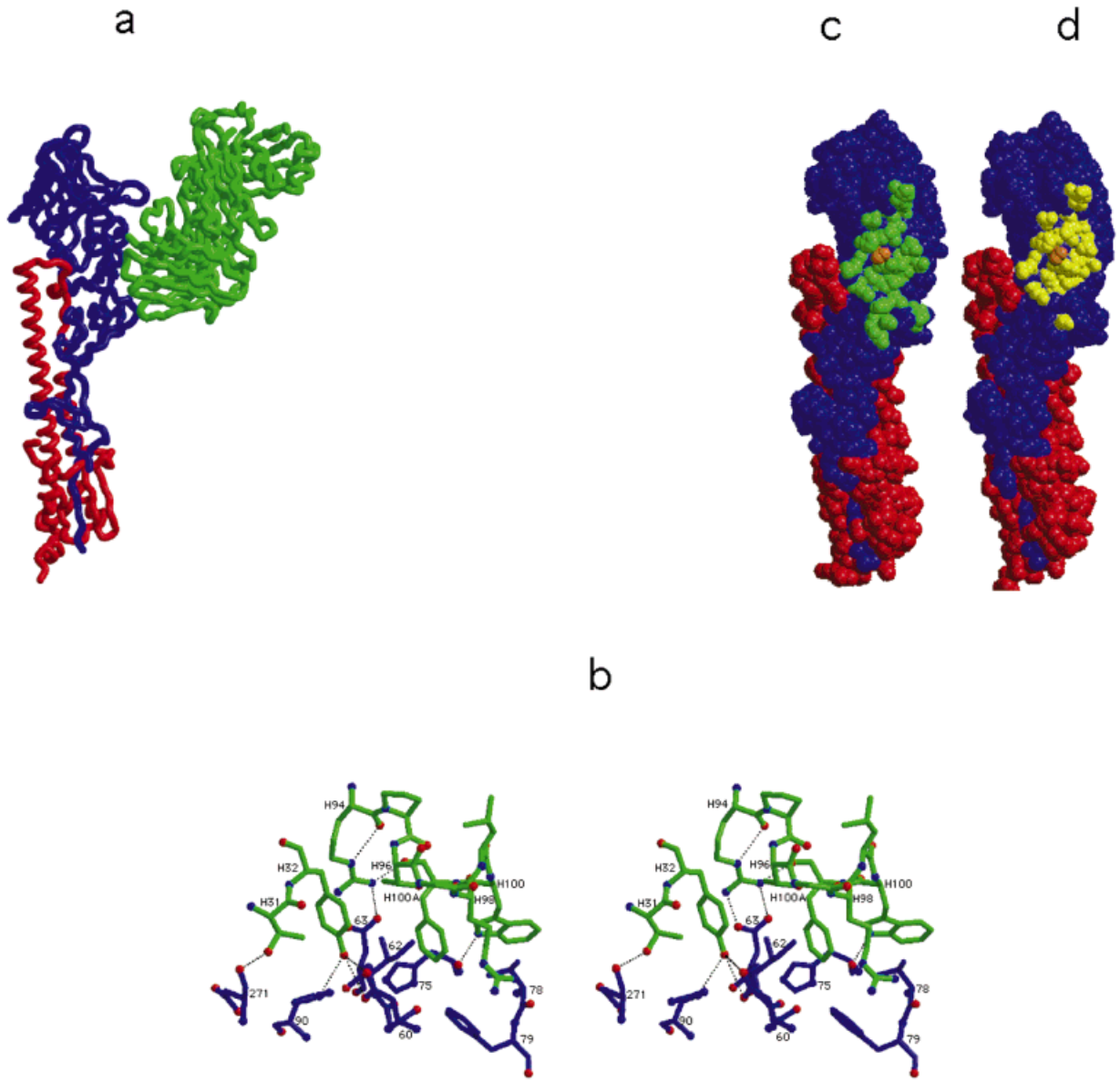

Fig. 1. X31 HA–BH151 Fab and X31 HA–HC45 Fab complexes. **a.** ribbon diagram of the BHA X31–BH151 Fab complex. One BHA monomer is shown (HA_1 in blue, HA_2 in red) and the Fab is in green. All figures were drawn with Molscript[43] and Raster3D.[44] **b.** stereo view of the BH151–HA interface: HA_1 residues are in blue and Fab residues are in green. Nitrogen and oxygen atoms are color-coded according to their atomic type. Hydrogen bonds between antigen and antibody, and those stabilizing the conformation of the side chain H94 (see text) are shown as dotted lines. For clarity, only those residues most buried at the antibody–antigen interface are displayed: they are involved in 70% of the atomic pair contacts between antibody and antigen. **c.** and **d**- HA epitopes recognized by HC45 and BH151. The BHA monomer (HA_1 blue, HA_2 red) is pictured as a CPK model. The epitopes (green and yellow) recognized by HC45 and BH151 are presented in (c) and (d), respectively. Residue HA_1 63 (common to both epitopes and involved in the sole salt bridge between the two complexes) is highlighted in orange.

in the BH151–X31 HA complex are identical in HC45 Fab–X31 HA, including the only salt bridge that links HA and the Fabs (HA_1 63 Asp–Fab H 94 Arg).

Comparison of the BH151 and Hc45 Epitopes

The HA residues that constitute the HC45 and BH151 epitopes are listed in Table III. BH151 interacts with 14 HA residues, 13 of which are also in the HC45 epitope. The common part of the two epitopes consists of three continuous segments of the HA_1 polypeptide chain. The first segment comprises residues 59–63 of HA_1; mutations of residue 63 to Asn or to Tyr allow the virus to escape neutralization by HC45 or BH151, respectively.[10] The other two segments comprise residues 74–79 and 90–94 of HA_1. The three segments account for 91% of the atomic pair contacts involved in the BH151–X31 HA interface.

TABLE III. Atomic Contacts Between Antibody and HA in the X31 HA · HC45 Fab and X31 HA · BH151 Fab Complexes†

HC45 Fab residues	Number of atomic contacts	BHA residues	Number of atomic contacts	BH151 Fab residues
H100a**I**	1	59**L**	1	H98**R**
H32**Y,** H100**T**	7	60**D**	5	H32**Y**
H32**Y,** H94**R,** H96**L**	6	62**I**	17	H32**Y,** H96**T,** H100a**F**
H2**V,** H94**R**	9	63**D**	11	H2**V,** H94**R,** H100a**F,** H102**Y**
L56**S**	1	74**P**	1	H100**W**
L56**S**	1	75**H**	4	H100**W**
L49**Y,** H100a**I,** H100b**I**	11	78**V**	19	L49**Y,** H98**R;** H100**W,** H100a**F**
H100a**I**	3	79**F**	3	H100a**F**
H31**T**	3	90**R**	4	H32**Y,** H31**T**
H27**Y,** H28**T**	2	92**K**	5	H26**G,** H27**Y**
H1**Q,** H2**V**	13	94**F**	9	H1**Q,** H2**V,** H26**G**
H31**T**	4	271**D**	5	H28**S,** H31**T**
H53**Y**	3	273**P**		
H99**T**	6	274**I**		
H99**T**	1	275**D**		
H53**Y**	2	49**G**		
H53**Y**	5	50**K**	1	H52**L**
L81**E**	1	143**P**		
		142**G**	1	L57**G**
Total number of contacts	79		86	

†The amino acids are represented in bold by the one-letter code.

The chemical, stereochemical and electrostatic environments presented to the two Fabs on HA are thus very similar.

The only difference observed between the HC45 and BH151 epitopes corresponds to antibody contacts with a fourth stretch of HA_1 polypeptide chain comprising residues 271–275; HC45 makes contacts with residues 271, 273, 274, and 275, whereas BH151 only contacts HA_1 271. Therefore the major part of the BH151 epitope on the hemagglutinin surface is included in the HC45 epitope (Fig. 1c,d); consequently, the HA surface buried upon complex formation is smaller in the case of BH151 (760 Å^2) than in that of HC45 (900 Å^2). The difference between the two epitopes albeit small has antigenic significance: a mutant influenza virus with a hemagglutinin carrying the HA_1 Pro273Ile substitution[27] is not recognized by the HC45 antibody whereas it still binds to BH151 (data not shown).

Comparison of the HC45 and BH151 Paratopes

The two paratopes are mostly composed of residues of the heavy chain complementarity determining regions (CDRs): residues of the light chain account for 2 contacts out of 86 in the BH151–X31 HA complex and for 6 contacts out of 79 in the HC45–X31 HA complex respectively. The light chain residues of the paratopes are on the edge of the L2 hypervariable loop[28] and, in the HC45 Fab, include residue L81, which is part of the framework.

Some residues of the paratopes (L49, H1, H2, H27, H31, H32, and H94) are strictly conserved in the two antibodies, spatially superimposable in the structures of the two complexes and involved in identical interactions with the antigen. Of these, residues H27, H31, and H32 belong to the heavy chain hypervariable loop H1 which adopts the same canonical structure in both complexes.[29,30] H94 Arg, which forms a salt bridge with HA_1 63 Asp in the two complexes, is very well conserved in murine antibodies and its position is very often fixed by an intra-chain salt bridge between H94 and H101, most often an aspartic acid in murine antibodies;[31,32] this is what is observed in the HC45 antibody. In the BH151 antibody, H101 is a serine; in this case, the position of the H94 Arg side chain is fixed by two hydrogen bonds the Arg H94 guanidium group establishes with H3 CDR main-chain atoms (H94 Arg Nε-H94 O (3.2 Å) and H94 Nη2-H100A O (2.6Å); Fig. 1b).

Despite the similarities that have been listed above, 70% of the interatomic contacts between X31 HA and BH151 involve residues of BH151 that differ in the two Fabs. Examination of the surface areas of the X31 HA molecule buried in the Fab complexes provides examples of the consequences of the different contacts in both complexes: in the BH151 Fab–X31 HA complex, HA_1 60 Asp is partially buried (accessible surface area (asa) = 25 Å^2) whereas this same residue is almost completely buried in the Hc45 Fab- X31 HA complex (asa = 3 Å^2); by contrast, HA_1 63 Asp is almost completely buried in the BH151 Fab–X31 HA complex (asa = 2 Å^2) but is more accessible in the Hc45 Fab–X31 HA complex (asa = 12 Å^2).

In complexes, both antibodies show very similar orientation and position with respect to HA (when HA molecules in the two complexes are superimposed, an 8.3° rotation relates BH151 and HC45 Fvs). Therefore, the differences in intermolecular interactions in the HC45–X31 HA and BH151–X31 HA complexes arise from differences in their CDRs and these can be classified in two categories. Firstly, there are amino-acid differences in the CDR loops that contact the antigen and have the same canonical structure in both Fabs (CDRS H1 and H2). Secondly, the conforma-

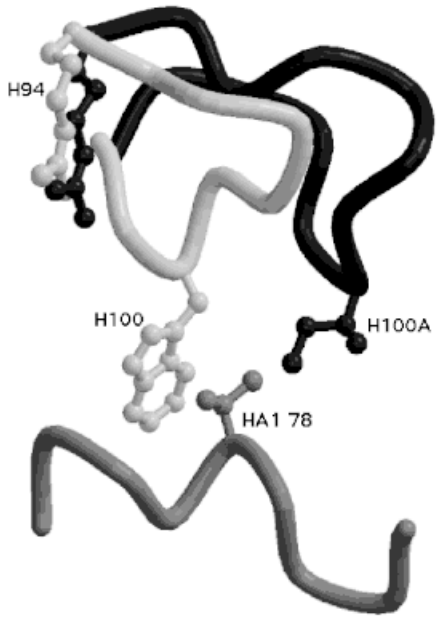

Fig. 2. Conformations of the H3 CDR in HC45 and BH151. H3 CDR polypeptide chains are shown in pale grey (BH151) and black (HC45) after superposition of the two complexes. Residue H94 Arg, on H3 CDR edge, conserved in the two Fabs and spatially superimposable in the two complexes is also displayed. Due to their different conformations, the H3 CDRs in the two Fabs interact differently with HA. Residue HA_1 78 (in grey, the HA_1 74–82 polypeptide chain is also displayed) provides an example of these differences: the Cαs of the Fab residues it interacts with predominantly are distant by 8 Å when the two complexes are superimposed.

tions and sequences of the H3 CDR loops (Fig. 2) differ; these contribute 33% and 25% to the Fab surface buried in the HC45 and BH151 complexes, respectively. As a result of these differences, the interactions that the H3 CDR loops make with HA differ completely: firstly, the only HA residue with which the two loops establish a hydrogen bond differs in the two complexes and secondly, although most of the Van der Waals contacts between the two loops and HA are with the same HA residues (HA_1 62 and HA_1 78), they are established by different H3 CDR residues: H100A Ile and H100B Ile in HC45–X31 HA complex; H100 Trp and H100A Phe in BH151–X31 HA complex.

DISCUSSION

Several instances of the binding of two proteins to nearly identical surfaces have been described structurally. Most of them concern complexes between antigen and antibody, where one of the partners is shown to be able to bind to structural variants of the other; in most cases, these variants differ from their wild-type counterpart by only one or a few amino-acid differences: examples include complexes between antibodies and antigenic variants of influenza virus hemagglutinin[33] and neuraminidase,[34] as well as complexes between anti-lysozyme antibodies and natural[35–37] or engineered[38–40] variants of lysozyme. In a few cases, authors have also examined structurally how two distinct antibodies—thus with much more different primary sequences than the aforementioned variants—can recognize highly overlapping epitopes on an antigen molecule; among these, Fields et al. have compared complexes of a single antibody with lysozyme and with an antiidiotype antibody and shown that the same atomic interactions are established in both instances with identical residues of the partner common to the two complexes;[4] because of the different folds of the lysozyme and the antiidiotypic antibody, it is not possible to establish a simple correspondence between the atoms that interact with a common atom of the antibody. In another instance, two antibodies have been found to contact influenza neuraminidase through a common set of residues; in this case a 72° rotation was required to relate the two antibodies in the two complexes and most of the interactions of neuraminidase residues differ sterically and chemically in both complexes.[3] In a third well-documented example,[2] two antibodies were shown to bind to very similar epitopes on HEL lysozyme: in that case, however, the affinities of the antibodies for the antigen differ by three orders of magnitude, though the structural reasons for this are not obvious. The case of the X31 HA complexes with HC45 and BH151 Fabs differs from the three aforementioned ones: the two antibodies have the same fold, bind to the same epitope in similar orientations and display the same affinity for their target antigen. Nevertheless, this result is obtained despite the fact that two thirds of the interactions are different in the two complexes.

The H3 CDR loops contribute a large proportion of the contacts that differ in the two complexes (44% in the HC45–X31 HA complex and 81% in the BH151–X31 HA complex). Since the Van der Waals interactions of the two H3 CDR loops, which have different conformations, are established with the same HA residues, one might expect that the surface of at least one of them is not complementary to the HA surface it contacts. Indeed, there are two solvent cavities in the HC45–X31 HA complex and these border H3 CDR loop residues. This lack of complementarity is also reflected by the value of the shape complementarity index:[41] 0.63 between HC45 H3 CDR and HA and 0.82 in the BH151–HA complex. It may be that the lack of complementarity between antibody and antigen surfaces that is apparent here allows some flexibility in the selection of antibodies that bind to a given antigen. This flexibility might in turn provide redundancy in the humoral immune response, as shown in this paper.

Antibodies arise through affinity maturation of the recombination products of germline genes. In this process redundancy may be unavoidable and it may also provide advantages to the host. Some of these would result from features of the antibody–antigen interactions such as those we observed in the BH151 and HC45 complexes; since these antibodies recognize their HA epitope in different ways, they are tolerant to different mutations in it and the resulting humoral immune response may allow broader and longer lasting protection against viral infection than if a single highly specific antibody had been produced. Inter-

estingly, this redundancy might not be restricted to the humoral immune response: in a recent paper, Ding et al.[42] have shown that two different T cell receptors can recognize the same MHC/peptide complex in structurally very similar ways—a situation very much alike the one described in this paper for antigen–antibody complexes. It is thus conceivable that the advantages provided by redundancy are profitable both to the B-cell and to the T-cell arms of the immune system.

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REFERENCES

1. Bentley GA, Boulot G, Riottot MM, Poljak RJ. Three-dimensional structure of an idiotope-anti-idiotope complex. Nature 1990;348: 254–257.
2. Braden BC, Souchon H, Eiselé JL, et al. Three-dimensional structures of the free and the antigen-complexed Fab from monoclonal anti-lysozyme antibody D44.1. J Mol Biol 1994;243:767–781.
3. Malby RL, Tulip WR, Harley VR, et al. The structure of a complex between the NC10 antibody and influenza virus neuraminidase and comparison with the overlapping binding site of the NC41 antibody. Structure 1994;2:733–746.
4. Fields BA, Goldbaum FA, Ysern X, Poljak RJ, Mariuzza RA. Molecular basis of antigen mimicry by an anti-idiotope. Nature 1995;374:739–742.
5. Wiley DC, Skehel JJ. The structure and function of the haemagglutinin membrane glycoprotein of influenza virus. Annu Rev Biochem 1987;56:365–394.
6. Fleury D, Barrere B, Bizebard T, Daniels RS, Skehel JJ, Knossow M. A complex of influenza hemagglutinin with a neutralizing antibody that binds outside the virus receptor binding site. Nat Struct Biol 1999;6:530–534.
7. Skehel JJ, Schild GC. The polypeptide composition of influenza A viruses. Virology 1971;44:396–408.
8. Skehel JJ, Bayley PM, Brown EM, et al. Changes in the conformation of influenza virus haemagglutinin at the pH optimun of virus mediated membrane fusion. Proc Natl Acad Sci USA 1982;79:968–972.
9. Bizebard T, Mauguen Y, Petek F, Rigolet P, Skehel JJ, Knossow M. Crystallization and preliminary X-ray diffraction studies of a monoclonal antibody Fab fragment specific for an influenza virus hemagglutinin and of an escape mutant of that hemagglutinin. J Mol Biol 1990;216:513–514.
10. Gigant B, Fleury D, Bizebard T, Skehel JJ, Knossow M. Crystalization and preliminary X-ray diffraction studies of complexes between an influenza hemagglutinin and Fab fragments of two different monoclonal antibodies. Proteins 1995;23:115–117.
11. Otwinowsky Z, Minor W. Processing of X-ray diffraction data collected in oscillation mode. In: Carter CWJ, Sweet RM, editors. Methods in enzymology. New York: Academic Press; 1997. p 307–325.
12. Navaza J. AMoRe: an automated package for molecular replacement. Acta Crystallogr A 1994;50:157–163.
13. Bernstein FC, Koetzle TF, Williams JGB, et al. The protein data bank: a computer-based archival file for macromolecular structures. J Mol Biol 1977;112:535–542.
14. Wilson IA, Stanfield RL. Antibody-antigen interactions: new structures and new conformational changes. Curr Opin Struct Biol 1994;4:857–867.
15. Brünger AT. X-PLOR (Version 3.1) Manual. New Haven: Yale University; 1992.
16. Jones TA, Zhou J-Y, Cowan SW, Kjeldgaard M. Improved methods for building protein models in electron density maps and the location of errors in these models. Acta Crystallogr A 1991;47:110–119.
17. Laskowski RA, McArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Cryst 1993;26:283–291.
18. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495–497.
19. Sambrook J, Ftitsch EF, Maniatis T. Molecular cloning, a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1989. 1223 p.
20. Jones ST, Bendig MM. Rapid PCR-cloning of full-length mouse immunoglobulin variable regions. Bio/Technology 1991;9:88–89.
21. Gleeson TJ, Staden R. An X-windows and UNIX implementation of our sequence analysis package. Comp Appl Biosci 1991;7:398.
22. Karlsson R, Michaelsson A, Mattsson L. Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. J Immunol Methods 1991;145:229–240.
23. Shrake A, Rupley JA. Environment and exposure to solvent of proteins atoms. Lysozyme and insulin. J Mol Biol 1973;79:351–371.
24. Kabsch W. A solution for the best rotation to relate two sets of vectors. Acta Crystallogr A 1976;32:922–923.
25. Davies DR, Chacko S. Antibody structure. Acc Chem Res 1993;26: 421–427.
26. Vakser IA. Evaluation of the GRAMM low-resolution docking methodology on the hemagglutinin-antibody complex. Proteins 1997;Suppl 1:226–230.
27. Smith CA, Barnett BC, Thomas DB, Temoltzin-Palacios F. Structural assignment of novel and immunodominant antigenic sites in the neutralizing antibody response of CBA/Ca mice to influenza hemagglutinin. J Exp Med 1991;173:953–959.
28. MacCallum RM, Martin ACR, Thornton JM. Antibody-antigen interactions: Contact analysis and binding site topography. J Mol Biol 1996;262:732–745.
29. Chothia C, Lesk AM. Canonical structures of hypervariable regions of immunoglobulins. J Mol Biol 1987;196:901–917.
30. Chothia C, Lesk AM, Tramontano A, et al. Conformations of immunoglobulin hypervariable regions. Nature 1989;342:877–883.
31. Kabat E. Sequences of proteins of immunological interest. Washington, DC: National Institute of Health; 1991. 1857 p.
32. Hsu E, Steiner LA. Primary structure of immunoglobulins through evolution. Curr Opin Struct Biol 1992;2:422–431.
33. Fleury D, Wharton SA, Skehel JJ, Knossow M, Bizebard T. Antigen distortion allows influenza virus to escape neutralization. Nat Struct Biol 1998;5:119–123.
34. Tulip WR, Varghese JN, Webster RG, Laver WG, Colman PM. Crystal structures of two mutant neuraminidase-antibody complexes with amino acid substitutions in the interface. J Mol Biol 1992;227:149–159.
35. Chitarra V, Alzari PM, Bentley GA, et al. Three-dimensional structure of a heteroclitic antigen-antibody cross-reaction complex. Proc Natl Acad Sci USA 1993;90:7711–7715.
36. Chacko S, Silverton E, Smith-Gill S, et al. Refined structures of bobwhite quail lysozyme uncomplexed and complexed with the HyHEL-5 Fab fragment. Proteins 1996;26:55–65.
37. Braden BC, Fields BA, Ysern X, et al. Crystal structure of the complex of Fv D1.3 and turkey egg white lysozyme: a novel conformational change in antibody CDR-L3 selects for antigen. J Mol Biol 1996;257:889–894.
38. Chacko S, Silverton E, Kam-Morgan L, Smith-Gill S, Cohen G, Davies D. Structure of an antibody-lysozyme complex: unexpected effect of a conservative mutation. J Mol Biol 1995;245:261–274.
39. Fields BA, Goldbaum FA, Dall'Acqua W, et al. Hydrogen bonding and solvent structure in an antigen-antibody interface. Crystal structures and thermodynamic charcterisation of three Fv mutants complexed with lysozyme. Biochemistry 1996;35:15494–15503.
40. Dall'Acqua W, Goldman ER, Lin W, et al. A mutational analysis of binding interactions in an antigen-antibody protein-protein complex. Biochemistry 1998;37:7981–7991.
41. Lawrence MC, Colman PM. Shape complementarity at protein/protein interfaces. J Mol Biol 1993;234:946–950.
42. Ding YH, Smith KJ, Garboczi DN, Utz U, Biddison WE, Wiley DC. Two human T cell receptors bind in a similar diagonal mode to the HLA-A2/Tax peptide complex using different TCR amino acids. Immunity 1998;8:403–411.
43. Kraulis P. MOLSCRIPT: a program to produce both detailed and schematic plots of proteins structures. J Appl Crystallogr 1991;24: 924–950.
44. Merritt EA, Bacon DJ. Raster3D: Photorealistics molecular graphics. In: Carter CWJ, Sweet RM, editors. Macromolecular crystallography (Part B). New York: Academic Press; 1997. p 505–524.