Chapter 19
Antigen–Antibody Complexes

A. Brenda Kapingidza, Krzysztof Kowal and Maksymilian Chruszcz

Abstract In vertebrates, immunoglobulins (Igs), commonly known as antibodies, play an integral role in the armamentarium of immune defense against various pathogens. After an antigenic challenge, antibodies are secreted by differentiated B cells called plasma cells. Antibodies have two predominant roles that involve specific binding to antigens to launch an immune response, along with activation of other components of the immune system to fight pathogens. The ability of immunoglobulins to fight against innumerable and diverse pathogens lies in their intrinsic ability to discriminate between different antigens. Due to this specificity and high affinity for their antigens, antibodies have been a valuable and indispensable tool in research, diagnostics and therapy. Although seemingly a simple maneuver, the association between an antibody and its antigen, to make an antigen–antibody complex, is comprised of myriads of non-covalent interactions. Amino acid residues on the antigen binding site, the epitope, and on the antibody binding site, the paratope, intimately contribute to the energetics needed for the antigen–antibody complex stability. Structural biology methods to study antigen–antibody complexes are extremely valuable tools to visualize antigen–antibody interactions in detail; this helps to elucidate the basis of molecular recognition between an antibody and its specific antigen. The main scope of this chapter is to discuss the structure and function of different classes

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U. Hoeger and J. R. Harris (eds.), *Vertebrate and Invertebrate Respiratory Proteins, Lipoproteins and other Body Fluid Proteins*, Subcellular Biochemistry 94,
https://doi.org/10.1007/978-3-030-41769-7_19

of antibodies and the various aspects of antigen–antibody interactions including antigen–antibody interfaces—with a special focus on paratopes, complementarity determining regions (CDRs) and other non-CDR residues important for antigen binding and recognition. Herein, we also discuss methods used to study antigen–antibody complexes, antigen recognition by antibodies, types of antigens in complexes, and how antigen–antibody complexes play a role in modern day medicine and human health. Understanding the molecular basis of antigen binding and recognition by antibodies helps to facilitate the production of better and more potent antibodies for immunotherapy, vaccines and various other applications.

Keywords Antigen · Antibody · Antigen–antibody interface · Antigen recognition · CDR · Framework region · X-ray crystallography · NMR · CryoEM · Proteins · Superantigens · Allergic diseases

Introduction to Antibodies

Immunoglobulins

Immunoglobulins (Igs), also known as antibodies, play an integral role in the armamentarium of immune defense in vertebrates. The presence of these protective molecules mainly found in the blood stream and other body fluids, was first identified by von Behring and Kitasato in 1890 (Schroeder and Cavacini 2010). The duo reported the existence of an agent in the blood that could neutralize diphtheria toxin. Later, it was noticed that the agent could discriminate between two immune substances. Through the years, it was discovered that antibodies eradicate and neutralize extracellular pathogens including viruses and bacteria (Schroeder and Cavacini 2010). More than 120 years of research and investigations have not only demystified the function, structure and the immunological role of these protective agents, but also emphasized the antibodies' complex nature. A significant fraction of all studies in this area focus on human and murine antibodies, so in this chapter we will mainly concentrate on antibodies from these two organisms.

Antibodies are secreted by B lymphocytes, specifically differentiated B cells called plasma cells. When antibodies are secreted into blood and tissue fluids, they can exist as soluble proteins in the blood plasma, or as membrane bound proteins, adhered to the surface of B cells as B-cell receptors (BCR). These BCRs facilitate the activation of the B cells including their differentiation into memory B cells that recognize "second-time" antigen offenders to launch a better immune response, or antibody producing plasma cells (Borghesi and Milcarek 2006). However, for a full immune response to be achieved, usually B cells should interact with T-helper lymphocytes, upon antigen binding, leading to antibody production (Parker 1993).

Antibodies, except for those observed in sharks and camelids (Stanfield et al. 2004; Hamers-Casterman et al. 1993), are hetero-oligomeric glycoproteins that belong to the immunoglobulin superfamily. Visualized simply, the shape of antibodies basic

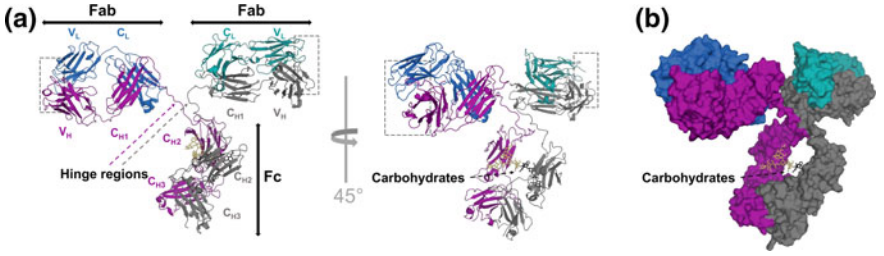

Fig. 19.1 Structure of murine IgG1. **a** Cartoon representation of IgG1 (PDB code: 1IGT (Harris et al. 1997)) with light chains (blue and teal) and heavy chains (purple and gray) marked in different colors. Domains forming both light and heavy chains, as well as Fc and Fab fragments are labeled. Antigen binding regions are marked with boxes. Carbohydrate residues are shown in stick representation. **b** Surface representation of IgG1. Orientation of the molecule is the same as for the neighboring carton representation. The figure indicates that the carbohydrate residues are located in cavity between two C_H2 domains

structure can be compared to the letter "Y" (or "T" or a Y/T hybrid; Fig. 19.1) (Harris et al. 1998; Tian et al. 2015). Both extremities of these Y-shaped proteins are important for their function (Janeway et al. 2001; Huber 1980). The top part of the Y, the antibody fragment (Fab), has the antigen binding site called the paratope which is specific to one part of the antigen referred to as an epitope. The lower part of the Y consists of the crystallizable fragment (Fc), which binds to specific receptors to communicate with other components of the immune system (Maverakis et al. 2015). There are five types of Fc regions that enable antibodies to bind to different Fc receptors thereby initiating various immune responses depending on the receptor bound. These differences in the Fc regions give rise to five antibody classes (Fig. 19.2), (Arnold et al. 2007): IgM, IgG, IgA, IgD and IgE. IgG and IgA are further divided into subclasses IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2 respectively (Spiegelberg 1989; Leder 1982).

More specifically, the structure of all antibodies consists of two heavy (H) and two light (L) polypeptide chains that have an NH_2 terminal variable-domain (V) and a COOH-constant domain (C) (Fig. 19.1). The variable domains bind antigens and the constant domains specify effector functions such as binding to Fc receptors (Spiegelberg 1989). The five different types of Fc regions on the heavy chain, α (alpha), γ (gamma), δ (delta), ε (epsilon), and μ (mu), give rise to IgA, IgG, IgD, IgE, and IgM antibody classes respectively. Both L chains have one identical constant domain; whereas both H chains have three, or four such domains. There are two different light chains, κ (kappa) and λ (lambda), which are characterized by different physicochemical properties (Townsend et al. 2016). There is no evidence, however, that the light chain constant region determines, or affects the effector function of an antibody. Variable domains can be further divided into three regions of sequence variability, complementarity determining regions (CDRs), and four regions that have comparatively constant amino acid sequences named the framework regions (FRs) (Schroeder Jr and Cavacini 2010; Spiegelberg 1989). A paratope is, more often than

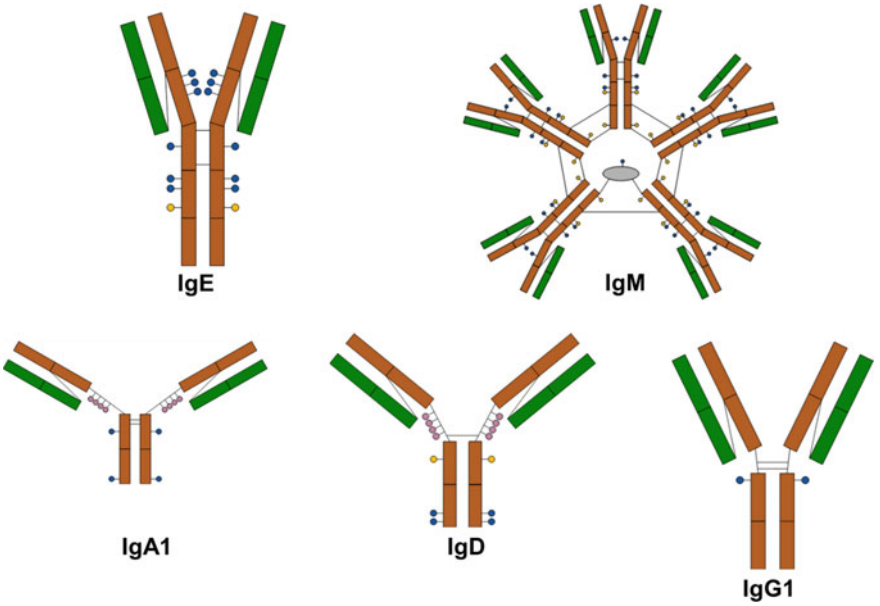

Fig. 19.2 Diagrammatic representations of antibody classes IgE, IgM, IgA1, IgD, and IgG1. Light chains are in green, heavy chains-brown, inter-chain disulfide bridges-black and IgM J chain-grey. N-glycosylation sites are presented in blue, oligomannose glycans in yellow and O-glycans in pink (Arnold et al. 2007)

not, composed of amino acids originating from the three CDRs of the heavy chain and the three CDRs of the light chain (Fig. 19.3). The antigen binding site is extremely variable and this enables antibodies to recognize millions of different antigens even within the same antibody class (Market and Papavasiliou 2003).

Despite their different classes and subclasses, antibodies implement prominent modes of action, to either eradicate or mark the pathogen for devastation by immune cells. Antibody action on pathogens includes, but is not limited to, neutralization, opsonization, antibody dependent cellular cytotoxicity (ADCC) and agglutination (Abbas et al. 2014). Pathogens use molecules in their cell walls, or envelopes to bind the host cell and gain entry into it. Antibodies like IgA and IgG attach to these pathogen molecules thereby blocking entry into the host cell. Antibodies also coat pathogens for degradation in a process called opsonization (Fig. 19.4). These antibody-coated pathogens are marked for phagocytosis by neutrophils and macrophages. In ADCC, antibodies like IgG coat the surface of a cell and generate signals that activate natural killer cells to discharge their toxic granule proteins on an infected cell thereby killing the cell (Abbas et al. 2014). Another way of eradicating pathogens that antibodies employ is agglutination. In this mechanism, antibodies glue pathogens together into clumps. The clumped cells simultaneously become inactive and are susceptible to phagocytosis or, become a target for the complement system (Elek et al. 1964). Antibodies execute their protective roles in the immune system

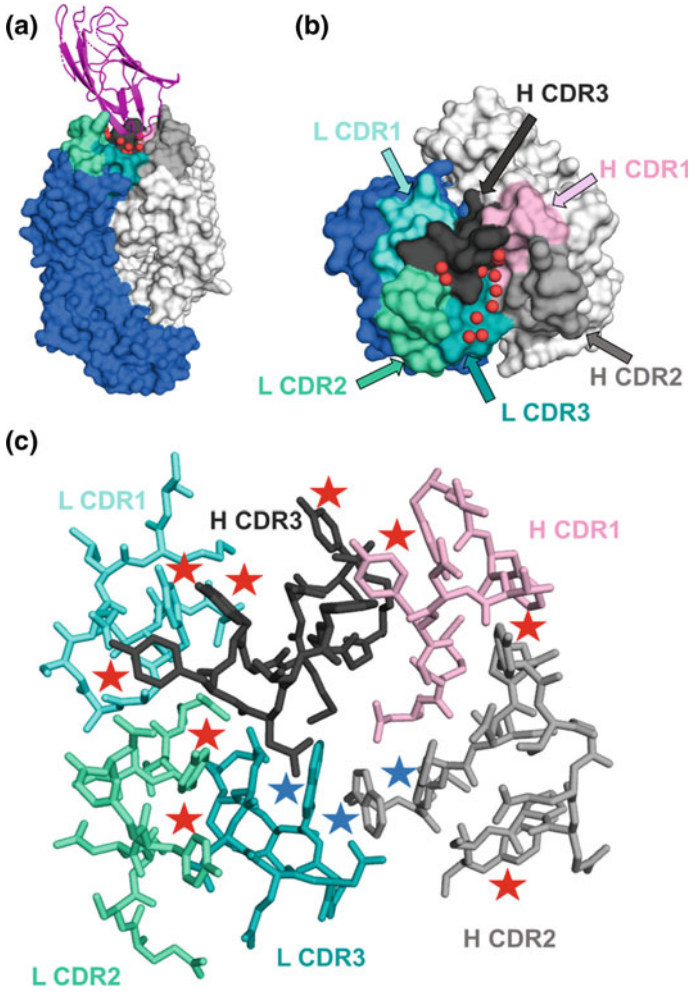

Fig. 19.3 Structure of a complex between Fab fragment of murine antibody 7A1 (IgG) and house dust mite allergen Der p 2 (PDB code: 6OY4 (Glesner et al. 2019)). **a** Overall structure of the complex. Der p 2 is shown in carton representation (magenta) and Fab fragments shown in space filling representation. Water molecules buried between the antibody and antigen are represented by red spheres. **b** CDRs of 7A1 mapped on the antibody surface and shown in different colors. **c** CDRs shown using stick representation. Red stars mark position of tyrosine residues and blue stars mark position of tryptophan residues

by employing various mechanisms, and all of these functions include some form of interaction between an antibody and its antigen. For clarification, there are at least two definitions of an antigen. The first definition is that an antigen is a substance that induces an immune response, and the second states that anything that binds to immunoglobulins, or T-cell receptors is an antigen. For the purpose of this chapter,

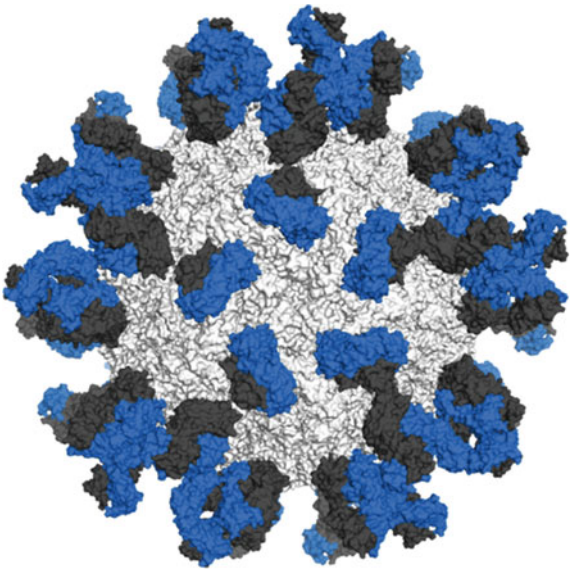

Fig. 19.4 Complex of foot-and-mouth disease virus capsid (light gray) with Fab fragments of neutralizing antibody SD6 (PDB code: 1QGC (Hewat et al. 1997)). Heavy chain of the antibody is presented in dark gray and light chain of the antibody is shown in blue

the second definition will be adopted. Irrespective of the antibody class, the antibody should somehow interact with its antigen to elicit an immune response. For example, in neutralization, the immune response is heavily dependent on the interaction of an antibody's variable region with its antigen. However, other mechanisms like ADCC, depend on the interaction between the antibody's constant domain with immune cells through Fc receptors and/or complement proteins (Pollara and Tay 2019).

Antibody Classes and Functions

Although the general structure of all antibody classes is very similar, each antibody class has unique biological characteristics, functions and elicits specific immune responses. Starting with immunoglobulin M, IgM, is the most conserved antibody class expressed first in immune-development and it is found in all vertebrates (Boes 2000). A naïve B cell, never exposed to an antigen, expresses cell surface monomeric IgM in bound form (here by "monomer" we mean a basic antibody unit composed of two heavy and two light chains). Most circulating IgM is "natural" IgM; thus, IgM is produced spontaneously without a known exogenous antigenic, or microbial stimulus. There are three main known forms of secreted IgM: monomeric, pentameric, and hexameric. In healthy people, circulating IgM exists predominantly in the pentameric form. Pentameric IgM is formed in the endoplasmic reticulum, contains 5 monomers of IgM linked together by a J-(joining) chain (Fig. 19.2). The multimeric IgM has high avidity despite low affinity of its individual components. This makes IgM very efficient in the coating and neutralization of antigens. In addition, upon

binding to an antigen, polymeric IgM potently activates the complement system. IgM is also associated with primary immune response and is involved in immunoregulation (Boes 2000; Grönwall et al. 2012). The relatively low affinity IgMs are also known as natural antibodies. These natural antibodies play a role in augmenting the clearance of dying cells, as well as reinforcing the mechanisms that protect the body from the development of autoimmune diseases (Grönwall et al. 2012).

Even though a naïve B cell expresses IgM, it also expresses cell surface immunoglobulin D (IgD). IgD mainly functions as an antigen receptor on naïve B cell surfaces, although it is also believed to play a part in the production of antimicrobial factors by activating mast cells and basophils to release pro-inflammatory cytokines (Chen et al. 2009b). The co-expression of both IgM and IgD renders the B lymphocyte ready to respond to an antigen. When the cell bound antibody binds to an antigen, this activates the B cell causing it to divide and differentiate into an antibody producing "factory", a plasma cell (Geisberger et al. 2006). The antibodies produced by the plasma cell are not only specific for the bound antigen but, are also secreted and no longer membrane bound. Intriguingly, some daughter cells from the activated B cell, are subjected to class switch recombination and somatic hypermutation to help generate a diverse and high-affinity repertoire of antibody classes and subclasses (Durandy 2003). These two processes cease the production of IgM, and/or IgD and start the production of other antibody classes like IgG, IgE and IgA leading to a specific effector function needed for each specific antigenic challenge (Geisberger et al. 2006; Stavnezer and Amemiya 2004).

Immunoglobulin G, IgG, does not only have the longest half-life in the serum, but is the most predominant antibody in the body. In all its four forms, IgG (IgG1, IgG2, IgG3 and IgG4) provides the majority of immunity in antibody-based immunity and is the only antibody class that is able to cross the placenta to give passive immunity to a fetus (Schroeder and Cavacini 2010). IgG subclasses were identified due to functional, structural and antigenic differences in the heavy chain constant region particularly C_H1 and C_H3. These C_H1 regions control the mobility and flexibility of the antibody. Hence, IgG subclasses exhibit different functional activities (Schroeder and Cavacini 2010). Structural differences in their heavy chains determine the ability to activate the complement system and to bind to IgG receptors such as CD64 (FcεRI), CD32 (FcεRII) and CD16 (FcεRIII). For example, it was suggested that IgG1 and IgG3 are mainly induced in response to protein antigens, however, IgG2 is usually activated in response to polysaccharide antigens and IgG4 antibodies are often formed after a long-term, or repeated exposure to an antigen in a non-infectious setting like in allergic diseases (Schroeder and Cavacini 2010; Vidarsson et al. 2014). IgG subclasses also have similar functions such as participation in secondary immune response, trans-placental transport as well as neutralization of toxins and viruses. However, subclasses still impact the outcome of the immune response. For example, IgG3 antibodies were observed to be more effective in neutralizing the HIV virus than IgG1 (Cavacini et al. 2003). IgG antibodies, especially IgG1 and IgG4, have also been shown to influence antigen–antibody complex formation and binding to B cells in food allergies (Meulenbroek et al. 2013).

Immunoglobulin E, IgE, although the least abundant antibody class in the serum with the shortest half-life, it is a very potent antibody which is a troublesome instigator of allergic reactions (Platts-Mills 2001; Burton and Oettgen 2011). However, the major role of IgE involves immunity to parasites through its binding to eosinophils. IgE also binds to mast cells and basophils with high affinity but, it is of rudimentary importance to highlight that the IgE receptor on eosinophils is seen only in some clinical conditions, such as, parasite infestations (Gounni et al. 1994). IgE immune responses are called "immediate hypersensitivity". This term does not only denote the extreme nature of IgE sensitivity to antigens, but also the tremendous speed in which the immune response takes place (Burton and Oettgen 2011). IgE has an extremely high affinity for FcεRI receptors expressed on mast cells, basophils, eosinophils and Langerhans cells (Schroeder and Cavacini 2010). When an allergen enters the human body, it binds IgE on mucosal mast cells, or if administered parenterally, it also binds to basophils circulating in the blood. However, eosinophils and Langerhans cells are not involved in immediate hypersensitivity. They participate in the late phase response which occurs 3–8 h after the initial challenge. IgE strongly binds to FcεRI receptors after which an antigen crosslinks two IgE molecules leading to degranulation of mast cells and the release of histamine and other pro-inflammatory mediators. Immediate hypersensitivity is manifested in one, or several symptoms like urticaria in the skin, food-induced diarrhea and anaphylaxis of the gut, bronchospasms and so on depending on the organ where sensitization occurred (Schroeder and Cavacini 2010; Burton and Oettgen 2011).

Immunoglobulin A, IgA, the most abundant antibody at mucosal surfaces and in secretions, is the first line of defense in the immune system armamentarium against ingested and inhaled antigens like toxins, viruses, and bacteria at mucosal surfaces (Underdown and Schiff 1986). These mucosal surfaces include the gut, the urogenital tract, and the respiratory tract. IgA has also been shown to be found in saliva and breast milk. IgA serum levels are higher than IgM, but lower by considerable magnitudes compared to IgG. Generally, IgA is found in monomeric form in the serum, but can assume different oligomeric states at the mucosa. Termed secretory IgA (sIgA) at the mucosa, the antibody mainly exists in dimeric form. Dimeric sIgA was shown to be more effective in preventing damage (by toxins) to epithelial cells than monomeric (Schroeder and Cavacini 2010). IgA is found in two main subclasses, IgA1 and IgA2. The difference between the two subclasses is mainly in their hinge region, with IgA1 having a longer hinge region. The elongated IgA1 hinge region consists of repeated amino acids that increases the antibody's propensity to bacterial protease degradation. For that reason, 90% of serum sIgA is IgA1, and IgA2 predominates on the mucosal surface. IgA fights against viruses and bacteria by neutralizing, or preventing the binding of the pathogens on the mucosal surface (Schroeder and Cavacini 2010; Underdown and Schiff 1986).

Antigen–Antibody Complexes

Since 1890, when the existence of antibodies was first defined as part of the immune system armament against pathogens, scientists went on a quest to demystify and understand the interactions between an antibody and its antigen. Antigen–antibody complexes became an extremely valuable tool in understanding detailed antigen–antibody interactions and elucidating the basis of molecular recognition between an antibody and its specific antigen (Davies et al. 1988). Antigen–antibody complexes have not only helped to predict the biological function of particular antigens, or proteins, but also explained the mechanisms by which antigens can elicit an immune response. For example, it was found that in allergic diseases some allergens promote sensitization through their proteolytic activity, or by mimicking certain proteins in signaling pathways (Karp 2010). The main aim of this part of the chapter is to discuss various aspects of antigen–antibody interactions including antigen–antibody interfaces, methods used to study these complexes, antigen recognition by antibodies, types of antigens in complexes and how antigen–antibody complexes play a role in modern day medicine and human health.

Structural Methods to Study Antigen–Antibody Complexes

In modern day research, various ways of studying antigen antibody-complexes have been developed. Among all those methods, X-ray crystallography, nuclear magnetic resonance (NMR) and Single Particle Cryogenic Electron Microscopy (CryoEM) have been the most widely used. Of the three methods, X-ray crystallography championed in the Protein Data Bank (PDB) with almost 94% of antigen–antibody complex structures determined using this method. X-ray crystallography is followed by NMR and CryoEM that contributed approximately 6% of the reported structures of antigen–antibody complexes in the PDB (Berman et al. 2000). Structural models of antigen–antibody complexes generated using these methods are not only interesting from the scientific point of view, but their analysis is also performed for various medicinal and other commercial purposes. In studying antigen–antibody complexes, all the afore-mentioned methods have their merits and demerits. A brief summary of the method as well as its advantages and disadvantages will be discussed below.

X-Ray Crystallography

Since most B cell epitopes (fragments of antigens interacting with paratopes) are conformational (Van Regenmortel 2009), to date, X-ray crystallography remains the gold standard that provides a detailed three-dimensional structure of an antigen–antibody complex. X-ray crystallography is able to produce a snapshot of an antigen–antibody complex at high resolution that makes it possible to visualize the specific

interactions between the antigen and antibody (Sheriff et al. 1987; Jeffrey et al. 1995). However, due to antibodies large size, flexibility and glycosylation, it is extremely difficult, to determine their structure by X-ray crystallography (King and Brooks 2018). In fact, currently there are only three available crystal structures of intact antibodies (Harris et al. 1997, 1998; Saphire et al. 2001), and there is no single structure of a whole antibody in complex with an antigen. Fortunately, antibody variants like antibody fragments (Fab), single chain variable fragments (ScFv) and other various antibody formats are easier to crystallize and therefore, they are used for determination of antigen–antibody structures using X-ray crystallography.

X-ray crystallography comprises three basic steps (Chruszcz et al. 2008). The first step is to obtain an X-ray diffracting crystal. This step is usually the bottle-neck for using this method in studying antigen–antibody complexes. The second step is obtaining the crystal X-ray diffraction pattern. Structure determination using X-ray crystallography depends exclusively on diffracted beams that are produced when X-rays interact with the crystal (King and Brooks 2018; Wlodawer et al. 2008). The diffraction data collected is combined and processed computationally to produce an electron density map that contains information about the chemical structure and orientation of the atoms in the molecular structure under study. Finally, the atomic model generated from the electron density map is refined using several software programs that employ various parameters important for structure determination, to yield the final crystal structure (Wlodawer et al. 2008).

Nuclear Magnetic Resonance

Although NMR does not give as exhaustive information on antigen–antibody binding as X-ray crystallography, this method is able to map residues forming epitopes and/or paratopes, and shed light on intermolecular interactions (Zuiderweg 2002; Wider and Wüthrich 1999). The biggest advantage of NMR over X-ray crystallography is that it does not need the formation of a crystal, so it is usually used for those proteins that are hard to crystallize. The basic idea employed in NMR epitope mapping and antigen–antibody interactions studies is that when an amino acid on an antigen binds to an antibody, the amino acid's chemical environment changes. The chemical environment of residues on the antigen–antibody interface changes because protein-solvent interactions are replaced by protein-protein interactions (Zuiderweg 2002). The NMR signal that a residue emits unbound and bound to an antibody changes, and it is these changes in NMR signal that are studied to deduce residues that are involved in antibody binding. By studying the differences in NMR spectra of an antigen before and after binding an antibody, epitope residues can be easily identified. Retrospectively, if two different antibodies exhibit the same changes in the NMR signals, then they are regarded as sharing the same epitope (Simonelli et al. 2018). However, for the NMR signals to be generated, the antigen has to be labelled by either ^{15}N, or ^{13}C. Therefore, expression systems like *E. coli,* or yeast are required which might not be practical for some proteins.

^{15}N-Heteronuclear Single Quantum Coherence (^{15}N-HSQC) is usually the most used NMR method for studying antigen–antibody interactions. The N–H of each amino acid residue generates an NMR signal. So sensitive are the positions of these signals to the protein conformation that ^{15}N-HSQC is often referred to as the protein fingerprint. NMR use in structure determination and antigen–antibody interactions is readily applicable to proteins that are 40–50 kDa. However, NMR signal intensity is greatly weakened by the increase in molecular weight of the complex. Intense reduction in signal, for bigger proteins and complexes, from 60–100 kDa, almost renders NMR inapplicable. Consequently, for larger antigen–antibody complexes, Transverse Relaxation Optimized Spectroscopy (TROSY) is used (Zuiderweg 2002; Wider and Wüthrich 1999). Although a very powerful tool, NMR requires high amounts of pure homogeneous sample of 0.5 mM or, more. Detailed and comprehensive discussion on NMR usage in antigen–antibody interactions research is found in these excellent articles (Simonelli et al. 2018; Rosen and Anglister 2009).

Single Particle Cryogenic Electron Microscopy

Although X-ray crystallography and NMR are the fundamental techniques for structure determination at atomic resolution, over the past decade, CryoEM has been intrinsically gaining popularity in the field of structural biology. This is mainly because in CryoEM large multi-subunit complexes of viruses, bacterial appendages, eukaryotic ribosome and cellular organelles are able to be visualized without the need for crystallization (Chen et al. 2009a; Donnarumma et al. 2015). Currently, structures of small proteins, or other antigen of similar size cannot be analyzed using CryoEM, however this problem can be mitigated by forming antigen–antibody complexes. In this manner structures of complexes as small as 65 kDa can be determined. This approach could be the solution to solving antigen–antibody structures with full IgE or IgG, since these proteins are very flexible and hard to crystallize (Donnarumma et al. 2015; Wu et al. 2012).

In CryoEM, a small amount of sample suspended in buffer solution is quickly frozen to form a non-ctystalline glass-like specimen called vitreous ice. This vitrified sample is then exposed to high frequency electrons which scatter through the specimen due to electrostatic interactions with the sample's atoms. Scattered and unscattered electrons form an interference pattern that results in 2D projection images which are recorded. These 2D projections are recorded from viewing the specimen from different angles. For structure determination, the combined 2D projections are further digitally processed and analyzed taking into account all orientation parameters (Skiniotis and Southworth 2016). The final structure is then solved enabling visualization of biological complexes under close to physiological conditions. Unfortunately, quite often this technique provides low-resolution structural information, and it has to be combined with other methods to provide more detailed information on epitopes and paratopes involved in antigen–antibody interactions. For a further thorough discussion of CryoEM in structural biology, see the following articles (Skiniotis and Southworth 2016; Boekema et al. 2009).

Other Methods to Study Antigen–Antibody Interactions

Although the three methods discussed above are the "go-to" techniques for mapping antibody epitopes in studying antigen–antibody interactions, they do possess major restrictions. Hence, several other relatively simpler methods are employed in B-cell epitope mapping (Potocnakova et al. 2016). Examples of such methods are mutagenesis, hydrogen–deuterium exchange coupled with mass spectroscopy, and peptide-based approaches. In the mutagenesis approach, to putatively determine antibody epitopes, binding of the antibody to the antigen mutants is analyzed. A reduction in antibody binding is deciphered as an indication that the mutated amino acid residues form part of the epitope. Using "shotgun mutagenesis", or Ala scanning, thousands of proteins can be screened simultaneously (Abbott et al. 2014). In the other method used, hydrogen-deuterium exchange coupled to mass spectrometry, the rate of deuteration is slowed down if some part of the antigen is interacting with an antibody. After deuteration, the level of deuteration for the pepsin digested antigen is analyzed by mass spectrometry. This technique is applicable to most proteins with a moderate level of purity and even impure antibodies can be used (Wei et al. 2014). One more common method in epitope mapping is the peptide-based approach. A number of overlapping peptides covering the whole primary sequence of an antigen are synthesized. These peptides are immobilized onto a solid matrix and their binding to the antibody of interest is analyzed in an ELISA-like format. The peptide(s) that bind to the antibody are then sequenced and the mimotopes deciphered. This technique is applicable to linear epitopes, quick and easy to perform. Huge peptide libraries can be generated allowing for fast and comprehensive epitope mapping (Potocnakova et al. 2016; Ahmad et al. 2016). There are many more techniques, be it computational and in vitro, or in vivo that this chapter cannot comprehensively cover. Other methods not discussed here are reviewed by Ahmad et al. (2016).

Antigen–Antibody Interactions

Complementarity Determining Regions

Just as their name suggests, the six hypervariable loops of the heavy and light chain known as the complementarity determining regions (CDRs) (Fig. 19.3), have been solely designated to be responsible for direct antigen binding. Hence, in studying the structural and molecular basis for antigen recognition, finding the CDR boundaries and the residues involved in antigen binding have been the focus of many studies. Several methods to decipher CDR boundaries have been developed over the years like the Kabat, Clothia and IMGT numbering nomenclatures (Al-Lazikani et al. 1997; Chothia and Lesk 1987; Chothia et al. 1989; Sela-Culang et al. 2013). However, as mentioned earlier, the general structure of an antibody comprises of the Fab regions and the constant region(s). The Fab region contains two variable domains from the

light and heavy chain and two constant domains (Fig. 19.1). Each of the variable domains contains three hypervariable loops (CDRs). Therefore, there are three CDRs from the heavy chain (H1, H2 and H3) and the three from the light chain (L1, L2 and L3) (Sela-Culang et al. 2013; Schroeder and Cavacini 2010). The folding of the light and heavy variable domains brings the hypervariable loops together allowing them to form an antigen binding site, also known as the paratope. The hypervariable loops are supported by framework of beta-sheets that are the basic building blocks of the characteristic immunoglobulin domains (Schroeder and Cavacini 2010).

Paratopes and Epitopes

Identification of CDRs is used as the starting point in identifying paratopes, since the residues that make contact with the antigen are quite often found at the center of the region formed by the CDRs' residues (Sela-Culang et al. 2013). Interestingly though, 3D structure analysis of antibodies showed that only 20–33% of the CDRs' amino acid residues actually take part in antigen binding and these are the most variable within each CDR (Padlan et al. 1995). This also suggests that CDRs of a particular antibody may form several paratopes that are able to recognize various epitopes. For example, such a situation was observed in the case of a bH1 antibody that is able to recognize fragments of two completely different proteins (Fig. 19.5a), (Bostrom et al. 2009). This scenario is an example of the so-called true cross-reactivity, in which an antibody raised against one antigen also binds to another unrelated antigen. True cross-reactivity like this, also shows that the antibody may be poly-specific (Van Regenmortel 2014). Additionally, the presence of such cross-reactivity also highlights the importance of distinguishing between immunogenic and antigenic properties of molecules. A single antibody may recognize two unrelated antigens;

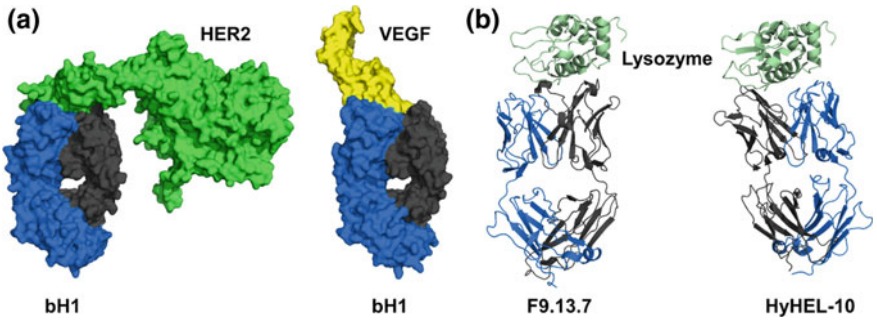

Fig. 19.5 **a** Structures of the same antibody (bH1) with two completely different proteins (PDB codes: 3BE1 (Bostrom et al. 2009) and 3BDY (Bostrom et al. 2009)). **b** Structures of two unrelated antibodies binding to the same epitope on lysozyme surface (PDB codes: 1FBI (Lescar et al. 1995) and 3HFM (Padlan et al. 1989)). Fab fragments of antibodies were used for structural studies. Light chains are shown in blue and heavy chains are shown in dark gray

however, it is also likely that two unrelated antibodies are binding to the same epitope. This is illustrated in Fig. 19.5b (Pons et al. 2002), where the same epitope located on a lysozyme protein molecule is recognized by two different paratopes originating from two different antibodies. The second situation corresponds to the presence of an immunodominant epitope that is recognized by various polyclonal antibodies. This phenomenon was vividly demonstrated in one study whereby allergenic epitopes of bovine α_{s1} casein, a major allergen from cow milk, were identified using 188 overlapping peptides. All sera from bovine α_{s1} casein allergic patients bound to three common regions on the allergen surface. These regions showed the most reactivity to the polyclonal antibodies from the patients' sera, although the antibodies also bound to other regions. Indeed, the three regions were immunodominant epitopes compared to the other epitopes (Spuergin et al. 1996).

True cross-reactivity is observed less frequently than the so-called shared reactivity (Van Regenmortel 2014; Berzofsky and Schechter 1981). In shared reactivity two antigens possess a common fragment in their epitopes that is recognized by the same antibody. The case of two-house dust allergens, Der f 1 and Der p 1, that both bind to a cross-reactive antibody, 4C1, provides an excellent example to illustrate shared reactivity (Fig. 19.6), (Chruszcz et al. 2012). Der p 1 and Der f 1 share approximately 80% sequence identity, and 4C1 (murine IgG) binds to epitopes that are almost identical in both allergens. The residues forming the core of the 4C1 epitopes on Der f 1 and Der p 1 are not only conserved in terms of their sequence, but the side chains of the conserved residues also adopt very similar conformations. The 4C1 epitopes on Der f 1 and Der p 1 are examples of conformational and discontinues epitopes, as they are composed of residues that are distant when considering the proteins' amino acid sequences (primary structure of the proteins), however, the residues forming the epitopes are brought in vicinity of each other when the proteins are folded. Such epitopes can be destroyed by denaturation of the proteins (loss of the tertiary and secondary structure), or by modification of the proteins' surfaces.

A second example of shared reactivity was demonstrated by Stanfield et al. (2006a). The authors elucidated the binding of human immunodeficiency virus type 1 (HIV-1) neutralizing antibody 2219, that is able to bind three peptides derived from V3 fusion protein (Fig. 19.7). The three peptides binding to 2219 have approx-

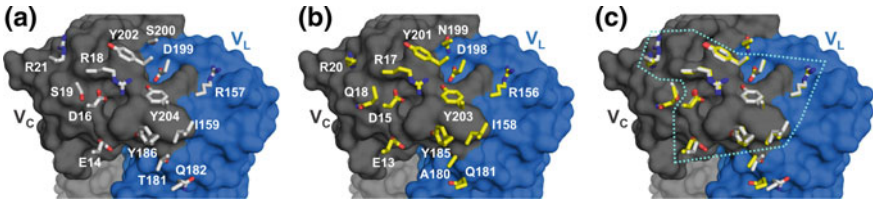

Fig. 19.6 Comparisons of 4C1 binding epitopes on Der f 1 (PDB code: 5VPL (Chruszcz et al. 2012)) (**a**) and Der p 1 (PDB code: 5VPG (Chruszcz et al. 2012)) (**b**). The residues forming the epitopes are shown in stick representation. Antibody is shown in space-filling representation with the heavy chain in gray and light chain in blue. **c** Superposition of Der f 1 and Der p 1 epitopes. The residues inside of the marked regions are identical for both Der f 1 and Der p 1

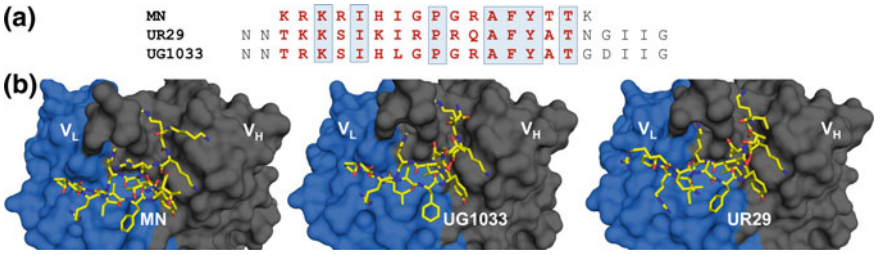

Fig. 19.7 **a** Three different peptides derived from third hypervariable loop of HIV gp120 that bind human monoclonal antibody 2219. Residues in dark red are ordered in the crystal structures shown below. Amino acids highlighted with blue boxes are conserved in all three peptides. **b** Crystal structures of antibody 2219 in complex with peptides MN (left; PDB code: 2B0S (Stanfield et al. 2006a)), UG1033 (middle; PDB code: 2B1A (Stanfield et al. 2006a)) and UR29 (right; PDB code: 2B1H (Stanfield et al. 2006a))

imately 50% sequence identity, however, they all adopt a very similar β-hairpin conformation. Therefore, these peptides can be simultaneously described as both being linear and conformational epitopes. A third example of shared reactivity is related to interactions of antibodies with some polysaccharides that are N- or O-linked to proteins. These alleged cross-reactive carbohydrate determinants (CCDs) are important from the perspective of allergy diagnostics (Mari et al. 1999) because the presence of IgEs directed against CCDs may result in some false positives and suggests a polysensitization.

Structural Characterization of Paratopes and Epitopes

Numerous structural studies of antigen–antibody complexes allowed for more detailed characterization of paratopes and epitopes. It was shown that the five out of the six hypervariable regions adopt a relatively small number of main chain conformations (Al-Lazikani et al. 1997; North et al. 2011), and only H CDR3 displays a pronounced conformational variability (Weitzner et al. 2015). It was also observed that of all the CDR chains, L CDR3 and H CDR3 residues dominated in antigen binding (Padlan et al. 1995; Padlan 1994). A number of research studies revealed that each CDR consists of distinct and unique amino acid residues different from other CDRs (Kunik and Ofran 2013; Zhao and Li 2010; Raghunathan et al. 2012). Furthermore, according to the so-called hotspot hypothesis, only a few very specific residues on the antigen–antibody interface within each paratope and epitope are critical for antigen recognition and binding (Bogan and Thorn 1998). Site directed mutagenesis on epitope residues have supported this hypothesis. Mutations of one, or two epitope residues significantly reduced, or even abrogated antibody binding showing that only a few residues dominated the energetics of the antigen–antibody interactions (Li et al. 2001; Glesner et al. 2017). It was also shown that paratopes

are enriched in some residues, like aromatic amino acids (Phe, Trp and Tyr), with tyrosine being the most important (Birtalan et al. 2008; Peng et al. 2014; Robin et al. 2014). This is shown in Fig. 19.3c, which clearly illustrates the enrichment of CDRs in tyrosine residues. While enrichment of paratopes in some amino acids can be easily demonstrated, such effects are not observed for proteinous epitopes, and their composition is generally the same as the composition of the protein surface (Kunik and Ofran 2013; Kringelum et al. 2013).

Structural studies also demonstrated that quite often water molecules are trapped between paratopes and epitopes (Braden et al. 1995; Bhat et al. 1994). Water molecules enhance antigen–antibody interactions and stability by improving charge complementarity through hydrogen bonding, and increasing packing density and paratope-epitope complementarity by filling "empty" cavities at the interface (Braden et al. 1995; Mariuzza and PoIjak 1993). Generally, surfaces corresponding to paratopes may be divided into concave, ridged, planar, and convex (MacCallum et al. 1996). Research showed that the concave paratopes are mainly observed for complexes of antibodies with haptens (Fig. 19.8), ridged for complexes with peptides (Fig. 19.7) and planar for proteinous antigens (Fig. 19.6). It was also observed that

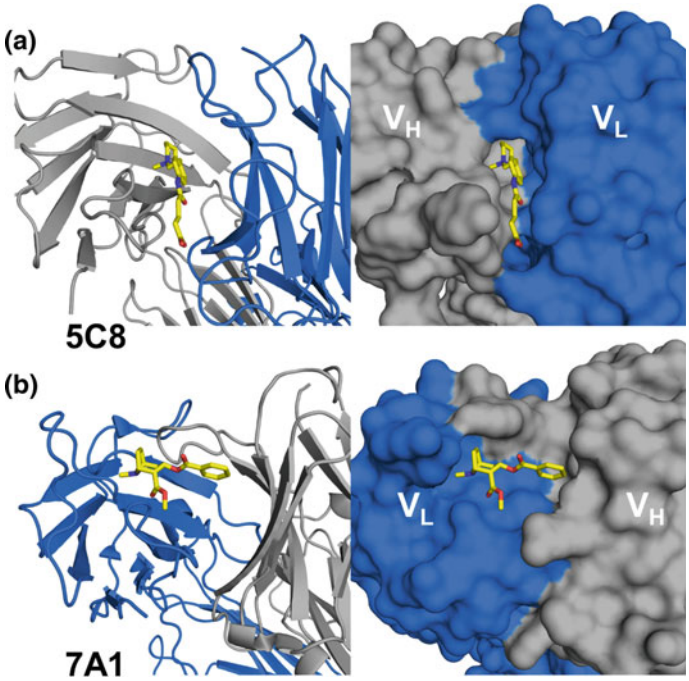

Fig. 19.8 Crystal structure of Fab fragments with haptens. The binding sites are shown in two representations—carton (left) and surface (right). Haptens are shown in stick representation. **a** Complex of catalytical antibody 5C8 with a transition state analog (PDB code: 25C8 (Gruber et al. 1999)). **b** Complex of 7A1 antibody with cocaine (PDB code: 2AJV (Zhu et al. 2006))

the concave paratope topology is characteristic for antibodies binding disordered antigens (MacRaild et al. 2016). Convex paratopes were perceived for dromedary heavy-chain antibodies (Fig. 19.9a), (De Genst et al. 2006). Carbohydrate antigens are mainly recognized by ridged paratopes (Dingjan et al. 2015; Haji-Ghassemi et al. 2015). However, structural studies revealed an interesting mode of antibody binding for carbohydrates. Namely, in the case of an anti-carbohydrate HIV neutralizing antibody in complex with a hexasaccharide, the antibody Fab forms an unusual dimer with heavy chains swapped (Fig. 19.10) (Stanfield et al. 2006a). Usually the affinities of anti-carbohydrate antibodies are lower than those observed for protein or, peptide antigens (Haji-Ghassemi et al. 2015), therefore, the unusual mode of binding observed for the mentioned anti-carbohydrate HIV neutralizing antibody may compensate for the lower affinity by forming contacts with the two antigens. In this situation, instead of affinity, the term avidity should be used to describe the overall strength of the binding between the antibody with multiple binding sites and antigen(s).

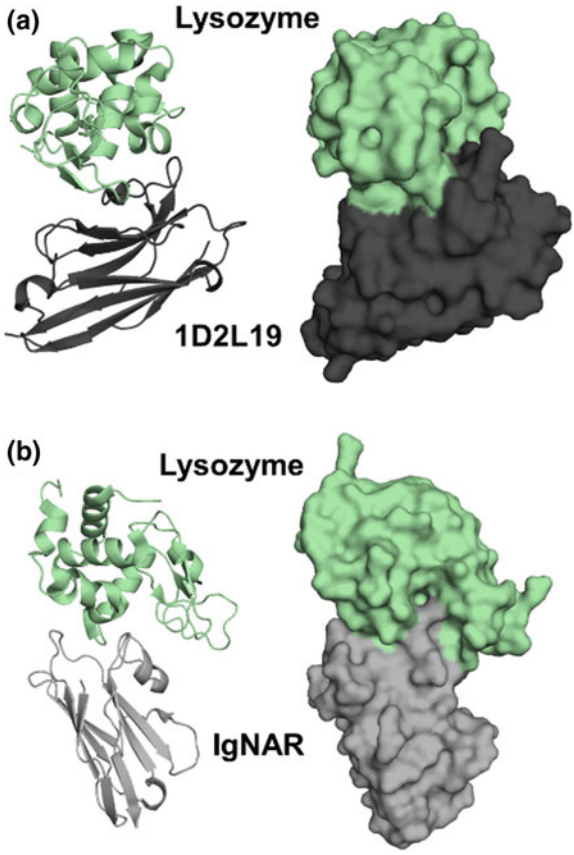

Fig. 19.9 Structures of lysozyme with variable domains of single chain antibodies. The complexes are shown in cartoon (left) and surface representation (right). **a** Complex with camelid antibody 1D2L19 (PDB code: 1RI8 (De Genst et al. 2005)). **b** Complex with shark IgNAR (PDB code: 1SQ2 (Stanfield et al. 2004))

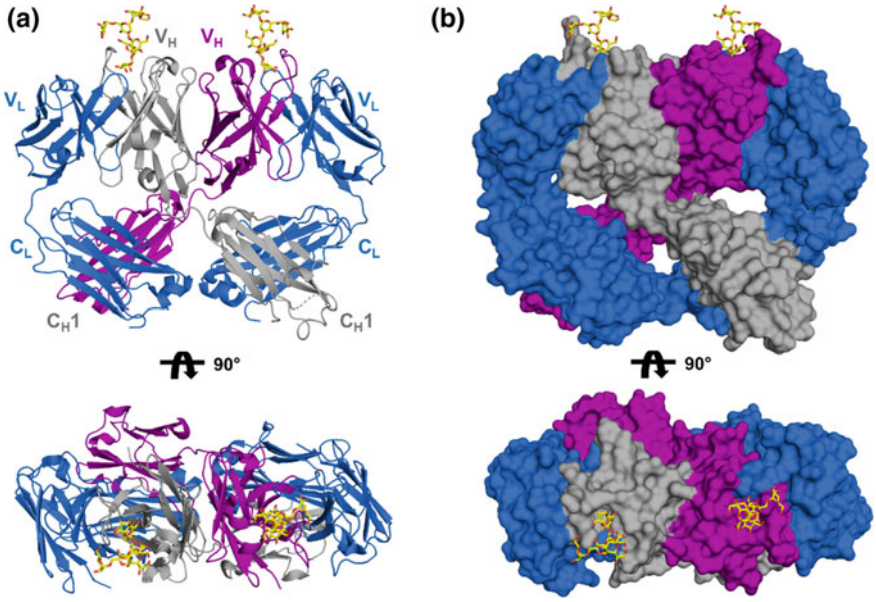

Fig. 19.10 Structure of a complex between a dimeric Fab 2G12 and Rv3 hexasaccharide (PDB code: 4RBP (Stanfield et al. 2015)) in cartoon (**a**) and surface (**b**) representations. The swapped heavy chains are shown in gray and purple, while light chains are shown in blue. The antigen is shown in stick representation (carbon atoms in yellow and oxygen atoms in red)

As implied above, the stability of macromolecular assemblies, like antigen–antibody complexes, is also facilitated by the same molecular interactions that stabilize correctly folded native proteins. Hence, just as the chemical environment affects the three-dimensional state of a protein, the pH, ionic strength and solvent also affect antigen–antibody complexes (Torres and Casadevall 2008). These chemical environmental effects can influence the distribution of charges and conformational effects thereby affecting binding of antibodies to their antigen. The major contributions to the free energy of an antigen–antibody binding comes from hydrophobic and hydrogen bonding interactions. (Torres and Casadevall 2008; Webster et al. 1994). However, in the fine tuning of antibody specificities charge–charge interactions, like salt bridges or cation-π interactions, play very important roles (Dalkas et al. 2014).

Other Non-CDR Components Important for Antigen Binding

As discussed previously, the constant domains have long since been believed to be exclusively responsible for mediating effector functions. However, there is a growing body of evidence suggesting that constant regions, framework regions (FRs) and other residues that are not part of the CDRs play a crucial role in antigen binding (Torres and

Casadevall 2008; Sela-Culang et al. 2013). Framework regions are part of the variable regions providing support to the CDRs to assume the right conformation for antigen binding (Sela-Culang et al. 2013). They are mainly believed to be scaffolds for the CDRs, but during murine antibody humanization when FR residues were omitted, substantial decrease, or complete abrogation of antigen binding was observed. When some of the FR residues were mutated back into the murine antibody sequence antigen binding was retained (Kettleborough et al. 1991). This highlighted the importance of FR residues in antigen binding. In fact, some of the FR residues due to their proximity to the CDRs can actually bind the antigen (Xiang et al. 1995, 1999). Interestingly, some FR residues that are further from the CDRs in sequence, but closer in 3D structure were also found to be involved in antigen binding especially heavy chain FR-3 residues, that account for 1.3% of human antigen–antibody contacts (Raghunathan et al. 2012; Capra and Kehoe 1974). FR residues that do not make contact with the antigen help maintain the CDR structural confirmation and orientation needed for antigen binding (Haidar et al. 2012).

Constant regions mainly deemed to be responsible for antibody effector functions, like Fc receptor binding and avidity, have been recently proven to be involved in antibody specificity and affinity for its antigen (Torres and Casadevall 2008). Although antibody classes have identical variable domains, but different constant domains, it was observed that they bind the same antigen with different affinities (Casadevall and Janda 2012; Tudor et al. 2012; Adachi et al. 2003; Xia et al. 2012). This phenomenon has also been shown in antigen–antibody complexes, whereby the dissociation constant of a single chain variable fragment antibody in complex with its antigen was different from a Fab format of the same antibody. Consequently, the importance of constant domains in antigen binding is undeniable (Adachi et al. 2003). Allosteric effects of the constant domains on the variable domain conformation have been attributed to be the cause of the differences perceived in antibody class affinity and specificity (Xia et al. 2012). Conclusively, constant regions are indeed important players in antigen–antibody complexes. Hence, the antibody class in antibody engineering for either research, vaccines or, immunotherapy should be carefully chosen, because the affinity, specificity and the epitope bound can be easily affected by the class used.

Other than constant and framework regions, research shows that the glycosylation of antibodies plays a pivotal role in antibody function and antigen binding. Although all immunoglobulins are glycosylated, they exhibit substantial diversity both in the number and location of the conserved glycosylation sites be it on the variable, or constant regions (Fig. 19.2). Glycosylation accounts for 12–14% of the total molecular weight of IgE, IgM and IgD. Interestingly, IgG glycosylation is only 2–3% of its total molecular weight (Arnold et al. 2007). Antibody glycans play multiple roles from maintaining antibody effector functions by enhancing the binding of the Fc regions to its receptors (Mimura et al. 2001) to facilitating subcellular transport, secretion and clearance (Gala and Morrison 2002). Most significantly, antibody glycans play important structural roles by maintaining antibody conformation, as well as stability (Mimura et al. 2000), and they also participate in binding events (Malhotra et al. 1995). For example, in one study the removal of glycans from IgG antibodies led to

the reduction, or complete abolition of IgG binding to its receptors, FcγRs, demonstrating the importance of glycans in IgG antibody function (Schroeder and Cavacini 2010). Not only that, both antibody-dependent cellular cytotoxicity and complement dependent cytotoxicity were shown to be heavily influenced by the glycosylation and glycan composition of IgG antibody (Schroeder and Cavacini 2010). For an exhaustive discussion on the importance of antibody glycosylation, please consult the review by Arnold et al. (2007).

Conformational Changes Associated with Antigen–Antibody Binding

Perspectives on antigen–antibody interactions have changed over time. Initially it was believed that these interactions could be described using the "lock and key" model proposed by Fisher. However, as more and more structural data became available it became clear that in many cases the "lock and key" model did not properly describe the antigen–antibody interaction (Wilson and Stanfield 1994), and the so-called "induced fit" model provided a significantly better picture of antigen and antibody binding. As demonstrated by structural studies, this induced fit model was made possible due to antibody flexibility (Stanfield et al. 2006b), that allows for profound conformational changes in paratope regions upon antibody–antigen complex formation. It was also stressed that proteins (including antibodies) are highly dynamic molecules, which in normal physiological conditions undergo continuous conformational changes involving both main and side chain domains. This dynamic nature of proteins suggests that in solution immunoglobulins adopt various conformations and there is a pre-existing equilibrium between various (sometimes very similar) conformations of these molecules (Keskin 2007). This pre-existing equilibrium model explains how antibody flexibility may lead to promiscuity, or cross-reactivity (Van Regenmortel 2014); the same set of amino acids may adopt various conformations enabling them to recognize and interact with various epitopes. In fact, the antigens are also dynamic, hence, epitopes may also undergo various conformational changes (Liang et al. 2016). The plasticity of the epitopes and paratopes can be illustrated using the idea of adjustable locks and flexible keys that was proposed by Khan and Salunke (2014).

The analysis of antigen–antibody complexes and flexibility of the molecules also provides an interesting insight into antibody maturation process. Namely, structural studies have proposed that for antibodies that exhibit higher affinity for their antigens, "lock and key" type of binding is observed and that the binding is mainly driven by ionic and polar interactions. These interactions contribute towards higher binding energy between the antibody and its antigen by maintaining the rigidity of the antigen binding site (Wedemayer et al. 1997; Sinha and Smith-Gill 2002; Chong et al. 1999). Therefore, it was suggested that during the process of maturation, the flexibility of the paratope forming region of the antibody is reduced, and the reduction of this region's

plasticity is accompanied by changes that allow the formation of more specific polar interactions, like hydrogen bonds, or charge–charge interactions (Pons et al. 2002; LeBrasseur 2003; Cauerhff et al. 2004; Sinha et al. 2002; DeKosky et al. 2016). At the same time, the increase in antibody affinity is driven by the expansion of the paratope area and the formation of additional non-polar interaction (Li et al. 2003).

The orientation of the CDRs and paratope forming residues is affected by the orientation of variable regions of an antibody. One may expect that the same variable regions may have a different relative orientation dependent on the constant region of a particular antibody (Torres and Casadevall 2008; Narayanan et al. 2009). Therefore, class-switching combined with flexibility of the antigen binding site may results in an improved fit to the antigen.

Antigen Recognition and Interactions in Heavy Chain-Only Antibodies

As discussed earlier, the conventional antibody paratope is made up of six CDRs, three from the variable domain heavy chain and another three from the light chain. Interestingly though, there are species that deviate from this norm (Hamers-Casterman et al. 1993). Camelid and shark antibodies lack the light chain domain making the antigen binding site composed of only the heavy chain CDRs (Hamers-Casterman et al. 1993; Greenberg et al. 1995). The variable domains (antigen binding domains) of these heavy chain-only antibodies are usually referred to as single-domain antibodies (sdAbs). As warranted by their lack of light chain CDRs, sdAbs have smaller paratope diameter and surface area. Compared to conventional antibodies, the size of sdAbs paratopes is about half (Henry and MacKenzie 2018). On the sdAbs paratopes, the heavy chain CDR3 is unusually long, folding over the area supposedly to have been for the light chain interface, thereby interacting more with the cognate antigen. Despite these differences, the heavy chain CDRs of sdAbs are made up of similar amino acids compositions, like conventional antibodies (Henry and MacKenzie 2018; Harmsen et al. 2000; Muyldermans et al. 1994), although it was also suggested that these paratopes display a greater diversity in comparison with conventional antibodies (Mitchell and Colwell 2018). Consequently, sdAbs also implement the same antigen–antibody interactions and can bury similar solvent accessible areas as conventional antibodies although the energetics are more pronounced on the sdAbs small epitopes producing high affinity interactions (Henry and MacKenzie 2018). Merited by their small molecular size, sdAbs are claimed to be able to access recessed sites on antigen interfaces like enzyme active sites and viral glycoproteins (Rouet et al. 2015; Jahnichen et al. 2010). However, the dominant mechanism employed by conventional antibodies in antigen–antibody complexes with haptens, small molecule lipids as well as oligosaccharides, is the same as sdAbs exhibit in antigen–antibody complexes (Henry and MacKenzie 2018; Fanning and Horn 2011).

Antigen–Antibody Complexes in Human Health

The binding of an antigen to its specific antibody, a presumably simple immunological event, dramatically changes the properties of the bound antigen leading to various immune responses including antigen presentation and processing, inflammatory responses and receptor signaling (Wen et al. 2016). Modern day medicine has exploited this immunologically simple event to innovate new drugs, immunotherapy and biomedical technologies in the treatment of not only cancer, but autoimmune diseases, antibody deficiencies and pediatric infections (Wen et al. 2016; Kholodenko et al. 2019). Although full antibodies are used in immunotherapy (Jolles et al. 2005; Wood 2012), due to the relatively large size of antibody molecules, for example IgG~150 kDa, they cannot effectively penetrate into some tissues like tumors and cross the blood–brain barrier to gain access to the central nervous system (Kholodenko et al. 2019). Hence, in antigen–antibody complexes used in health care today, antibody fragments are usually preferred (Nelson 2010; Strohl and Strohl 2013). Antibody fragments still possess the same antigen specificity as full-length antibodies. They also have other unique characteristics that are important for both diagnostic and therapeutic purposes: antibody fragments have a shorter serum circulating half-life, are easy to manufacture and manipulate, can be engineered to be multi-specific and due to the absence of constant regions, they do not trigger cytotoxic immune responses, or antibody dependent cell-mediated cytotoxicity which can lead to adverse side effects (Strohl and Strohl 2013). However, in some cases the presence of the Fc fragment is indispensable. This is the case when an antibody employs the complement system, or enhanced phagocytosis, both processes are dependent on the presence of the Fc fragment. A good example of this scenario is in anti-CD20 therapy (Freeman and Sehn 2018).

In human health, the association of an antibody with its specific antigen leads to a cascade of events that either result in a beneficial, or sometimes detrimental immune response to the host. Taking HIV and cancer as examples, use of antibody complexes in vaccine development and immunotherapy for these two incurable diseases has been promising. For example, in one study on HIV in mice, antibodies recognizing the V3 region of the envelope glycoprotein, gp120, elicited production of strong neutralizing antibodies against the virus (Visciano et al. 2008). This immune response, caused by an antibody binding to its epitope, was merited to the exposure of a concealed V3 region which is usually inaccessible without antibody binding (Pan et al. 2015). Other antibodies generated for binding gp120, but outside the CD4 binding region, also induced production of neutralizing antibodies against several HIV strains (Liao et al. 2004). These and other findings are crucial in the design of an HIV vaccine. In cancer and tumor immunotherapy, one common method of treatment is targeting surface factors crucial for tumor cell growth (Nimmerjahn and Ravetch 2005). When antibodies recognize and bind to these factors, like epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF), forming an antigen–antibody complex, ADCC is triggered which leads to tumor lysis (Moalli et al. 2010). Apart from triggering ADCC, binding of antibodies to antigens

on the surface of tumor cells can also induce tumor cell death mainly via apoptosis (Ludwig et al. 2003). Additionally, another study showed that the formation of these antigen–antibody complexes facilitates tumor suppression by employing therapeutic, prophylactic and memory-inducing methods (Rafiq et al. 2002). All these efforts and many more not mentioned here, have led to several antibodies approved by the FDA for use in cancer therapy (Kholodenko et al. 2019). A comprehensive discussion of antigen–antibody complexes use in the study and treatment of other diseases is found here (Wen et al. 2016).

On the negative side, the formation of antigen–antibody complexes has been linked to the development of various autoimmune and allergic diseases (Theofilopoulos and Dixon 1980). One prominent example is the major role played by immunoglobulin E, IgE, in what has been termed type 1 hypersensitivity. IgE is implicated in hypersensitivity reactions stemming from food allergies, allergic asthma, atopic dermatitis, allergic rhinitis to some types of drugs, and sting allergies (Platts-Mills 2001). In allergic diseases, antigen presenting cells present the antigen to T helper cells, Th2 cells. The activated Th2 cells releases interleukins, like interleukin 4 and 13, which leads to the upregulation of IgE production. This antigen specific IgE attaches itself to the high affinity FcεRI receptors on mast cells, or basophils through CH3 domain of its constant regions. At this juncture, IgE is geared for attack upon a subsequent antigen exposure (Presta et al. 1994). When an antigen binds to IgE variable regions of two adjacent antibodies, formation of this antigen–antibody complex cross-links the receptors. This crosslinking together with the uptake of calcium ions into the mast cell, or basophil results in cell activation with rapid release of mediators such as histamine, or tryptase from cell granules as well as newly-synthesized such as leukotrienes, prostaglandins and cytokines. These mediators are indicted to be responsible for both the immediate and late allergic reactions that can be as severe as anaphylaxis (Oettgen and Geha 1999; Williams and Galli 2000).

Superantigens

Of all the antigens discussed in this chapter, superantigens are one of the most, if not the most, potent antigens capable of inducing massive immune responses and wreaking havoc in the human immune system (Fraser 2011). In comparison to a normal antigen-induced T-cell response where only 1 in 100,000 (0.001%) of the body's T-cells are activated, superantigens are capable of activating up to 1 in 5 (20%) of the body's T-cells (Van Kaer 2018). Attributed to this tremendous immune response, superantigens have been implicated in diverse human diseases ranging from food poisoning, toxic shock syndrome, autoimmune diseases, allergic diseases to HIV-1 (Van Kaer 2018; Marone et al. 2006).

The ability of superantigens to evoke such a strong immune response compared to conventional antigens, lies in their capability to bind both the Major Histocompatibility complex (MHC) class II molecules on antigen presenting cells and T-cell receptors on T-cells (Fraser 2011). In a normal setting, microbial antigens are

engulfed and digested by antigen presenting cells (APC) to yield small peptides that are presented on the APC cell surface bound together MHC class II molecules. The MHC and peptide complex are consequently recognized by T-cell receptors on T cells, and an immune response specific to that peptide antigen is launched (Fraser 2011). Research has shown that superantigens, unlike conventional antigens, by-pass antigen presentation stage and bind both MHC and T-cell receptors as an intact protein (Van Kaer 2018). This T-cell activation results in the excessive production of cytokines, interleukin-2 and tumor necrosis factor alpha, which elicits the massive immune responses associated with different diseases (Fraser 2011).

Other antigens that do not solely elicit T-cell mediated immune response also exhibit similar binding characteristics (Marone et al. 2006). For example, Protein Fv that is both a superallergen and superantigen, is produced in the human liver and is associated with Hepatitis A, B, C and E. It binds with high affinity to the human antibody heavy chain variable domain regardless of the antibody class, or subclass. Protein Fv is a superantigen because it binds to the VH3 fragment of different human antibodies. The protein also acts as a superallergen because it binds to a variable region of IgE (but not IgM or IgG) present on mast cells and basophils through its high-affinity interaction with IgE VH3+ (Bouvet and Marone 2007). This binding is done outside of the conventional antigen binding region. Intriguingly, one Fv protein molecule can bind up to 12 antibody fragments of human IgG, IgE and IgM (Bouvet et al. 1990). Other superallergens like Protein A associated with *Staphylococcus aureus* also possess two binding sites, one that binds to the Fab region of IgG, IgM, IgA and IgE and another binding site that interacts with receptors. For IgE, binding of its receptors lead to mast cells and basophils degranulation which is a hallmark of allergic diseases. HIV-1 gp-120 superallergen also cause basophil and mast cells degranulation in the same manner (Marone et al. 2006).

Interestingly though, not only superallergens can possess these dual binding sites. For example, Phl p 7, an important calcium binding allergen from *Phleum Pratense* (Timothy grass), can bind a human IgG antibody in a canonical way by interacting with five of the hypervariable loops, but could simultaneously interact in a non-canonical way with CDR-L2 and framework residues (Fig. 19.11). This phenomenon, technically makes Phl p 7 a superantigen (Mitropoulou et al. 2018). Additionally, two molecules of Phl p 7 could interact with two antibody molecules contradicting the general dogma that only dimeric antigens can interact with two antibody identical molecules at a specific time. When anti-Phl p 7 IgG antibody was switched to IgE, only one molecule of Phl p 7 cross-linked and signaled effector functions. These findings further confirmed the unusual antibody binding for Phl p 7 (Mitropoulou et al. 2018). For a detailed discussion of superantigens and superallergens and their role in human diseases please consult this excellent book by Marone (2007).

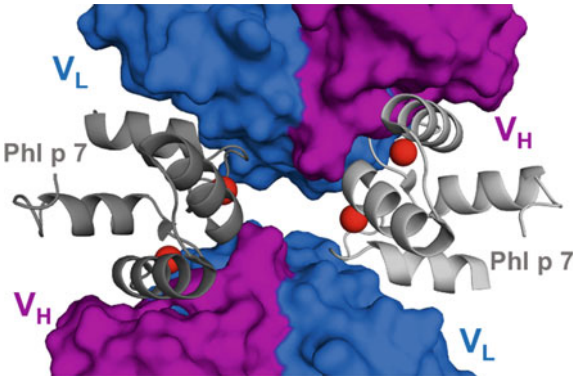

Fig. 19.11 Crystal structure of the complex between two Fab fragments of a human antibody and two molecules of timothy grass pollen allergen—Phl p 7 (PDB code: 5OTJ (Mitropoulou et al. 2018)). The antibody is binding two molecules of the monomeric allergen using conventional and superantigen-like recognition. Phl p 7 molecules are shown in cartoon representation (gray) and the antibodies are shown in surface representation (light chains in blue and heavy chains in purple). Calcium ions bound by Phl p 7 are presents as red spheres

Closing Remarks

As one of the most versatile natural biological sensors, antibodies have been extensively used in numerous settings in research, disease diagnostics and therapy. Antibodies protect vertebrates against invasion by harmful microbes by binding to antigens to launch an immune response; also by activating other components of the immune system to annihilate the invaders. More than a century of research and investigations have greatly improved our understanding of the function, structure and the immunological role of these protective agents, and also emphasized the complex nature of antibodies.

To launch an immune response after encountering an antigen, antibodies recognize and bind to their specific antigens making antigen–antibody complexes. Although the central dogma states that in antigen–antibody complexes CDR amino acid residues hold the keys to antigen recognition, research has shown that non-contacting residues, on both constant and framework regions, are responsible for preserving the structural conformations needed for the CDRs to make antigen binding possible. By affecting the molecular flexibility of the antibody, non-contacting residues can induce substantial impact in antigen binding especially in the affinity and specificity of the antibody. Moreover, some residues on the framework regions actually make contact with the antigen and substantially contribute to the energetics needed for antigen binding. Binding of an antigen to its specific antibody dramatically changes the properties of the bound antigen leading to various immune responses including antigen presentation and processing. The binding of superantigens to antibodies compared to conventional antigens irrefutably illustrates the importance of antigen–antibody complexes in immunity and explains the molecular basis of diseases in human health.

Modern day medicine, has exploited antigen–antibody complexes to innovate new drugs, immunotherapy and biomedical technologies in the treatment of various diseases like cancer, autoimmune diseases, antibody deficiencies and pediatric infections. Studying the structural and molecular mechanisms by which antibodies recognize and bind to their specific antigens helps to understand the basis of immunity, facilitate disease diagnostics and enable better design of vaccines and immunotherapy.

Acknowledgements The authors would like to thank Dr. Tapiwa Pekoyo for insightful comments and discussions. This work was supported by grant R01AI077653 from National Institute of Allergy and Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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