Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY REVIEW ARTICLE

Discovery of protective B-cell epitopes for development of antimicrobial vaccines and antibody therapeutics

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doi:10.1111/imm.12213
Received 20 October 2013; revised 05 November 2013; accepted 08 November 2013.

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Summary

Protective antibodies play an essential role in immunity to infection by neutralizing microbes or their toxins and recruiting microbicidal effector functions. Identification of the protective B-cell epitopes, those parts of microbial antigens that contact the variable regions of the protective antibodies, can lead to development of antibody therapeutics, guide vaccine design, enable assessment of protective antibody responses in infected or vaccinated individuals, and uncover or localize pathogenic microbial functions that could be targeted by novel antimicrobials. Monoclonal antibodies are required to link *in vivo* or *in vitro* protective effects to specific epitopes and may be obtained from experimental animals or from humans, and their binding can be localized to specific regions of antigens by immunochemical assays. The epitopes are then identified with mapping methods such as X-ray crystallography of antigen–antibody complexes, antibody inhibition of hydrogen–deuterium exchange in the antigen, antibody-induced alteration of the nuclear magnetic resonance spectrum of the antigen, and experimentally validated computational docking of antigen–antibody complexes. The diversity in shape, size and structure of protective B-cell epitopes, and the increasing importance of protective B-cell epitope discovery to development of vaccines and antibody therapeutics are illustrated through examples from different microbe categories, with emphasis on epitopes targeted by broadly neutralizing antibodies to pathogens of high antigenic variation. Examples include the V-shaped Ab52 glycan epitope in the *O*-antigen of *Francisella tularensis*, the concave CR6261 peptidic epitope in the haemagglutinin stem of influenza virus H1N1, and the convex/concave PG16 glycopeptidic epitope in the gp120 V1/V2 loop of HIV type 1.

Keywords: antibodies; antigens/peptides/epitopes; human; structural biology/crystallography.

Introduction

Antibodies participate in the immune response to microbes by interacting with surface or secreted microbial antigens. Each antibody binds to an epitope, defined as the three-dimensional structure of the amino acids, sugars or other residues in an antigen that can be contacted by the variable (V) regions of an antibody.[1,2] The most protective antibodies against viruses and toxins target those epitopes on microbial antigens that interact with host receptors to invade host cells, and block invasion. They are therefore neutralizing antibodies.[3–7] The most protective antibodies against extracellular microbes target carbohydrate epitopes on capsular or other cell surface

Abbreviations: bNAb, broadly neutralizing antibody; cryoEM, cryoelectron microscopy; DXMS, deuterium exchange/mass spectrometry; HIV, human immunodeficiency virus; IC_{50}, half-maximal inhibitory concentration; LPS, lipopolysaccharide; NMR, nuclear magnetic resonance; RSV, respiratory syncitial virus; STD, saturation transfer difference; scFv, single-chain variable fragment

polysaccharides,[7] enabling microbe-killing through Fc-mediated effector mechanisms: complement-dependent killing, phagocytosis, and antibody-dependent cellular cytotoxicity.[8] These antibody functions are major determinants for the success of vaccination at preventing infectious disease.

Currently licensed vaccines are directed against: viruses, including measles, mumps, rubella, rabies, poliovirus, varicella virus, papillomavirus; extracellular bacteria, such as *Streptococcus*, *Meningococcus* and *Haemophilus influenzae* type b; and bacterial toxins, such as diphtheria and tetanus toxins (http://www.cdc.gov/vaccines/schedules/).[7] These vaccines work by inducing microbe-specific or toxin-specific protective antibodies,[3–7] although T cells are required for somatic hypermutation to produce high-affinity IgG and IgA antibodies, and are important for the generation of B-cell memory.[9]

The essential role of antibodies against infectious diseases is further evidenced by the effectiveness of passively administered intravenous immunoglobulin for long-term treatment of immune deficiencies like X-linked agammaglobulinaemia and hyper-IgM syndrome.[10] Specific immunoglobulins, such as hepatitis B immunoglobulin (http://www.cdc.gov/mmwr/preview/mmwrhtml/00022736.htm), tetanus immunoglobulin[11] and rabies immunoglobulin[12] are successfully used as post-exposure prophylaxis. In recent years, monoclonal antibodies (mAbs) have been used for prophylaxis against infections with respiratory syncytial virus (RSV)[13] and rabies,[12] and for treatment of inhalational anthrax.[14]

Despite the clinical success of licensed vaccines and passively administered antibody preparations, the development of effective vaccines and therapeutic antibodies against viruses and extracellular bacteria that exhibit high antigenic variation, against non-viral intracellular pathogens like fungi and intracellular bacteria, and against microbes with multi-stage life-cycles like protozoan and metazoan parasites has proven to be challenging.[6,7] Furthermore, vaccines, immunotherapeutics and other antimicrobials are needed for prophylaxis and treatment of diseases caused by emerging and re-emerging infectious agents and potential agents of bioterrorism, including naturally evolving or intentionally engineered drug-resistant variants (http://www.niaid.nih.gov/topics/emerging/pages/list.aspx).

Development of vaccines and antibody therapeutics is greatly aided by identification of microbial epitopes targeted by protective antibodies – protective B-cell epitopes. This knowledge can lead directly to development of therapeutic antibodies, as it has for infections with RSV,[13] rabies[12] and anthrax.[14] It could also guide the design of subunit vaccines to include protective epitopes and exclude any identified pathogenic epitopes that might induce cross-reactive autoimmune[15] or infection-enhancing antibodies.[16–18] Furthermore, known protective B-cell epitopes could be used to monitor the quality of antibody responses in infected or vaccinated individuals.[19–21] Lastly, identification of protective B-cell epitopes may uncover or localize pathogenic microbial functions which, as has been suggested[22,23] and demonstrated,[24] may lead to the development of novel antimicrobials. We review here recent approaches to discovery of protective microbial B-cell epitopes, based largely on examples tabulated at the end of the article.

Strategies to identify and characterize antimicrobial protective mAbs

Identification of protective B-cell epitopes requires protective mAbs which, through their interaction with antigen, prevent or contribute to prevention of microbial pathogenesis. How are protective mAbs obtained? In some cases one or more protective antigens in a given microbe are known and mAbs to a target antigen, or fragments thereof, are generated and tested for efficacy against the microbe *in vivo* and/or *in vitro*. In other cases no knowledge of protective microbial antigens is available, or such knowledge is ignored, and a collection of mAbs to microbial surface components and/or secreted products is first generated and divided into groups in which all group members bind to the same antigen in immunoassays. The target antigens are identified and representative mAbs against each antigen are then tested for efficacy against the microbe.[25]

The mAbs are derived from lymphocyte-containing samples obtained from immunized experimental animals, most often mice, usually by hybridoma production,[25–32] or from naturally infected or vaccinated humans by one of several methods. These include: cloning of V region genes from bone marrow or peripheral blood B cells or plasma cells into phage-display[22,33,34] or yeast-display[35] vectors, *in vitro* activation and expansion of memory B cells,[21] cloning of the V region genes from single memory B cells[21,22,36–40] or plasma cells[41,42] into IgG expression vectors, or Epstein–Barr virus transformation of B cells[40,43] optionally followed by fusion with myeloma cells.[44] The target antigen of each mAb is identified by immunochemical assays including ELISA and Western blot analysis on purified candidate microbial antigens.[25,45,46] Whether or not purified (native or recombinant) antigen is available, the protein and/or carbohydrate nature of the target epitope can be determined by pre-treatment of the antigen or antigen mixture with proteases[25] or glycosidases[37,47,48] in ELISA or Western blot. Protein antigens can be identified by proteome microarray analysis, in which reactivity of each mAb to the recombinantly expressed microbial proteins is assessed;[25] or by mass spectrometric analysis of an SDS–polyacrylamide gel band immuneprecipitated from a microbial extract by the mAb[49] or of a mAb-reactive spot

on a two-dimensional gel.[50] Alternatively, mAbs are screened directly in a functional assay such as neutralization,[21,36–38] or memory B cells are selected by FACS for binding to the antigen but not to an antigen variant in which the epitope region of interest has been deleted.[39]

Monoclonal antibodies specific for the same microbial antigen can be subgrouped by the germline genes that encode their VH and VL regions[22,29,38,42,44,51] and by their ability to block each other's binding to the antigen in competition immunoassays.[29,37–40,45,46] Hence, sharing of the same VH, D, JH, VL and JL genes, or even just the same VH and VL genes is reflected in high amino acid sequence homology (http://www.imgt.org/) and indicates specificity for the same epitope. For antigens with sequentially repeating epitopes, as often found in capsular and outer-membrane microbial polysaccharides, mAbs that block each other's binding to the antigen target the same or an overlapping epitope.[29,32,52] Although this is also true for protein antigens, some anti-protein mAbs that block each other's binding to the antigen may do so not by targeting an overlapping epitope but by interfering with each other's binding sterically or allosterically.[38] However, in a group of mAbs specific for the same antigen, those mAbs that do not block each other's antigen-binding, or that show different cross-blocking profiles with third-party mAbs, define different epitopes.

In vivo testing of mAbs for anti-microbial efficacy is done in animal models of infection, including mice,[22,28,29,33,53–55] rats,[36] guinea pigs,[56] non-human primates,[57,58] and humanized mice,[59] in which protective mAbs are identified by their ability to confer or prolong survival or reduce microbial burden. In addition, or alternatively, mAbs are functionally evaluated *in vitro* for their ability to cause killing of target microbes or a reduction in the microbes' replication,[27,30] block host-cell invasion by microbes or their products (neutralize),[21,22,33,36–39,42,44,46,56,58,60–63] block the binding of microbial factors to host components,[30,33] or interfere with assembly of microbial toxins.[64] Use of human immune components in these *in vitro* assays, such as human cell lines,[33,36–38,46,55] can validate results obtained *in vivo* in animal models. Although efficacy indicates that the targeted epitope is protective, lack of efficacy does not necessarily mean that the epitope is non-protective because in addition to epitope specificity the protective efficacy of mAbs depends on their avidity and isotype.[29,52,54] A flow diagram of general strategies for generation and identification of protective mAbs is shown in Fig. 1.

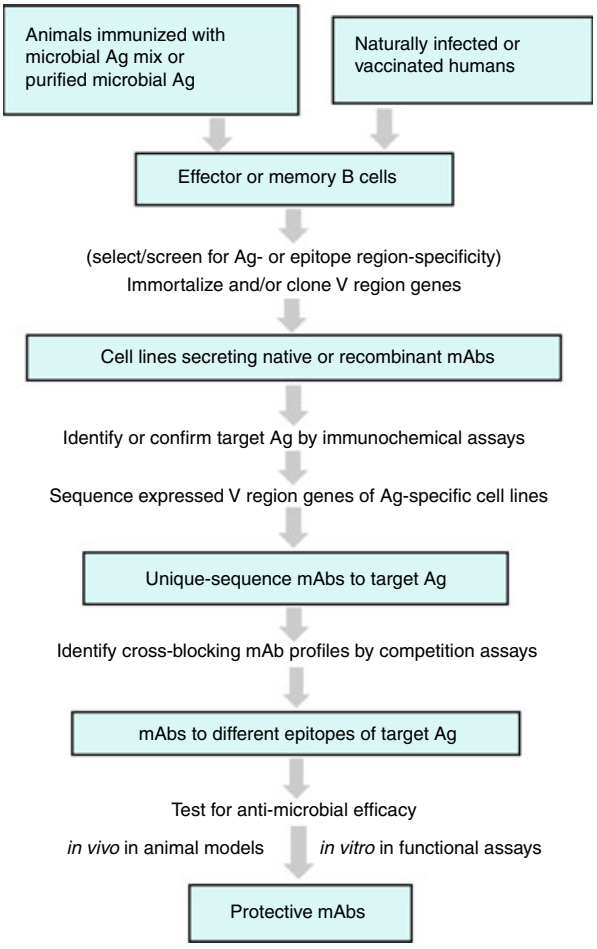

Figure 1. Flow diagram of general strategies for identification of protective monoclonal antibodies (mAbs).

Types, definitions and documentation of microbial B-cell epitopes

Microbial B-cell epitopes are located mainly on the exposed parts of microbial antigens,[65] generally protein and carbohydrate components, and may consist entirely of amino acids in peptidic epitopes, sugar residues in glycan epitopes, or combinations thereof in glycopeptidic epitopes. Many glycan epitopes and a small minority of peptidic epitopes consist of contiguous residues and are, therefore, referred to as continuous (or linear). However, the vast majority of peptidic epitopes are discontinuous, consisting of one to several amino acids that are separated by a few or many non-contact amino acids in the primary sequence but are brought together by the folding of the protein.[2] Therefore, the existence of discontinuous peptidic epitopes depends on a supporting scaffold of non-contact residues. The minimal contiguous amino acid sequence containing all residues of a discontinuous epitope, which is required for proper conformation of the contact residues, has been termed the 'contact residue span' and may range from 20 to 400 amino acids (most frequently 50–79) in native proteins.[66] Hence, a B-cell epitope is defined both by the identities of the contact residues and by their conformation, which is determined by the three-dimensional fold of the contact residue span.

Information on published and investigator-submitted microbial B-cell epitopes can be found at the Immune

Epitope Database and Analysis Resource (IEDB) (http://www.iedb.org/), which houses B-cell and T-cell epitope data and makes them accessible and searchable.[1,67] This includes the contact residues of the epitopes, antibody-binding and protection assays, and tools for mapping of discontinuous epitopes onto three-dimensional antigen structures and for epitope prediction.[67] Among the tens of thousands of documented microbial B-cell epitopes, the vast majority were defined based on antigen-derived synthetic peptides used to induce antibodies in experimental animals. Some of the antibodies to these peptides cross-react with the native and/or recombinant microbial antigen (http://www.iedb.org/), but their affinities for the native microbial antigen are generally several orders of magnitude lower than those of antibodies induced by immunization with the native antigen.[2] Among the discontinuous peptidic and the non-peptidic epitopes only some have been shown to be protective, and no or few B-cell epitopes have been studied for several of the microbes considered to be emerging or re-emerging infectious agents or potential agents of bioterrorism (http://www.iedb.org/).

Methods of B-cell epitope mapping

Epitope mapping refers to identification of the antigen residues that are contacted by a complementary antibody during antigen–antibody binding. As a first step in epitope mapping, the binding of protective mAbs (often referred to as just antibodies) can be localized to specific regions of antigens by testing the reactivity of the mAbs to antigen subunits or fragments.[26,30,68,69] For microbial carbohydrate chains with repeating units, antibodies that bind to unique epitopes at the non-reducing end can be distinguished from those that bind to repeating internal epitopes by their Western blot patterns, where the binding intensity of the latter but not the former increases with increasing chain size. This is exemplified in Fig. 2 for *O*-antigen chains that comprise a capsular polysaccharide and part of the lipopolysaccharide of *Francisella tularensis*, a potential bioterrorism agent.[29,32]

Finer mapping methods for peptidic, glycan and glycopeptidic epitopes include: X-ray crystallography of antigen–antibody (Ag–Ab) complexes, antibody inhibition of hydrogen-deuterium exchange in the antigen, antibody-induced alteration of the nuclear magnetic resonance (NMR) spectrum of the antigen, glycan microarray probing of the antibody, oligosaccharide (glycan) competition with antigen for antibody-binding, selection or screening of antigen-derived proteolytic fragments or peptides for antibody-binding, testing the Ag–Ab reactivity of site-directed (antigen or antibody) or random (antigen) mutants, electron and cryoelectron microscopy of Ag–Ab complexes, and experimentally validated computational docking of Ag–Ab complexes.

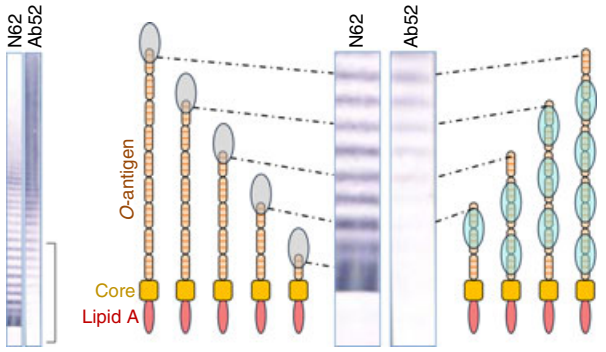

Figure 2. Western blot distinction between unique terminal and repeating internal epitopes of microbial carbohydrate chains with repeating units. The N62 monoclonal antibody (mAb), specific for a terminal epitope in the *O*-antigen (O-Ag) of the *Francisella tularensis* lipopolysaccharide (which consists of variable numbers of a tetrasaccharide repeat, represented as ABCD) binds with equal intensity to short and long lipopolysaccharide chains (relative to the abundance of each chain). But the binding intensity of the Ab52 mAb, specific for a repeating internal epitope, increases with increasing chain length, as more mAb molecules are bound. The basis for the differential binding pattern is illustrated schematically for the (bracketed) lower part of the Western blot, with N62 represented by grey ovals and Ab52 by cyan ovals. Western blot lanes from Lu *et al.*[29]

X-ray crystallographic analysis of Ag–Ab complexes reveals the identities of the contact residues and conformations of both the epitope and the complementary binding-site of the antibody.[21,30,33,42,48,52,62,70–75] It can be applied to any type of antigen, including protein and carbohydrate antigens. In the first step of this method, favourable conditions for nucleation and growth of high-quality crystals of the Ag–Ab complex are identified by testing a wide variety of crystallization solutions. The crystal of choice is exposed in multiple orientations to a beam of monochromatic X-rays, which are scattered by the electrons of its atoms, and the resulting diffraction patterns of spots (or reflections) are recorded. The information in the diffraction data is then used to calculate a three-dimensional electron-density map of the molecules that make up the crystal, in which the known (non-hydrogen) atoms of the antigen and antibody (from primary sequence analysis) are positioned to create a model of the complex. The contact residues that comprise the epitope can be defined by identifying all antigen residues within 4–5 Å of the antibody, a distance used by the Molecular Modeling Database to define contacts.[76,77] X-ray crystallography requires large amounts of highly pure antigen and antibody, usually Fab antibody fragment, and is limited by inability to obtain Ag–Ab co-crystals with some antigens, especially membrane proteins.

Antibody inhibition of hydrogen–deuterium exchange in the antigen, which involves the deuterium exchange – mass spectrometry (DXMS) technology,[78,79] reveals small segments containing contact residues in

protein antigens.[30,44] DXMS exploits the continuous reversible exchange of peptide-amide hydrogens in proteins with water hydrogens, the exchange rate of each hydrogen correlating directly with the extent to which it is exposed (accessible) to solvent. The exchange rates are determined by incubating the protein in buffer with deuterated water (D_2O) for graded time periods followed by proteolysis into overlapping peptides, which are separated chromatographically. The deuterium content of each peptide is then analysed by mass spectrometry to obtain a 'heat' map of the exchange rate for the entire protein. Subtraction of the DXMS heat map of the antibody-bound antigen from the DXMS heat map of the free antigen reveals the sequences(s) of the antigen where deuterium exchange was inhibited by antibody binding. Although the spatial resolution of DXMS is not at the single residue level, the antigen segments comprising the epitope are localized to within a few amino acids.[79] DXMS can be performed with lower-purity preparations of antigen and antibody than X-ray crystallography, does not require crystals, and intact antibody molecules, rather than Fab fragments, can be routinely used.

Antibody-induced alteration of the NMR spectrum of the antigen reveals contact residues of both protein and carbohydrate antigens.[48,80–83] NMR spectroscopy is based on the ability of the nuclei of some isotopes, like ^{1}H, ^{2}H, ^{13}C and ^{15}N, to absorb and re-emit electromagnetic radiation at a specific resonance frequency when placed in a magnetic field (http://www.cis.rit.edu/htbooks/nmr/). The resonance frequency of different nuclei of the same isotope is altered by the electrons of neighbouring atoms. This results in different signals (chemical shifts) relative to a standard reference molecule, generating a spectrum that yields information about the chemical environment of each nucleus, from which its identity can be determined. The complexity of NMR spectra makes it difficult to determine the structure of large Ag–Ab complexes. However, the antigen residues within 5 Å of the antibody (the contact residues) can be determined if the antigen spectrum does not overlap with the antibody spectrum, as will be the case for carbohydrate antigens or for recombinant protein antigens that have been labelled, for example, with ^{2}H, ^{13}C and ^{15}N.[81,84] Subtraction of the NMR spectrum of the antibody-bound antigen from the NMR spectrum of the free antigen is then used to identify contact residues by the changes in chemical shifts that occur when residues exposed to solvent in the free antigen are buried in the Ag–Ab complex. This technique, called saturation transfer difference (STD) NMR, is similar in principle to DXMS except that both peptidic and non-peptidic epitopes can be mapped and the actual contact residues are identified in STD NMR. However, only relatively small antigen fragments can be used for epitope mapping by STD NMR, and labelled recombinant protein fragments are needed.

Oligosaccharides, synthesized or purified from microbial sources, can be used to probe the binding of antibody in glycan microarrays[37] or in competition immunoassays.[29,37,54,80,85] For glycan microarray probing, large numbers of different glycans are immobilized (printed) on glass slides in microspots, and antibody-binding is detected with fluorescent reagents, which are quantified in a fluorescence scanner.[86] For oligosaccharide competition, short antigen-derived oligosaccharides of different length and structure are used as competitors in immunoassays that measure binding of the antibody to the antigen. Per cent binding-inhibition versus competitor concentration is plotted for each competitor and the most potent competitor, which requires the lowest concentration for half-maximal binding-inhibition, is deduced to comprise the glycan epitope. These methods are limited by the difficulty of synthesizing some oligosaccharides or purifying sufficient quantities from microbial sources. Furthermore, the spatial resolution will not be at the single residue level if oligosaccharides that differ by single sugar residues are not available.[29,54]

Antigen-derived overlapping or non-overlapping proteolytic fragments and peptides can be displayed on the surface of phage particles[30] or yeast cells[26,68] for selection by the antibody or can be used in Western blot or ELISA or microarrays to screen for antibody binding (peptide scanning).[21,30,40,75] This approach is limited by the dependence of many epitopes on the three-dimensional structure of the larger antigen[75] and the likelihood of identifying only partial epitopes.[2,30]

Site-directed mutations (point mutations, insertions, deletions) can be introduced into recombinant versions of either the antigen[21,38,40,43,44,62,87] or the antibody[21,48,52,74] and the mutants can be tested for loss of Ag–Ab binding or other functions. Mutants are often obtained by changing entire segments of the antigen or specific residues to alanine (alanine shaving)[88] and verifying proper folding of the mutant proteins by their binding to mAbs that target other epitopes and/or to polyclonal antibodies.[39,46] Alternatively, systematic random mutations to alanine can be introduced along an entire protein antigen (alanine scanning) and mutants probed for antibody binding, to obtain partial epitope information.[21,26,38,46,48,87]

Electron microscopy (EM) allows visualization of Ag–Ab complexes.[44,52] The Ag–Ab mixtures are spread on a metal grid and introduced into a high-vacuum column in the electron microscope. There the sample is exposed to and diffracts an electron beam, which is then focused by electrostatic and electromagnetic lenses to yield electron-density maps that can be converted into an image. Stains and fixatives are used to protect the sample from radiation damage, but these alter the fine structure of the macromolecules. In a modification called cryoEM, Ag–Ab complexes can be observed in physiological

buffers, without stains and fixatives, by preserving the complexes in a frozen hydrated state by rapid freezing at near liquid nitrogen temperatures, which protects the sample from radiation damage. The resolution of most EM and cryoEM methods is too low to reveal contact residues and, therefore, Ag–Ab models obtained by other methods are used to interpret the EM and cryoEM maps.

Computational docking of Ag–Ab complexes can be used to predict the contact residues using Ag–Ab models from either homology modelling or, preferably, X-ray crystallography of both or at least one of the partners. In the docking protocol, the antigen and antibody structures are tested in a large number of orientations and then each orientation is scored by energy, seeking the global minimum orientation. The scoring function can be greatly enhanced in accuracy if experimental data from the methods discussed above are used to guide (constrain) the selection, referred to as experimentally validated computational docking.[20,40,43,44,54,80,82,83]

Because epitope information from different mapping methods differs in extent and resolution (summarized in Table 1), multiple mapping methods are often used to obtain complementary or supporting data. As exemplified in Fig. 3 for the mapping of a protective peptidic B-cell epitope in *Neisseria meningitides* factor H binding protein,[30] X-ray crystallography usually provides the most complete highest-resolution map. But other methods such as mutational analysis of both antigen and antibody contact residues and testing the binding of intact antibody to antigen under physiological conditions may be needed to reveal the critical contacts, without which Ag–Ab binding is abolished or greatly reduced, or those that make one antibody more potent or more cross-reactive than another at a particular function like neutralization.[19,27,33,35,38,56,60,61,71] Furthermore, the epitope core responsible for a pathogenic function may be shared by antibodies that target overlapping epitopes with slightly different registers of contact residues.[81] Therefore, for initial identification of protective B-cell epitope cores, the lower resolution maps obtained using DXMS for peptidic epitopes, and oligosaccharide competition and/or glycan microarray probing for glycan epitopes, may suffice.

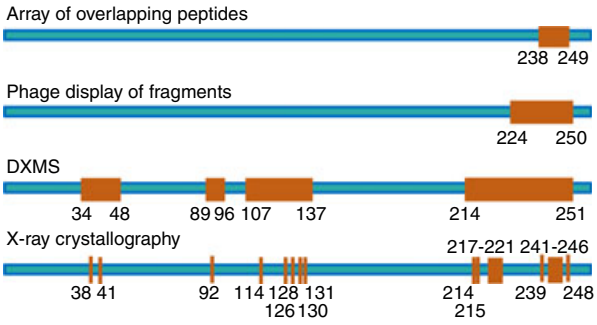

Figure 3. Linear representation of mapping results for a B-cell epitope in *Neisseria meningitides* factor H binding protein, obtained using different methods. Figure adapted with modifications from Malito *et al.*[30] Epitope segments and contact residues are indicated by amino acid numbers and represented in orange.

Table 1. Information obtained from different epitope mapping methods

Method	Epitope type(s) mapped	Extent and resolution of map
X-ray crystallography of Ag–Ab complex	any[1]	entire epitope, contact residues, epitope conformation
DXMS (Ab-inhibition of hydrogen-deuterium exchange in the Ag)	peptidic	entire epitope, small segments containing the contact residues
STD NMR (Ab-induced alteration of the NMR spectrum of the Ag)	any	contact residues
Glycan array probing or oligosaccharide competition for Ab-binding	glycan and glycopeptidic	entire epitope (glycan) or partial epitope (glycopeptidic), contact residues if oligosaccharides differing by one sugar are available; otherwise – small segments containing the contact residues
Peptide scanning (of Ag peptides) or testing of Ag fragments for Ab binding	peptidic	usually partial epitope, small segments containing some of the contact residues
Mutagenesis (alanine shaving, alanine scanning, point or deletion mutations) and testing for Ag–Ab binding	peptidic and glycopeptidic	partial epitope, critical contacts
EM or cryoEM of Ag–Ab complex	any	epitope region
Experimentally validated computational docking of Ag–Ab complex (constrained by data from other methods)	any	entire epitope, contact residues, epitope conformation

Ab, antibody; Ag, antigen; cryoEM, cryoelectron microscopy; DXMS, deuterium exchange/mass spectrometry; EM, electron microscopy; STD NMR, saturation transfer difference nuclear magnetic resonance.

[1]Including peptidic, glycan, lipid, nucleic acid, or combinations thereof.

Relation of shape, size and structure of microbial B-cell epitopes to protective antibody function

B-cell epitopes come in a variety of shapes and sizes, reflecting the complementary shapes and sizes of the binding-sites of antibodies, as illustrated in Fig. 4 for five protective epitopes and outlined in Table 2 for one or more protective epitope examples from each microbe category including bacteria, fungi, parasites and viruses. Epitopes can be convex, concave, relatively flat (with minor protrusions and depressions), or mixtures thereof, vary in surface area from < 200 to > 1500 Å^2, and consist of from fewer than two to more than 34 residues (Fig. 4 and Table 2). They can be glycan, peptidic or glycopeptidic, include secondary structure segments of α-helices, β-strands and loops, and encompass or depend on the conformation of multiple antigen subunits (quaternary epitopes[72,73]) (Fig. 4 and Table 2), or even span two antigens (hybrid epitopes[43]).

A terminal and a repeating-internal epitope in *F. tularensis* O-antigen exemplify convex glycan epitopes, the former a 185-Å^2 two-sugar epitope that fits in a cavity-type antibody-binding site[29] and the latter a 308-Å^2 six-sugar V-shaped epitope that fits in a groove-type binding site that has a small central pocket that anchors the vertex of the epitope[89] (Fig. 4a and Table 2). The protective efficacy of the two targeting antibodies is probably due mainly to mediation of effector functions. A 680-Å^2 20-amino acid hydrophobic pocket formed by parts of a highly conserved α-helix and loop in the stem of influenza virus haemagglutinin is a concave peptidic epitope, which interacts with a convex binding-site formed by an exceptionally long CDR3 in the VH region of a broadly neutralizing Ab (bNAb).[71] This bNAb can inactivate six of the 16 influenza virus haemagglutinin subtypes by blocking the low pH-induced conformational change required for fusion of the virus with the host cell endocytic membrane during infection[71] (Fig. 4b and Table 2). A 1013-Å^2 18-amino acid epitope with convex and concave parts, consisting of both helical and loop segments of the trimeric RSV fusion (F) protein in its pre-fusion conformation, interacts with a complementary antibody that locks the F protein in the pre-fusion conformation, preventing the rearrangement required for exposure of the fusion peptide and fusion of the viral and host cell membranes[73] (Table 2 and Fig. 4c; the post-fusion conformation of the F protein is shown for comparison). A 1535-Å^2 glycopeptidic epitope with both convex and concave parts, and consisting of β-strand amino acids and both high mannose-type and complex-type N-linked glycans in gp120 of the HIV-1 envelope spike, interacts with a bNAb[38,48] (Table 2 and Fig. 4d). The bNAb can inactivate 73% of 162 strains representing major HIV-1 clades,[48] possibly by preventing envelope conformations that allow binding to the host cell receptor CD4.

Clinical applications or implications of protective microbial B-cell epitopes

Translation of protective B-cell epitope mapping results into clinical applications is occurring and will continue to occur. As outlined for the examples given in Table 2, the influenza A bNAb CR6261, specific for a conserved epitope in the haemagglutinin stem, is in Phase I clinical trials as a therapeutic, and sera from patients with tularaemia,[20] malaria[19] and HIV infection[21] have been tested for the presence of antibodies to mapped protective epitopes by competition ELISA for antigen-binding with the protective mAbs. Two antigens for which protective epitopes have been mapped are in Phase I–II clinical trials as malaria vaccines (Table 2), and one is a major component of a meningococcal serogroup B vaccine that has been recently licensed for use by the European Medicines Agency[90] (see Table 2). The sera of vaccinees could be tested for the presence of antibodies to mapped protective epitopes as a correlate of vaccine protection.

Other protective antibodies could also be developed as therapeutics, especially for infections with drug-resistant microbes like methicillin-resistant *S. aureus*[33] or Oseltamivir-resistant H1N1 influenza viruses,[42] or for long-term vectored immunoprophylaxis against HIV infection by injection of adeno-associated virus transduced with an expression vector encoding several non-overlapping bNAbs.[59] In addition, or alternatively, the mapped epitopes of protective antibodies could be engineered into new or improved vaccine designs. For example, an influenza virus haemagglutinin comprised of the stem only, without the highly variable head domain, is efficacious in mouse models and has been proposed as a candidate for a universal flu vaccine.[91,92] Development of engineered improved versions of bNAbs could be considered for therapeutic or prophylactic treatment of dengue virus infection, if combined with modifications to the Fc region to reduce interaction with Fcγ receptors and hence the possibility of antibody-dependent enhancement of infection, which occurs at sub-neutralizing antibody concentrations.[46,55] And, in view of the high number of somatic mutations found in the V regions of HIV-1 bNAbs[21,93,94] and the discovery that the germline V region progenitors may not bind the same antigen,[39] the development of HIV vaccines that would guide the immune system, stepwise, from immunoglobulin gene rearrangement, through affinity maturation and production of bNAbs, has been proposed[6,74,95] (see Table 2).

Concluding remarks

Mapping the protective B-cell epitomes of microbes, the totality of protective epitope cores on microbial antigens, will continue to provide the best mAb candidates to be used for antimicrobial therapy and prophylaxis. Further-

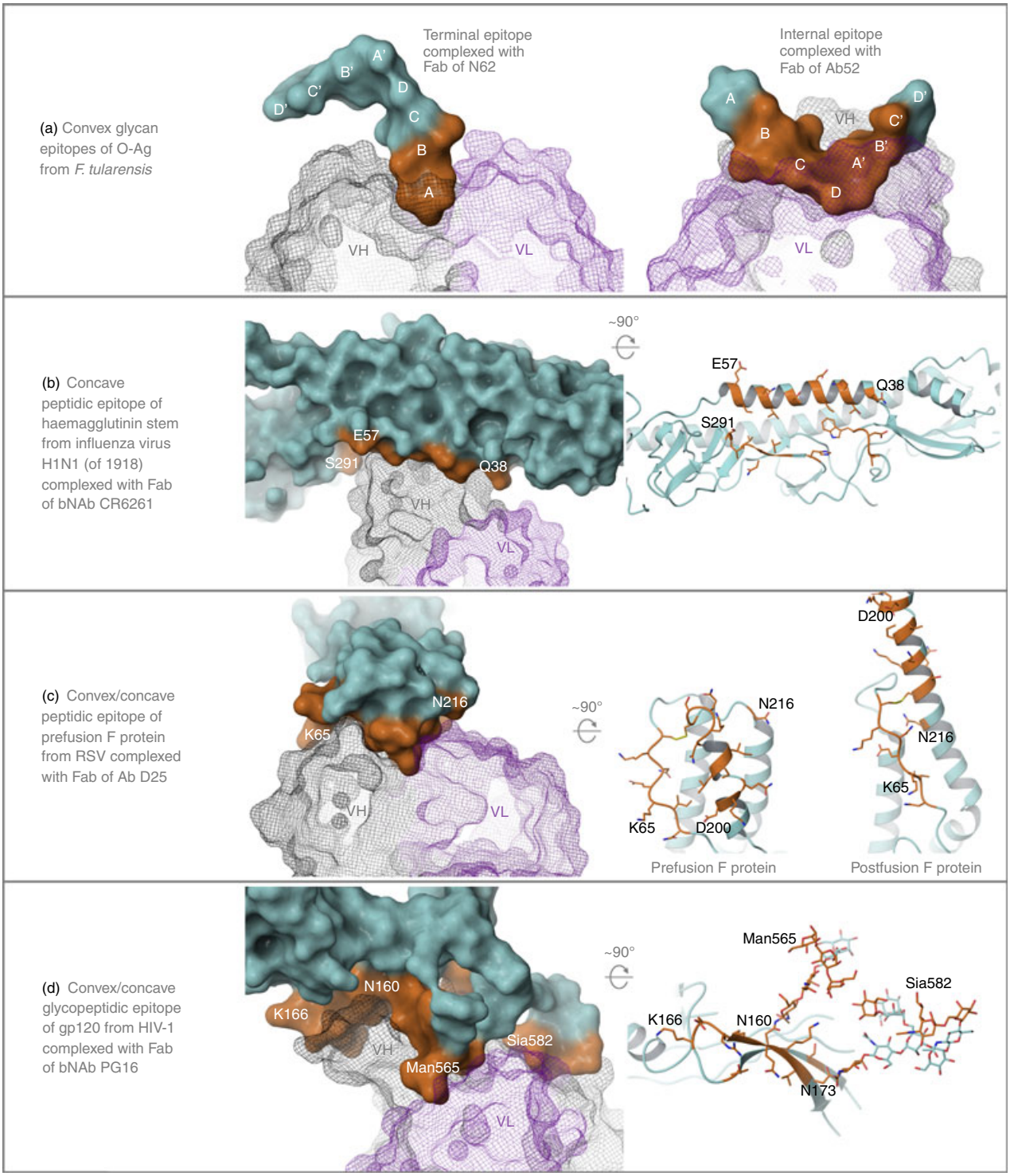

Figure 4. Examples of protective B-cell epitope structures. Antigens are represented as solid molecular surfaces (left panels and top right) and ribbon diagrams (right panels) rotated away from the reader approximately 90° about the horizontal axis, coloured with cyan carbons except for contact residues which are coloured orange. Antibody V regions are shown as wire-mesh molecular surfaces coloured grey (VH) and purple (VL). Images are clipped front and back to more clearly show interactions of antibodies and antigens. Selected contact residues are indicated in each panel for reference. The four sugars of the tetrasaccharide repeat in *Francisella tularensis* O-Ag[108] are represented as ABCD for one and A'B'C'D' for the other of the two repeats shown. S, serine; E, glutamic acid; Q, glutamine; K, lysine; N, asparagine; D, aspartic acid; Man, mannose; Sia, sialic acid. Images were generated using Maestro (version 9 3 5, Schrödinger, Inc., New York, NY).

Table 2. Examples of protective B-cell epitope discovery

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
Bacteria								
Neisseria meningitides; Gram-negative; septicaemia, meningitis	Factor H binding protein (fHbp); in outer membrane; inhibits phagocytosis and complement-mediated killing by binding and recruiting human factor H (fH), a complement down-regulator, to the bacterial surface[97, 98]	28 000 mol. wt membrane-anchored surface-exposed lipoprotein, approximate β-barrel N-terminal domain and β-barrel C-terminal domain; three main variant groups[99]	12C1, mouse IgG2b; hybridoma obtained by immunization of CD1 mice with a recombinant fusion protein containing the three main variants of fHbp (var1, -2, and -3)[30]	Inhibits binding of fHbp to fH and exhibits bactericidal activity against serogroup B *N. meningitides* strain MC58 (var1)[30]	• fHbp synthetic peptide array • Phage display of fHbp fragments • DXMS of fHbp–Ab complex • X-ray crystal of fHbp –Fab complex • Single point mutagenesis in fHbp[30]	Peptidic concave/convex; from three loops and seven β-strands distributed on both N- and C-terminal domains; ~ 1000 Å^2, 23 amino acids[30]	*Q38*, *R41*, *E92*, *I114*, *K126*, *Q128*, *R130*, I131, Y214, N215, A217, *E218*, *K219*, *G220*, *S221*, *E239*, ***K241***, T242, V243, N244, G245, *I246*, *H248*; epitope overlaps the fH binding-site and shares 15 of its 52 residues (italic) – Ab interferes with fH-fHbp binding[30]	fHbp is a major component of the meningococcal serogroup B vaccine 4CMenB that has been recently licensed for use by the European Medicines Agency[90] – sera of vaccinees could be tested for 12C1-like Abs
			502, mouse IgG2a; hybridoma obtained by immunization of CD1 mice with a recombinant var1 fHbp[27]	Does not inhibit binding of fHbp to fH but has strong bactericidal activity against the serogroup B MC58 strain[27]	• STD NMR of Fab in complex with ^{1}H-^{15}N-labelled C-terminal domain of fHbp (fHbp$_c$) • Site-directed mutagenesis of fHbp • Experimentally validated computational docking of Fv and fHbp$_c$[82]	Peptidic from three loops and β-strands	F141, K143, G148, R149, A174, K199, K203, **R204**, A206, V207, F227, G228, E233[27, 82]	

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
Staphylococcus aureus; Gram-positive, group II re-emerging pathogen; skin infections, toxic shock syndrome, food poisoning	α-haemolysin; secreted toxin; at low concentrations, binds and activates the metalloprotease ADAM10 on host cells, inducing pro-inflammatory cytokines and promoting epithelial breach;[100] at high concentrations, forms pores in host cell plasma membranes, disrupting ion gradients and causing lysis[101]	33 000 mol. wt monomer with a stem domain topped by a β-sandwich cap domain and a rim domain under the cap; seven monomers assemble on the host cell membrane to form a mushroom-shaped lytic pore with a membrane-spanning β-barrel[102]	LTM14, recombinant human IgG; V regions derived from a single-chain variable fragment (scFv) combinatorial phage-display library built from 654 healthy human donors[33]	Inhibits binding of α-haemolysin to ADAM10 and its lysis of human lung epithelial and T-cell lines; protects prophylactically against methicillin-resistant (and -sensitive) *S. aureus* in pneumonia, skin, and bacteraemia mouse models of infection, and therapeutically up to 18 hr post-infection in the pneumonia model[33]	• X-ray crystal of Fab in complex with recombinant *S. aureus* α-haemolysin H35L, a non-toxic mutant[33]	Peptidic convex/flat; from five loops and four β-strands located between the cap and rim domains; 881 Å^2, 17 amino acids[33]	K30, E31, Q64, Y65, *R66*, V67, Y68, S69, *E70*, G72, A79, K205, A207, D208, P213, R253, W274 with R66 as the centre of the epitope;[33] *R66*, *E70*, R200, D255, D276 involved in α-haemolysin–membrane interaction[103] – Ab neutralizes α-haemolysin by preventing its binding to the membrane of host cells[33] (shared residues in italic)	Ab has therapeutic potential either alone or in combination with antibiotics, especially for treatment of methicillin-resistant *S. aureus* (MRSA)[33]

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
Francisella tularensis; Gram-negative, facultative intracellular, category A priority pathogen, tier 1 select agent; tularaemia	O-Ag; in outer membrane lipopolysaccharide (LPS) and in capsule[104–106]; inhibits phagocytosis and complement-mediated killing[107]	Variable numbers of repeats of the (ABCD) tetrasaccharide [2)-β-D-4,6-dideoxy-4-formamido-D-glucose(1→4)-α-D-2-acetamido-2-deoxy-D-galacturonamide (1→4)-α-D-2-acetamido-2-deoxy-D-galacturonamide(1→3)-β-D-2-acetamido-2,6-dideoxy-D-glucose(1→] abbreviated as Qui4NFm-GalNAcAN-GalNAcAN-QuiNAc[108]	Ab52, mouse IgG2a; hybridoma obtained from a BALB/c mouse immunized with the attenuated *F. tularensis* strain LVS and *F. tularensis* LPS[32]	Prolongs or confers survival in a mouse model of respiratory tularaemia with the virulent *F. tularensis* strain SchuS4 or with LVS, respectively, and reduces blood and spleen bacterial counts[54]	• Oligosaccharide competition (with defined O-Ag repeat length) • X-ray crystal of Fab • Experimentally validated computational docking of the Fab and a two-repeat O-Ag constrained by above and other immunochemical data[29, 89]	Glycan convex V-shaped, repeating internal; 308 Å^2, six sugars[89]	BCDA'B'C' GalNAcAN-GalNAcAN-QuiNAc- Qui4NFm-GalNAcAN-GalNAcAN[89]	Sera from tularaemia patients inhibit Ag-binding by Ab63 (below) and Ab52, but show 75-fold variation in the ratios of Ab63 to Ab52 inhibitory potencies, indicating different distributions of terminal- versus internal-binding O-Ag Abs in different individuals[20]
			N62 or Ab63, mouse IgG2b or IgG3, encoded by the same combination of germline VH and VL region genes; hybridomas obtained from BALB/c mice immunized with preparations of *F. tularensis* LVS[29]	Prolong survival in a mouse model of respiratory tularaemia with the virulent *F. tularensis* strain SchuS4, and reduce blood and spleen bacterial counts[29]	• Oligosaccharide competition (with defined O-Ag repeat length) • X-ray crystal of the N62 Fab • Experimentally validated manual docking of the N62 Fab and the 2-repeat O-Ag model generated for the Ab52 mAb (above)[29]	Glycan convex unique non-reducing terminus; 185 Å^2, two sugars[29]	AB Qui4NFm-GalNAcAN[29]	
Fungi								
Candida albicans; thrush, vaginal yeast infections, systemic candidiasis	β-mannan; in cell wall; suppresses cell-mediated immunity[109] and functions as adhesin during yeast cell attachment to splenic marginal zone macrophages[53]	Consists of (1→2) β-linked mannose oligomers linked to the major α-mannan component of the cell wall either directly or via a phosphodiester bond;[110] helical conformation[85]	C3·1, mouse IgG3; hybridoma obtained from a BALB/cBy J mouse immunized with a mannan-liposome preparation[28]	Prolongs survival and reduces kidney yeast counts in a mouse model of disseminated candidiasis, and reduces vaginal yeast counts in a mouse model of vaginal *Candida albicans* infection[53,28]	• Oligosaccharide competition[80,85] • STD NMR • Experimentally validated computational docking with above constraints[80]	Glycan helical, repeating internal two to three sugars[80,85]	β-D-mannopyranosyl-(1→2)-β-D-**mannopyranosyl-(1→2)-** mannose unit at the reducing end most important; Ab binds better to di- and trimannosides than to longer oligosaccharides[80, 85]	Small-size (1→2) β-linked mannose oligosaccharides might be better than longer chains in a *Candida albicans* conjugate vaccine[85]

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
Parasites								
Plasmodium falciparum; protozoan parasite, intracellular; malaria	Apical membrane Ag 1 (AMA1); in cell membrane of both sporozoite and merozoite stages; binds to host erythrocytes in complex with the merozoite RON2 protein to form a vacuole, triggering merozoite invasion of the erythrocyte[111]	69 000 mol. wt type I integral membrane protein with an extracellular region consisting of three domains; domains I and II are PAN domains (five β-sheets surrounding an α-helix); highly polymorphic loops from domains I and II surround a conserved hydrophobic trough[112,113]	1F9, mouse IgG2b; hybridoma obtained from mice immunized with recombinant, refolded *P. falciparum* AMA1[114]	Inhibits invasion of erythrocytes by strains 3D7 and D10 merozoites[60]	• X ray crystal of Fab in complex with AMA1 domains I+II • Point and deletion mutagenesis of AMA1[70]	Peptidic concave; formed by one half of the hydrophobic trough and surrounding loops, primarily three polymorphic loops from domain I; 1250 Å^2, 27 amino acids[70]	F183, T186, E187, P188, L189, M190, P192, M193, T194, D196, E197, R199, H200, F201, Y202, K203, D204, N205, V208, K209, G222, N223, M224, I225, N228, K230, K235[70] 2° structure annotation from Table S1[70]	AMA1 Malaria Vaccine Phase II clinical trial FMP2·1/AS02A (http://clinicaltrials.gov/ identifier NCT00460525); Abs to the 1F9 epitope in sera from *P. falciparum*-infected children, detected by competition ELISA, positively correlate with the parasite growth-inhibitory activity of serum Abs[19]
	Merozoite surface protein 1 (MSP1); in cell membrane of merozoite stage; involved in the initial binding of merozoites to erythrocytes; during the final stages of erythrocyte invasion, all but its C-terminal part is cleaved and shed[115]	~ 200 000 mol. wt GPI-linked membrane protein precursor; cleaved into four parts; during erythrocyte invasion three parts are shed, leaving the C-terminal 19 000, $MSP1_{19}$, which has two epidermal growth factor (EGF)-like-fold domains consisting of disulphide-bonded anti-parallel β-sheets[116]	12·8 or 12·10, mouse IgG2b or IgG1, respectively[81]	12·8 and 12·10, and their chimeric mouse-human IgG1 and IgG3 versions, inhibit MSP1 cleavage and invasion of rabbit erythrocytes by merozoites[61]	• STD NMR of each Fab with ^{2}H-, ^{13}C- and ^{15}N-labelled $MSP1_{19}$ expressed in yeast[81]	Peptidic; encompassing two loops and two β-strands in EGF-fold domain 1 12·8: convex nine residues; 12·10: convex/ concave 11 residues; overlapping epitopes	12.8: Q14, *N15*, *S16*, *G17*, *C18*, *F19*, E27, F87, D88 12·10: H5, Q6, C7, *N15*, *S16*, *G17*, *C18*, *F19*, R20, Q31, G89[81] shared residues in italic	AdCh63/MVA MSP1 vaccine Phase I/IIa clinical trial (http://clinicaltrials.gov/ identifier NCT01003314); FMP010 (MSP1 vaccine)/AS01B (adjuvant), Phase Ib clinical trial (http://clinicaltrials.gov/ identifier NCT00666380); sera of vaccinees could be tested for 12·8/12·10-like Abs

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
Viruses								
Influenza A virus; RNA-, enveloped, category C priority pathogen; flu	Haemagglutinin (HA); on viral envelope; binds to host-cell surface glycoproteins, mainly through sialic acid, and mediates fusion of the virus with the host cell membrane during infection[117] HA from H1N1 influenza virus	Trimeric glycoprotein of ~ 60 000 mol. wt monomer, cleaved during viral maturation into HA1 and HA2; 16 subtypes (H1–H16); two (outside the envelope) ectodomains [globular head (substrate binding, variable) and membrane-proximal stem (structural, conserved)]; undergoes low pH conformational change during membrane fusion[71,118]	CR6261, human IgG1; V region genes obtained from a combinatorial phage display library made from peripheral blood IgM^+ memory B cells of seasonally vaccinated individuals[22]	bNAb; neutralizes H1, H2, H5, H6, H8 and H9 influenza virus subtypes in *in vitro* infection of MDCK kidney dog cells; protects BALB/c mice from lethal intranasal challenge with H1N1 and H5N1 when administered intraperitoneally or intravenously before or up to 5 days post infection[22]	• X-ray crystal of Fab in complex with (recombinant fibritin-trimerized ectodomains of) HA trimer from H1N1 (A/South Carolina/1/1918) or H5 (A/Vietnam/1203/2004) subtypes[71]	Peptidic concave; includes helical part of stem and part of a loop in highly conserved hydrophobic pocket spanning HA1 and HA2;[71] 680 Å^2, 21 amino acids	H18, H38, V40, N41, L42, S291, L292, P293, T318, D19, G20, W21, Q38, T41, Q42, I45, D46, T49, V52, N53, I56 from HA1 till T318; rest from HA2;[71] Ab neutralizes the virus by blocking the low-pH-induced conformational change required for membrane fusion[71]	CR6261 is currently in Phase I clinical trial as therapeutic against influenza A virus (http://clinicaltrials.gov/ identifier NCT01406418); 'headless' HAs, containing only the conserved stem domain, may be good candidates for a universal flu vaccine, as shown in mouse models;[91,92] and new drugs that target the HA stem could be developed[24]
			CH65, human IgG1; V region genes derived from sorted single plasma cells from a subject 1 week after vaccination with the 2007 trivalent inactivated flu vaccine[42]	bNAb; neutralizes infectivity of MDCK cells by 30 out of 36 H1N1 strains tested[42]	• X-ray crystal of Fab in complex with HA trimer from H1N1 (A/Solomon Islands/3/2006)[42]	Peptidic concave/convex; includes α-helix and loop segments of receptor-binding pocket in head domain; 748 Å^2, 29 amino acids[42]	*Y95*, T131, T133, *G134*, *V135*, *S136*, *A137*, S145, *W153*, T155, G156, K157, N158, G159, L160, *H183*, *P186*, N187, I188, G189, *D190*, R192, A193, *L194*, H196, K222, *D225*, *R226*, E227; epitope overlaps the sialic acid binding-site and shares 12 of its 13 residues (italic) – Ab neutralizes by mimicking and blocking the interaction of sialic acid with HA[42]	Ab CH65 might be a useful template for a therapeutic Ab, especially for Oseltamivir-resistant HIN1 influenza viruses[42]

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
	HA from (highly pathogenic) H5N1 avian influenza virus		H5·3, human mAb; obtained by EBV-transformation of B cells from individuals vaccinated with an experimental H5N1 vaccine, followed by hybridoma generation[44]	Neutralizes influenza H5N1 viruses and pseudoviruses carrying respiratory droplet transmissible (rdt) HA variants that have mutations near the receptor binding-site of HA[44]	• Site-directed point mutagenesis of VN/1203 HA • X-ray crystal of Fab • EM of Fab complexed with VN/1203 HA trimer • DXMS of Fab complexed with VN/1203 HA head domain • Experimentally validated computational docking of the Fab and trimeric VN/1203 • HA crystal structures with the DXMS and EM constraints[44]	Peptidic concave; two sheets and a loop that form one wall of the sialic acid binding-site in the head domain of HA;[44] 12 amino acids[44]	*L133*, *G134*, *V135*, *S136*, *S137*, A138, Y141, Q142, G143, K144, S145, S146 epitope overlaps the sialic acid binding-site and shares five of its 15 residues (italic); Ab neutralizes pseudoviruses carrying rdt mutations near the sialic acid binding-site by inserting HCDR3 into the binding-site at an angle that avoids the rdt mutations[44]	Neutralizing Abs elicited by conventional subunit vaccines against highly pathogenic H5N1 avian influenza viruses may also neutralize rdt viral variants[44]
Respiratory syncytial virus (RSV); RNA+, enveloped; bronchiolitis	Fusion (F) glycoprotein; on viral envelope; catalyses fusion with host cell membrane during infection[119]	Trimeric (~ 70 000 mol. wt monomer), cleaved for activation into F1 and F2 subunits, undergoes major structural rearrangement during the membrane fusion process (to expose the fusion peptide) from a metastable prefusion conformation to a highly stable postfusion structure[73,120]	D25, human IgG1, prefusion-specific; V regions obtained from human memory B cells from a healthy donor and stimulated to proliferate by transduction with B-cell lymphoma (Bcl)-6 and Bcl-xL genes, and culture with CD40 ligand and interleukin-21[36]	Inhibits viral fusion with Hep-2 human cells; prevents viral replication in cotton rats when administered intramuscularly 1 day before intranasal challenge with the primary viral isolate RSV-X, at lower doses than the commercial RSV Ab palivizumab[36]	• X-ray crystal of recombinantly coexpressed D25 Fab and residues 1–513 of F glycoprotein fused to a C-terminal fibritin domain of bacteriophage T4 for trimerization (to capture F in its prefusion state)[73]	Peptidic convex/concave; quaternary epitope encompassing two subunits (F1 and F2) at the apex of the prefusion trimer, helical/loop region 1013 Å^2,[73] 18 amino acids	N63, I64, K65, E66, N67, K68, C69, D200, K201, Q202, L204, P205, I206, N208, K209, Q210, S211, N216 residues 63–69 from F1 and 200–216 from F2, in the prefusion conformation; Ab neutralizes the virus by locking the F protein into the prefusion conformation, preventing the rearrangement required for fusion of the virus with the host cell membrane[73]	Abs targeting the D25 epitope could be developed for prophylaxis of RSV-induced disease in neonates, and an F protein stabilized in the prefusion conformation, perhaps with disulphide bonds, may provide a new vaccine approach[73]

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
Dengue virus (DENV); RNA+, enveloped, transmitted by mosquitoes or ticks, category A priority pathogen; dengue fever	Glycoprotein E; on viral envelope; binds cell surface receptors and induces membrane fusion during infection[121,122]	Dimeric, ~ 60 000 mol. wt monomer with three domains: DIII (a β-sandwich with an internal hydrophobic core) binds to host cell receptors,[123] DII contains the fusion loop; low pH conformational change initiates membrane fusion and changes oligomerization state to trimeric, with the fusion loops forming the tip of a trimeric spike; four serotypes (DENV-1-4)[62,122]	4E11, mouse IgG2a;[63] hybridoma generated by immunization of mice with the envelope protein of DENV-1[31]	bNAb; neutralizes the ability of all four DENV serotypes to infect Huh 7·5 human liver cells;[63,124] reduces serum viral titer in AG129 mice when administered 1 day prior to DENV-2 challenge[55]	• Phage-display random peptide library (identified residues 306–314)[125] • X-ray crystal of recombinant 4E11 Fab or scFv complexed with recombinant DIII domain of glycoprotein E from each of four DENV isolates representing the four DENV serotypes[124]	Peptidic convex; common core epitope encompassing the edge of the β-sandwich of the DIII domain and its hydrophobic core; 750–950 Å^2, 17 amino acids[124]	T305, F306, V307, L308, K309, K310, E311, V312, E325, K327, K361, K385, L387, K388, I389, N390, W391; Ab neutralizes the virus by inducing premature exposure of the fusion loop[124]	Potential development of engineered improved versions of broadly cross-reactive Abs could be considered for therapeutic or prophylactic treatment of DENV infection, combined with modifications to the Fc region to reduce interaction with Fcγ receptors and, hence, the possibility of Ab-dependent enhancement of infection[55,46]
			1·6D, human IgG1; V region genes obtained by *in vitro* stimulation of peripheral blood B cells from a patient that had recovered from Dengue virus infection, followed by cloning and transfection into the human embryonic kidney cell line HEK-293[46]	bNAb; neutralizes the ability of all four DENV serotypes to invade monkey epithelial LLC-MK$_2$ cells, and enhances infection of Fc receptor II –bearing K562 human macrophage-like cells at sub-neutralizing concentrations[46]	• Point mutations library of DENV-3 generated with a PCR mutagenesis kit[46]	Peptidic; encompasses the fusion loop of the DII domain; partial epitope, two critical amino acids[46]	**W101**, **G109**; overlaps with the fusion loop (residues 98 to 109); – Ab interferes with fusion of glycoprotein E with the endosomal membrane of host cells, preventing release of the virus into the cytoplasm[46]	

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
Ebola virus; RNA-, enveloped, category A priority pathogen, tier 1 select agent; haemorrhagic fever	GP glycoprotein; on viral envelope; mediates attachment to host cells and membrane fusion[126,127]	Trimeric, 150 000 mol. wt monomer[128] activated by cleavage into GP1 and GP2;[129] three GP1 viral attachment subunits assemble into a chalice, cradled by the GP2 fusion subunits, and a glycan cap and mucin-like domain restrict access to the receptor-binding site in the chalice bowl;[72] rearrangement and further cleavage of GP occurs during fusion[130,131]	KZ52, human IgG1; V region genes obtained from a combinatorial Fab phage display library generated from the bone marrow of a human survivor of Ebola infection[34]	Neutralizes the ability of (Zaire) Ebola virus to infect Vero E6 monkey kidney epithelial cells, and confers or prolongs survival in guinea pigs against lethal subcutaneous challenge with guinea-pig-adapted Ebola virus when administered intraperitoneally before or up to 1 hr after challenge[56]; does not prolong survival or reduce plasma viraemia in rhesus macaques when administered intravenously at 50 mg/kg 1 day before and 4 days after intramuscular challenge with Ebola virus[58]	• X-ray crystal of recombinant Fab in complex with GP glycoprotein mutated to remove the mucin-like and transmembrane domains and two glycosylation sites (T42V and T230V)[72]	Peptidic convex; quaternary epitope at the base of the chalice formed by GP2 (mainly) and GP1, encompassing a β-strand, a loop, and a helix/loop; 894 Å^2, 15 amino acids[72]	V42, L43, V505, A507, Q508, P509, C511, P513, N514, H549, N550, Q551, D552, G553, C556 from GP1 till L43, rest from GP2;[72] Ab likely neutralizes by preventing GP rearrangement and blocking insertion of the internal fusion peptides into the cell membrane[72]	Lack of protection by the Ab in the monkey study, despite *in vitro* neutralization and protective efficacy in the guinea pig model, suggests that a single mAb will not suffice for post-exposure prophylactic treatment against Ebola virus[58]

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
Human immunodeficiency virus type 1 (HIV-1); RNA+, enveloped; acquired immunodeficiency syndrome	Envelope spike (Env), A-K clades (genetic subtypes), each with many strains; on viral envelope; binds to the primary receptor (CD4), and to the coreceptor (chemokine receptor CCR5 or CXCR4) on host cells and mediates fusion of virus and host-cell membranes[132, 133]	Heavily glycosylated trimer composed of three gp120 molecules, each non-covalently associated with a transmembrane gp41 molecule, which includes an N-terminal fusion peptide and a conserved membrane-proximal external region (MPER); gp120 consists of five conserved (C1–C5) and five variable (V1–V5) 'loops' (subdomains) comprising an inner and an outer domain; the domains are connected by a four-strand bridging (β) sheet, between the V1/V2 loop from the inner domain and the V3 loop from the outer domain; CD4 binds in a depression between the inner and outer domains and the bridging sheet, and triggers a conformational change that exposes the gp120 coreceptor binding-site and disrupts the gp120-gp41 interaction, leading to exposure of the gp41 fusion peptide; N-linked diverse glycans shield gp120 from Abs and consist of both high mannose-type oligosaccharides and complex-type oligosaccharides[95,132–134]	PG16, human IgG1; V region genes obtained from an activated/expanded memory B cell of an HIV-1 clade A-infected donor selected for broad and potent serum neutralizing activity against HIV-1[38]	bNAb; neutralizes 73% of 162 isolates representing major HIV-1 clades at $IC_{50} < 50$ μg/ml (infection assay with the coreceptor-transfected human glioblastoma cell line U87)[38]	• Alanine scanning and point mutations[38,48] • X-ray crystal of Fab in complex with the a glycosylated scaffolded V1/V2 loop, which was selected by Ab-binding site-directed mutagenesis of Ab • STD NMR characterization of glycan recognition by PG16 and site-directed mutants using commercially available glycans including biantennary complex-type and Man5GlcNAc2[48]	Glycopeptidic convex/concave; encompasses both high mannose- and complex-type N-linked glycans as well as a β-strand in the V1/V2 loop of gp120; 1535 Å^2, 11 amino acids and 10 sugars[48]	S158, **N160** (GlcNAc560, GlcNAc561, Man562, Man564, Man565, Man566), T163, K166, D167, R168, K169, Q170, K171, V172, **N173** (GlcNAc573, Man578, Gal581, **Neu5Ac582**); Ab may neutralize by preventing Env conformations that allow CD4 binding[48]	Vaccine design for V1/V2-containing immunogens may include both a high mannose-type glycan at amino acid 160 and a sialic acid-containing complex-type glycan at position 173[48]

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
			PGT128, human IgG1; V region genes obtained from an activated memory B cell of one of a few HIV-infected donors that scored the highest among 1800 donors on both breadth and potency for serum neutralization[37]	bNAb; neutralizes 72% of 162 isolates representing major HIV-1 clades at IC50 < 50 μg/ml, and 50% at IC_{50} < 0·1 µg/ml (infection assay with the coreceptor-transfected human glioblastoma cell line U87)[37]	• Glycan array probing • Oligosaccharide competition with defined-length oligomannosides • Alanine shaving in gp120[37] • X-ray crystal of Fab in complex with a glycosylated engineered gp120 outer domain with a mini V3 loop (eODmV3) • X-ray crystal of Fab in complex with a synthetic Man_9 glycan • Point mutations in Ab (of glycan-recognizing amino acids) • Alanine shaving of gp120 residues • EM of Fab in complex with soluble partially deglycosylated Env trimer and manual fitting of the Fab-eODmV3 X-ray crystal structure[52]	Glycopeptidic concave/convex; encompassing two conserved glycans and a β-strand segment at the C-terminus of the V3 loop; 1081 Å^2, 12 amino acids and 15 sugars[52]	T297, R298, N300, **N301** (GlcNAc300, GlcNAc301, Man302, Man303, Man304, Man307), R304, $I322_A$, I323, G324, D325, I326, R327, H330, **N332*** (GlcNAc200, GlcNAc201, Man232, Man233, Man234, Man237, Man238, Man214, Man215); *N332 is not a contact residue, but its N-linked glycan is; Ab recognizes and penetrates the gp120 glycan shield and potentially crosslinks Env trimers on the viral surface[52] – may neutralize by preventing Env conformations that allow CD4 binding	The PGT128 epitope may be a good candidate for inclusion in an oligoepitope Ab-based vaccine component[37,52]

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
			VRC01, human IgG1; V region genes obtained from a memory B cell isolated by fluorescence-activated cell sorting for binding to a gp120 core containing the CD4 binding-site and lack of binding to the gp120 core in which the CD40 binding-site had been deleted; memory B cells derived from a donor who has been infected with an HIV-1 clade B virus for more than 15 years and has not initiated antiretroviral treatment[39]	bNAb; neutralizes 91% of 190 isolates, representing major HIV-1 clades at IC_{50} < 50 μg/ml and 72% at IC_{50} < 1 μg/ml (infection assay with CF2Th/synCCR5, a dog thymocyte cell line transfected with human CCR5);[39] confers lifelong protection to humanized mice against systemic HIV challenge when administered as DNA in an adeno-associated virus expression vector as a single intramuscular injection [vectored immunoprophylaxis (VIP)][59]	• Point mutation D368R (CD4 binding-site knockout) of gp120[39] • X ray crystal of Fab in complex with a gp120 from the clade A/E recombinant 93TH057, consisting of its inner domain-outer domain core, with truncations in the V1/V2 and V3 loops as well as the N- and C-terminal regions that extend away from the main body of gp120 • Point and insertion mutations in the Ab, including reversion to germline sequence (with loss of binding)[74]	Glycopeptidic concave/convex; encompassing a loop in C1, the truncated V1/V2 loop, a loop in C2, a loop and a helix in C3, a loop and a β-strand in C4, the V5 loop, and a loop and a β-strand in C5 in the outer domain part of the CD4 binding-site, which is conformationally invariant, and extends past the functionally constrained CD4 binding-site into the conserved base of the V5 loop; 1249 Å^2, 34 amino acids and one sugar[74]	K97, *T123*, G124, G198, N276 (GlcNAc 776), T278, *N279*,* *N280*, *A281*, K282, *S365*, *G367*, ***D368***, *I371*, *W427*, *Q428*, *G429*,* *T430*,* *T455*, *R456*, *D457*, *G458*, *G459*, A460, N461, T463, N465, E466, T467, *R469*,* *G472*, *G473*, *D474*,* K476; epitope overlaps with the CD4 binding-site and shares 21 of its 27 residues [italic (* indicates sequence changes from[134])] – Ab neutralizes by blocking the binding of CD4 to gp120[74]	Vaccines could be developed that will guide the immune system from Ig gene rearrangement through affinity maturation to production of VRC01-like bNAbs;[74] VRC01 along with bNAbs directed to other conserved Env epitopes could be developed for vectored immunoprophylaxis against HIV infection in high-risk individuals[59]

Table 2 (*continued*)

Microbe; characteristics;[1] disease(s)	Ag characteristics		Ab characteristics		Epitope characterization			Clinical applications or implications
	Ag; location; function(s) in pathogenesis	Structural features	Ab; derivation	Performance in protection assays	Mapping methods	Type and shape; 2° structure and location; size[2]	Contact residues (annotated);[3] notes on mechanism	
			10E8, human IgG3; obtained (after *in vitro* activation and expansion) from a peripheral-blood memory B cell of a patient with neutralizing breadth and potency against a 20 cross-clade pseudovirus panel[21]	bNAb; neutralizes 98% of 180 isolates representing major HIV-1 clades at $IC_{50} < 50$ μg/ml, and 72% at $IC_{50} < 1$ μg/ml (infection assay with the human HeLa cell line TZM-bl, which expresses high levels of CD4 and CCR5)[21]	• Overlapping MPER peptide binding • Alanine scanning of MPER residues 671–683 • X ray crystal of Fab in complex with a peptide encompassing the entire 28-residue MPER (residues 656–683) • Alanine shaving of Ab contact residues[21]	Peptidic convex/flat; one face of the MPER of gp41, which forms two helices oriented 100° relative to each other; 773 Å^2, 15 amino acids[21]	L661, D664, K665, S668, L669, W670, N671, **W672**, **F673**, **T676**, N677, L679, W680, I682, **R683**; neutralization by the Ab may be due to interference with fusion of the viral and host cell membranes[21]	6 of 78 human sera from HIV-1-infected donors have 10E8-like Abs, as assessed by binding to HIV-2/HIV-1 chimeric viruses containing MPER subdomains and peptide inhibition, showing that such Abs are not rare and could be induced with a well-designed vaccine[21]

[1]Designations of microbes as group II re-emerging pathogen, or as (group III, agents with bioterrorism potential) category A, B or C priority pathogens are by the US, NIH, National Institute of Allergy and Infectious Diseases (http://www.niaid.nih.gov/topics/emerging/Pages/list.aspx). Designation as a tier 1 select agent is by the US Centers for Disease Control and Prevention (http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html).

[2]Shapes of epitopes are designated as concave, convex, flat, or combinations thereof by visual inspection of published structures with DeepView (http://www.expasy.org/spdbv/) if not specified in the primary reference.[135] The surface area of the epitope is from the PISA database (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html)[136] when not given in the primary reference. The surface areas of the *F. tularensis* O-Ag epitopes were calculated from the docked models.

[3]Contact residues are from the primary reference when listed, otherwise from IEDB (http://www.iedb.org/) or, if not listed in IEDB, are residues within 4 Å of the Ab as determined by DeepView (http://www.expasy.org/spdbv/).[135] Amino acid numbering system is from the primary reference. Sugar numbering system is from the entry in the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). Secondary structure is as determined by DeepView (http://www.expasy.org/spdbv/),[135] unless otherwise indicated. The first amino acid or sugar and discontinuous residues are underlined. Critical residues to Ag–Ab binding, determined by analysis of point mutants, are in bold. Secondary structure or sugar chain is indicated by colour code with two alternating hues of the same colour denoting independent structural elements: black/grey, β-strand; blue, α-helix; red/magenta, loop; green, sugar chain. Amino acid (one-letter code) abbreviations: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenyl alanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. Sugar abbreviations: Gal, galactose; GlcNAc, *N*-acetylglucosamine; Man, mannose; Neu5Ac, 5-acetyl-neuraminic acid (sialic acid).

more, such epitome knowledge will enable the design of subunit vaccines enriched in protective epitopes. Analysis of the molecular interactions between bNAbs and microbial antigens will inform the engineering of vaccine subunits to guide the immune system towards production of similar antibodies. Such vaccine strategies have a chance for success against pathogens of high antigenic variation that can replicate inside Fc receptor-bearing host cells if multiple protective epitopes are used[68,96] and, most importantly, if combined with strategies to elicit both cytotoxic and helper T cells that can kill or stimulate the microbicidal activity of microbe-infected host cells.

Acknowledgement

B-cell epitope discovery studies from the authors' laboratories, included in this review, were supported with Federal funds from the US National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272200900054C.

Disclosures

The authors have no potential conflicts of interest.

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