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Review

Anti-DNA antibodies: aspects of structure and pathogenicity

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Abstract. Anti-DNA antibodies contribute to the pathology of systemic lupus erythematosus. Their depositon in tissue lesions could result from localization of preformed immune complexes of antibodies with DNA or nucleosomes, or from cross-reaction of anti-DNA antibodies directly with tissue proteins. Structural analyses contribute to understanding their pathogenic potential. Primary structures of lupus immunoglobulin G double-stranded DNA-binding autoantibodies are determined by immunoglobulin genes with mutated variable region segments, indicative of selection by immunizing antigen. Arginine, lysine and asparagine residues in complementarity-determining region favor DNA binding. Heavy-chain variable regions make major contributions to DNA binding; affinity and specificity of binding are modulated or can be abrogated by the light-chain variable domain. Crytallographic structure is known for a few antibody-DNA complexes and several ligand-free Fab fragments. Computer modeling supplements this limited information. Structural information of lupus antibody interactions with both DNA and cross-reacting molecules will support use of ligands to inhibit tissue deposition of the antibodies and prevent lesion formation in lupus.

Key words. Antibodies; autoantibodies; lupus; SLE; immunoglobulin genes; pathogenicity; autoimmunity; crystallography; computer models.

Introduction

Autoantibodies that react with DNA are a prime feature of human systemic lupus erythematosus (SLE) and lupus diseases of MRL-*lpr/lpr*, (NZB/NZW)F1, (NZB×SWR)F1 and BXSB mice [1–4]. Normal sera contain low levels of anti-DNA antibodies, mainly of the immunoglobulin (Ig)M class. Sera of SLE patients and lupus mice, however, contain higher concentrations of anti-DNA antibodies, including IgG molecules with higher affinity for both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). Knowledge of the molecular structures of these antibodies is essential for insight into how they recognize DNA and how they may contribute to pathogenesis by binding DNA or cross-reacting molecules. Their binding mechanisms differ from those identified with many other DNA binding proteins, as anti-DNA antibodies do not have helix-containing structures or other known DNA binding motifs [5]. Their antigen-combining sites, like those of all antibodies, comprise peptide loops protruding from one end of a β-barrel framework structure [6]. The variable domains of heavy chain (VH) and light chain (VL) each contribute three loops from their complementarity determining regions (CDRs). Combinations of VH and VL residues determine antigen-antibody affinity and specificity, and replacement of either domain – or particular amino acids within the domains – can alter the binding. A large body of information on the primary structure of VH domains of anti-DNA antibodies has revealed diversity among their CDR sequences, but points to DNA-binding contributions of basic amino acids such as Arg and Lys and hydrogen-

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bonding amino acids such as Asn [7, 8]. Structural information provides detailed information on DNA binding for a few antibodies and models for others [9]. Despite a considerable body of knowledge, questions remain about diverse mechanisms of antibody interactions with DNA and cross-reacting molecules. This review considers research on the pathogenic role of anti-DNA antibodies and their primary structures, the contributions of VH and VL domains to antigen binding, and three-dimensional crystallographic structures and computer models of their interactions with DNA.

Correlates of pathogenicity of anti-DNA antibodies

Soon after anti-DNA autoantibodies were discovered in SLE sera in 1957, attention turned to evidence that they contribute to pathology and to clinical signs and symptoms of disease. Serum anti-DNA antibodies were not only selectively associated with the diagnosis of SLE; they were found much more frequently in patients with clinically active disease than in patients in remission (reviewed in [10]). Temporal correlation with clinically active disease was also seen with serial samples from individual patients [11]. Correlations were stronger for both diagnosis and clinical activity (especially nephritis) when antibodies to dsDNA rather than to ssDNA were detected (reviewed in [12]), and when the antibodies had complement fixing activity [13, 14]. Accumulated findings strongly suggested that some, but not all, anti-DNA antibodies are pathogenic, and raised questions whether pathogenicity is related to specificity, affinity or some other property of particular antibodies.

More direct early evidence for pathogenicity came from the discovery that antibodies to DNA and nucleoprotein, along with complement, are deposited in the glomeruli of patients with lupus nephritis [15, 16]. In both patients and lupus mice, measurements of circulating anti-DNA antibodies gave mixed answers to the question whether pathogenicity is related to antibody affinity [17–19]. However, antibodies eluted from glomerular deposits often have higher affinity for DNA than do those in serum at the time of active nephritis [20, 21]. Thus, high affinity for dsDNA has been identified as one of the correlates of pathogenicity.

When autoantibody-producing hybridomas were obtained in the early 1980s, it became possible to study panels of purified monoclonal antibodies and to define more precisely the antibody properties associated with pathogenicity. Some monoclonal antibodies, when injected into mice or produced in vivo by implanted hybridomas, are deposited in glomeruli, along with complement, and cause proteinuria. Many of these pathogenic anti-DNA antibodies are distinguished by their high isoelectric point and overall positive charge at physiological pH [19, 22]. Consistent with that relationship, their V regions have higher than usual numbers of basic amino acids such as Arg and Lys, especially in CDR sequences [7, 23]. These basic residues contribute to high affinity binding of DNA. Associations of high isoelectric point and high affinity with pathogenicity are concordant, but they are not universal. A different property, cross-reactivity, characterized some pathogenic monoclonal antibodies, including antibodies eluted from renal lesions, that did not have unusual charge or DNA-binding affinity [24, 25].

Mechanisms of deposition or formation of immune complexes in tissues

Although it is generally agreed that antibody and complement deposition play an important role in lupus nephritis, the mechanism of deposition is still a matter of study and debate. At first, the favored view was that circulating immune complexes are deposited in the glomeruli, as in a previously described experimental model of immune complex-mediated nephritis [26]. However, measurements of circulating DNA-anti-DNA immune complexes were variable [27, 28], and it was difficult to obtain direct evidence for deposition of complexes preformed in the circulation. A second proposal was that DNA becomes immobilized by binding to a glomerular membrane component [29, 30] and that antibody then binds to that DNA, activating complement and initiating inflammation. Another view considers the importance of DNA-histone associations, the fact that nucleosomes are early and important ligands for both anti-DNA antibodies and nucleosome-specific antibodies [31, 32], and the presence of nucleosomes and Ig-nucleosomes in the circulation of lupus mice [33]. Thus, it has been proposed that positively charged histones in antibody-DNA-histone or antibody-nucleosome complexes mediate binding of the complexes to negatively charged proteoglycan components of glomerular membranes [34]. Histones alone bind to glomerular basement membrane [35], and complexes of anti-DNA antibodies with DNA and histone bind to heparan sulfate, a basement membrane component [36]. Similarly, complexes of anti-DNA or anti-nucleosome antibodies with nucleosomes could bind to glomeruli, whereas free antibody did not [37]. Indeed, some experiments testing monoclonal anti-DNA antibodies actually have been done, unintentionally, with antibody-nucleosome complexes, because tissue culture medium is likely to contain nucleosomes released from dying cells [37, 38]. Even affinity-purified anti-DNA antibodies may contain antibody-nucleosome complexes. Nucleosome-free antibody can be obtained if culture fluid is applied to protein A-agarose and the column is then washed with a solution of high ionic strength, which dissociates nucleosomes and nucleosome-antibody complexes but favors

the IgG-protein A interaction, before elution of the antibody [37, 38].

A still different proposed mechanism for deposition is that anti-DNA antibodies cross-react directly with the renal membrane components themselves [39, 40] or skin [41], initiating complement activation without involving DNA or nucleosomes in the formation of immune complexes. Four pathogenic anti-DNA monoclonal antibodies, for example, were distinguished from nonpathogenic anti-DNA and anti-histone antibodies by their ability to react with a 100-kDa protein in the detergent-solubilized cytosol/membrane fraction of glomerular extracts [25]. In that group, the pathogenic antibodies were not distinguished by primary structure, isoelectric point or DNA-binding affinity. The 100-kDa protein was identified as α-actinin, an acidic protein that can be expressed on mesangial cell surfaces; immunofluorescence with anti-α-actinin antibody revealed an unusually high expression of α-actinin in glomeruli of lupus mice [42].

Laminin is another candidate for a glomerular cross-reaction related to pathogenicity of anti-DNA antibodies. Pathogenic serum or monoclonal anti-DNA antibodies bind to laminin [43, 44] and to a peptide representing a dominant epitope in the protein. Significant implications of this proposed mechanism include the possibility of using protein fragments or synthetic peptides to inhibit the deposition of pathogenic anti-DNA antibodies. Indeed, a peptide corresponding to a dominant laminin epitope for cross-reacting anti-DNA antibodies has been proposed for treatment of SLE [42].

Still other examples of peptide mimotopes that cross-react with anti-DNA antibodies were identified in a peptide phage display library [45]. Some peptides selected from the library were bound by anti-DNA antibodies and, when used as haptens, were able to induce formation of antibodies that react with DNA [46]. Such a peptide can also inhibit deposition of antibodies and nephritis in lupus mice [47]. Furthermore, anti-DNA antibodies that cross-react with the DNA-mimicking pentapeptide Asp/Glu-Trp-Asp/Glu-Tyr-Ser/Gly, a sequence that occurs in the extracellular domain of the (N-methyl-D-aspartate) receptor in brain, cause apoptotic death of neurons in vivo and in vitro [48]. Antibodies with that reactivity were detected in the cerebrospinal fluid of a patient with SLE; it has been proposed that they mediate some of the central nervous system manifestations of the disease [48].

Cross-reactions with other proteins present on the cell surface may also contribute to pathogenicity. Some monoclonal and affinity-purified serum anti-DNA antibodies that cross-react with the A and D polypeptides of SnRNP particles can bind to the surface of cells in culture and mediate complement-dependent killing of those cells [49]. Some anti-DNA antibodies can enter cells, affecting intracellular functions and survival [50–52]. The extent of cell damage in vivo and the contribution to clinical pathology by these mechanisms is unknown. Diverse mechanisms, including receptor-mediated transport [52], may mediate cell penetration.

Anti-DNA antibodies also cross-react with several other proteins and nonprotein substances, including bacterial phospholipid [53], polysaccharide [54], ribosomal P protein [55], ribosomal S1 protein [56], protein synthesis elongation factor [55, 57], cytoskeletal protein [58] and small haptens such as the dinitrophenyl group [59]. The extensive cross-reactivity of anti-DNA autoantibodies could have implications for the origins of the antibodies as well as their pathogenic mechanisms. Cross-reactivity is a property of natural autoantibodies that have not undergone extensive maturation through V gene mutation [60], and cross-reactivity between DNA and the Sm antigen has been observed in such antibodies [61, 62]. However, many anti-DNA antibodies are, in fact, IgG molecules coded by mutated V gene segments [7,8]; both of these properties indicate that they are products of antigen-driven B cell maturation. Perhaps these antibodies, though mutated and selected, have not evolved so far from original cross-reactive precursors as to lose that original property.

Primary structure and the genes for anti-DNA autoantibodies

With the isolation of hybridomas from lupus mice by the early 1980s, it became possible to study the primary structure of autoantibody V regions by sequencing of corresponding complementary DNA (cDNA). Excellent reviews of data gathered over the subsequent decade have summarized the major sequence features, with particular emphasis upon the VH region [7, 8, 63, 64]. The web site http://www.bioinf.org.uk/abs/ provides a query interface to the Kabat antibody sequence data, general information on antibodies including anti-DNA antibodies, and crystal structures and links to other antibody-related information.

VH regions of IgG anti-DNA autoantibodies contain substitutions based on mutations from germline genes, and mutations causing amino acid replacement are more frequent in CDR sequences than in framework sequences [64–66]. Thus, IgG antibodies result from a process of antigen-driven selection. Some IgM molecules also yield mutation-based evidence for selection [67]. Oligoclonal expansion in a given animal was revealed by detection of clonally related cDNA sequences [65, 66]. Among these related Ig VH sequences, increasing numbers of mutations are associated with increased affinity for ssDNA and appearance of dsDNA binding by the corresponding antibodies [65, 68]. This result provides evidence that DNA or a DNA-containing antigen plays a selecting role driving the formation of anti-DNA antibodies. A comparable analysis has not been done for reactivity with cross-reacting antigens.

Arg occurs in unusually high frequency in the CDR sequences of IgG anti-dsDNA autoantibodies, particularly in those that bind dsDNA with high affinity [7,68,69]. These Arg residues can result from single-base mutations, use of particular joining sites and untemplated bases in junctions between V region segments, and use of a particular reading frame in the DH segment. Arg is particularly suitable for DNA binding in antibodies and other DNA-binding proteins, as it can form bidentate hydrogen bonds with the bases and ionic bonds with the phosphate backbone. Asn, another hydrogen bonding amino acid, is also more frequent in anti-DNA antibody CDR sequences. The increased number of Arg and Lys residues is consistent with the relatively high isoelectric point of many pathogenic antibodies that bind DNA.

Although VH segments from diverse germ line genes are used, certain members of the J558, Q52 and 7183 families occur at a higher frequency in anti-DNA antibodies than would be predicted from random usage [7]. Also, the small V_H10 family occurs in several anti-DNA autoantibodies from NZB/NZW [70,71] and MRL-*lpr/lpr* [72] autoimmune mice as well as in immunization-induced antibodies to nucleic acid antigens such as Z-DNA [73], poly(dC) [74] and triplex DNA (Jel 318, GenBank M58456). The V_H10 family has few members in the germline, but they are highly represented in the preimmunization repertoire [75], and a GenBank search reveals that anti-nucleic acid antibodies account for a large fraction of V_H10 occurrences.

As is the case for H chains, usage of L chains is not highly restricted, but Vκ1 and Vκ8 genes occur at a much higher frequency than would be predicted for random usage [7, 8], as do certain VH-VL combinations [7, 8].

Primary structure of anti-DNA antibodies induced by experimental immunization

Primary structure analysis reveals a large overlap in V gene utilization between anti-DNA autoantibodies and anti-DNA antibodies resulting from experimental immunization. For example, Z22 and Z44 are highly selective anti-Z-DNA IgG molecules from responses to repeated immunization. For both the VH and VL domains of these antibodies, the most closely related GenBank sequences are V_H10-containing domains of anti-DNA autoantibodies [73]. Furthermore, the V domains of these IgG molecules have very few mutations from germline sequences. V genes of IgM antibodies formed by preautoimmune NZB/NZW mice immunized with bacterial DNA resemble those in anti-DNA autoantibodies [76]. So do the V genes of anti-dsDNA antibodies formed by nonautoimmune mice immunized with dsDNA-peptide antigen [77], or BK virus or BK virus DNA-methylated bovine serum albumin (BSA) complexes [78]. Secondary responses to immunization with poly(dC) included some IgM and IgG antibodies, related to autoantibodies in V region sequences, with few or no VH domain mutations, as well as some IgG antibodies with several VH domain mutations ([74] and unpublished data). Thus both spontaneously arising autoantibodies and immunization-induced antibodies to DNA might arise from a similar pool of precursor cells, some of which may be producers of natural anti-DNA autoantibodies. An underlying high frequency of ssDNA binders among natural IgM autoantibodies with unmutated V gene segments raises the possibility that they result from positive selection during B cell development [79].

H-Chain dominance in DNA binding

The requirement for both the H and L chains in formation of a complete antibody combining site was documented in early studies of chemically separated and recombined chains [80, 81] and by affinity labeling with haptens [82], and has been confirmed in many crystal structures of antigen-antibody complexes [6]. Still, most antibodies have more H chain contacts than L chain contacts with antigen [6], and the CDR3-VH makes more contacts than other CDRs. The separated H chain of a purified polyclonal antibody to *p*-aminophenyl-β-lactoside had an unusually large fraction (87%) of the affinity of the corresponding intact antibody [83]. The high antigen binding potential of H chains is also evident in camel serum antibodies, many of which are H chain dimers with no L chain [84]. Several antigen-binding VH domains, including lysozyme- and hemocyanin-binders, have been recovered from phage display libraries [85].

The importance of H chain in antigen binding by many anti-DNA antibodies, both autoantibodies and those induced by immunization, has been documented extensively by affinity labeling [86], transfection of VH domains into cells expressing a variety of VL domains [87], studies of recombined VH and VL domains [88] and measurement of DNA binding by isolated VH domains [89–94]. H chain dominance seems to be universal in mouse anti-DNA antibodies [94], however, significant DNA-binding activity of two human monoclonal antibodies, in fact, resides in the L chain [95].

Recombinant CDR variants of mouse lupus antibody DNA-1 and the much weaker antibody D5A provided a detailed analysis of the roles of individual VH CDRs in ssDNA recognition [96]. Segment swapping experiments indicated the importance of both CDR1-VH and CDR3-VH in DNA recognition by DNA-1 and further indicated that the specific type of nucleic acid recognition by a particular Fab can be drastically altered by CDR exchange. Critical residues in the CDR3-VH of DNA-1 determine the affinity and specificity of oligonucleotide binding [97].

The presence of Arg in CDR3 and net charge of V regions are determining factors for the involvement of V domains in DNA binding [64, 68, 94, 98–100]. Arg residues are found in CDR3 regions of both VH and VL domains of antibodies in which both domains are involved in DNA binding; they are present in the CDR3-VH, but not in CDR3-VL, of H chain dominant antibodies [94]. A suggested basis for the frequent occurrence of H chain dominance is that Arg residues are more frequent in VH than in VL domains, perhaps due to easy acquisition of Arg codons by mutation of VH germline codons, and a high probability that B cells with Arg-containing CDR-VH domains will become anti-DNA antibody-producers during clonal expansion [101].

Modulation of binding by L chain

While VL-free VH domains of anti-DNA antibodies can bind DNA, the association with VL domains modulates their binding. Some VL domains can completely block the DNA binding activity of VH domains [87, 91, 92, 102]. In the case of mouse lupus anti-dsDNA antibody 3H9, various L chains modify the specificity or affinity when they combine with the H chain [87]. Two other lupus mouse anti-DNA monoclonal antibodies sharing the same VH and VL sequence except for one amino acid in CDR3-VL have different binding specificities [94]. Thus, L chains play an important role in affecting the binding specificity and affinity and might also lead to recognition of additional antigenic determinants.

The roles of H and L chains have also been examined with recombinant VH, VL and Fv domains. The VH domain alone of antibody 2C10, a lupus mouse (MRL-*lpr*) monoclonal antibody that binds dsDNA with preference for AT-rich polynucleotides [103], binds DNA as well as or better than the VH plus VL combination in a scFv [92]. The negatively charged VL of 2C10 does not, by itself, bind DNA; it hinders but does not completely block the DNA binding of the VH [92]. Indeed, residues of both CDR3-VH and CDR3-VL contribute to binding specificity [100]. For example, the VH-VL combination in the 2C10 IgG, Fab or scFv binds equally well to poly(dAdC)·(dGdT) and helical B-DNA, and it binds very poorly to either the B-DNA or Z-DNA form of poly(dGdC)·(dGdC) [92]. However, the 2C10 VH domain alone, in the absence of the VL, prefers B-DNA to poly(dA-dC)·(dG-dT) and binds to Z-DNA nearly as well as to B-DNA [92]. An Fv combining the 2C10VH with the VL of anti-Z DNA antibody Z22 binds to Z-DNA better than to B-DNA. CDR3-VL residues responsible for the modulation of antigen binding and specificity have been identified as Asp92 and Asn93 [100]. Amino acid residues in FR-VH segments [104] as well as the first CH domain [105] could also affect the antigen-binding specificity in some cases.

The modulating role of L chain was also observed with antibody Z22 [91]; the Z-DNA binding activity of the H chain was retained in a scFv or Fab containing the Z22 VL domain but was blocked by nearly all other members (more than 1000) of a VL domain library; even one mutation in the Z22 L chain CDR3 caused a marked loss of affinity of a VH-VL domain for Z-DNA.

Modulation of binding by receptor editing

Developing B cells that express VH-VL combinations with high affinity for dsDNA may be eliminated in the bone marrow [106] or rendered anergic in the periphery [107–109], so that a state of tolerance for dsDNA is maintained. Still another mechanism for tolerance is receptor editing (reviewed by Nemazee and Weigert [110]), in which secondary VL rearrangement occurs, yielding a possibility for the VH to pair with a VL domain that blocks DNA binding. Certain Vκ gene segments are particularly effective editors but may still be unable to block VH domains that have very strong DNA binding activity [101]. The editing process may also revise VH domains, using sequences within the FR3-VH that resemble switch signaling sequences [111]. Replacement of the VH segment may yield either a nonfunctional gene or provide an opportunity to form a nonautoreactive antibody. It is difficult to know how frequently H chain revision occurs spontaneously, but clear examples have been observed in human tonsil [112] and rheumatoid synovial tissue [113]. Active editing occurs in mice predisposed to autoimmunity, but cells expressing anti-dsDNA or other autoreactive receptors escape the tolerance mechanisms and produce their pathogenic products in spite of it [114].

Crystallographic structures of antibody-DNA complexes

Crystallographic structural analysis of antibody Fab or Fv fragments to diverse antigens has provided important insights into H and L chain contributions to antigen binding and has revealed that, in most cases, all six CDR residues from both H and L chains contact antigens [6]. Only a few crystallographic structures of anti-DNA antibodies, with or without bound antigens/haptens, have been analyzed. Fab fragments of antibodies BV-04-01 and DNA-1, both of which bind poly(dT) and ssDNA, have been determined with and without bound oligo(dT) [115, 116], and a third Fab-thymine dimer complex (64M-2) has also been solved [117]. Structures of ligand-free Fab fragments of antibodies to ssDNA (Hed10) [118] and double- and triple-he-

lical DNA (Jel 72, Jel 318) [119, 120] have been determined and used for modeling interactions with helical nucleic acids.

Herron et al. described detailed interactions of ssDNA with the Fab from an NZB/NZW mouse autoantibody, BV04-01 [115]. Structural analysis of free BV04-01 Fab and an Fab-$(dT)_3$ complex revealed small adjustments in the orientations of VL and VH domains upon binding, and striking local conformational changes in the CDR1-VL and the CDR3-VH which, together with the domain shifts, yield improved complementarity of $(dT)_3$ and Fab. The most prominent contacts with BV04-01 involve the central thymine base, which is immobilized by cooperative stacking and hydrogen bonding interactions. The central thymine is intercalated between a Trp ring at position 100a of the H chain and a Tyr ring at position 32 of the L chain. Significant movement of the Trp residue from its position in the ligand-free Fab is required for this stacking. The resulting orientation of thymine is also favorable for the simultaneous formation of two hydrogen bonds with the backbone carbonyl oxygen and the side chain hydroxyl group of Ser L91. The dimensions of the antigen-binding groove of BV04-01 can accommodate ssDNA but not dsDNA. Although the central thymine is the dominantly interacting base, each thymine of the oligonucleotide interacts with H and/or L chains of BV04-01. These aspects of crystal structures are consistent with many features of chemical epitope mapping of BV04-01 [121] and with molecular modeling of V-88 (a mouse anti-ssDNA IgG/κ antibody) and its complex with DNA [104].

The ssDNA-binding Fab, DNA-1 originally isolated by phage display and selection [96,122], was also crystallized and analyzed with and without ligand [116, 123]. The 2.1-Å crystal structure of DNA-1 Fab complexed with $(dT)_5$ showed that DNA-1, like BV04-01, binds oligo(dT) primarily by sandwiching thymine bases between aromatic amino acids (in this case, all Tyr side chains) and allowing the bases to make sequence-specific hydrogen bonds with the protein. The critical stacking Tyr residues are at positions L32, L49, H100 and H100a, while His L91 and Asn L50 contribute hydrogen bonds (fig. 1). Comparison of the DNA-1 structure with other anti-nucleic acid Fab structures reveals a common ssDNA recognition module consisting of Tyr L32, a hydrogen-bonding residue at position L91, and an aromatic side chain from the tip of CDR3-VH. Arg side chains from CDR3-VH of DNA-1 appear to play a significant role in DNA binding. Rather than forming ion pairs with $(dT)_5$, Arg contributes to oligo$(dT)_5$ recognition by helping to maintain the structural integrity of the DNA-1 combining site. Other examples also show that some CDR residues not only participate directly in binding of antigen, but also influence the structure of the rest of the loop [124, 125].

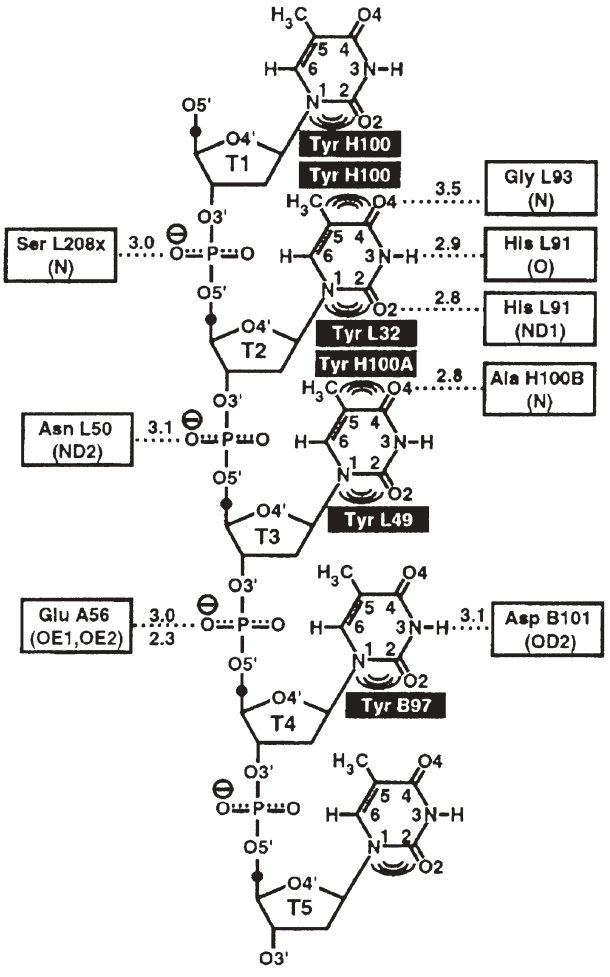

Figure 1. Schematic diagram of the Fab-$(dT)_5$ interactions in the DNA-1 structure. The broken lines denote hydrogen bonds and the arcs denote stacking interactions. The numbers above the dotted lines denote distances in angstroms. Ser L208x is a residue from a symmetry-related Fab. (Reprinted from [116], with permission of the publisher Academic Press/Elsevier Science.)

The crystal structure of the murine 64M-2 Fab, specific for DNA dT(6–4)dT photodimer, has been recently determined as a complex with its photodimer ligand, at 2.4-Å resolution [117]. Five CDRs of 64M-2, but not CDR2-VL, are involved in the ligand binding. The bound dT(6–4)dT ligand, adopting a ring structure, is fully accommodated in an antigen-binding pocket. The 3′-pyrimidone base is hydrogen-bonded to His H35, and is in contact with Trp H33. The sugar-phosphate backbone connecting the bases is surrounded by residues His L27d, Tyr L32, Ser L92, Trp H33 and Ser H58, but is not hydrogen bonded to these residues. Three water molecules are located at the interface between the bases and the 64M-2 Fab residues. Hydrogen bonds involving these water molecules also contribute to Fab recognition of the dT(6–4)dT bases.

Crystal structures of ligand-free antibodies and computer models of antibody-DNA complexes

The Fab structure of ligand-free NZB/NZW mouse anti-DNA antibody Hed10, which binds poly(dT), was determined to 3.0-Å resolution [118] and was the first known X-ray structure of an autoimmune Fab fragment. The V and C domain pairs of Hed10 Fab are connected by short polypeptide links, one within the H chain and one within the L chain, providing flexibility of the Fv domain within the Fab structure.

The structures of Fab Jel72, a murine monoclonal antibody specific for the right-handed duplex poly(dG) · poly(dC) and Fab Jel318, a murine monoclonal antibody reactive specifically with triple-stranded [poly(dTdm^{5}C) · poly(dGdA) · poly(dm^{5}CdT)] were determined at 2.6-Å resolution in the absence of ligands [126]. Later, their structures were refined and their interactions with duplex and triplex DNA were modeled by computer analysis [119, 120]. In the model with Jel72 Fab, residues in the CDR3-VH interact with DNA bases via the major groove of poly(dG) · poly(dC), whereas in the Jel318 Fab model, residues of CDR2-VH make contact with the DNA bases in the minor groove of the triplex DNA.

These crystallographic and computer-modeling analyses indicate that combining site structures of anti-ssDNA and -dsDNA are generally distinguishable from each other. Anti-ssDNA antibodies have deep clefts in which the antigen binds [115, 116, 118], whereas contact areas formed with dsDNA and triple-stranded DNA are flat surfaces which form more electrostatic contacts than occur in antibody-ssDNA complexes [119, 120]. In the case of anti-dsDNA antibodies, the modeled combining regions are generally bordered by projecting knob-like ridges, which possess positively charged amino acids, whereas there is generally an area of negative charge near the CDR3-VL. Models and epitope mapping also suggest that the protruding knobs would be in position to penetrate the major and minor grooves of the DNA and make specific contacts with the DNA bases [127, 128].

Computer models for both antibody and antibody-DNA complexes

Crystal structures are not known for most antibodies. With much primary structure information available, however, molecular models of the antibody combining regions have been generated by comparison with known crystal structures of antibodies of related primary structure [100, 129] (fig. 2). The models provide useful information on the nature of the interactions between antibodies and nucleic acids. In some cases, it has been possible to compare observed crystal structure and predicted computer models for the combining regions of anti-DNA antibodies [127].

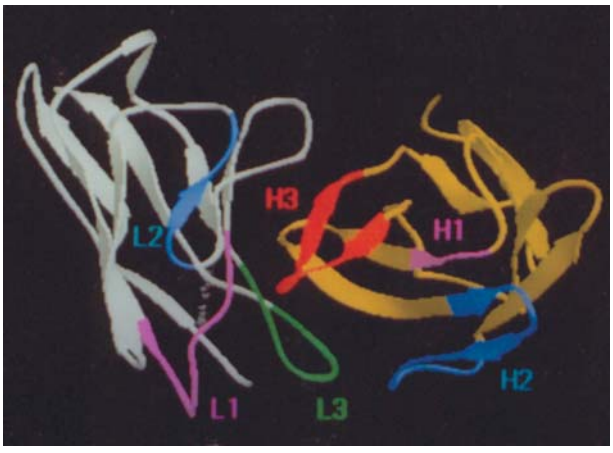

Figure 2. Molecular model of 2C10 Fv. The backbone and secondary structures are represented in a ribbon diagram. The H and L chains are colored yellow and white, respectively. The CDR regions are colored to illustrate the antigen-binding region with CDR1 regions purple, CDR2 regions blue, CDR3-VH red and CDR3-VL green. (Reprinted from [100], with permission of Elsevier Science.)

For antibodies Hed10, Jel72 and Jel318, root mean square (r.m.s.) deviations between the model and observed structures were between 1 and 2 Å for backbone and side-chain atoms of CDR loops other than the CDR3H, for which r.m.s. deviations were higher; 2.9 Å (Hed10), 5.9 Å (Jel72) and 3.5 Å (Jel318) [127]. In modeling of complexes, additional uncertainty enters if conformational change in the antibody combining site accompanies ligand binding, as it does for oligo(dT) binding by anti-ssDNA antibodies.

Study of computer-generated models supports the hypothesis that ionic and/or electrostatic interactions are prominent in binding of nucleic acids, as suggested by the anionic nature of DNA and the cationic amino acid sequences of the hypervariable regions [100, 130–132]. Figure 3 shows the electrostatic surface of antibody 2C10 Fv and its R98, R99, R100 and R102A multiple mutant.

Modeling also identifies potential targets for site-directed mutagenesis, an approach applied by Jang et al. to test predicted roles of particular amino acid residues in ds-DNA binding by antibody 2C10 [100]. Simultaneous replacement of four Arg residues with Ala residues in the CDR3H of 2C10 scFv abolished binding activity. With one exception, replacement of any one of these Arg residues reduced the activity to 20–50% of the unmutated 2C10 scFv activity. These results indicated that several CDR amino acids of antibody 2C10 contribute to DNA binding, without one residue dominating; and both VH and VL CDR3 domains contribute to binding, whereas the CDR1-VL hinders DNA binding. Molecular modeling and mutagenesis studies with antibody DNA-1 demonstrated that its CDR3-VH contributes significantly to antigen interaction [97]. Residues at the base (Arg98 and Asp108)

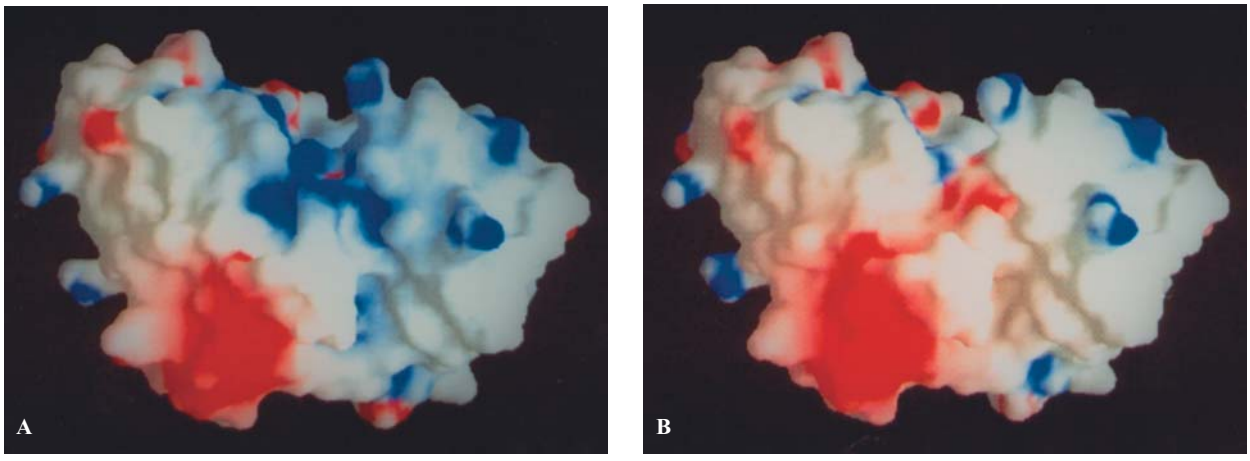

Figure 3. The electrostatic potential at the molecular surface of models of 2C10 Fv (*A*), and the multiple mutant in which several Arg residues of CDR3-VH were simultaneously mutated to Ala (R98, 99, 100, 102A) (*B*). Electrostatic potential is represented on a color scale from blue for positive potential, to white for neutral, to red for negative potential. (Reprinted from [100], with permission of Elsevier Science.)

and in the middle (Tyr101-Arg-Pro-Tyr-Tyr105) of CDR3-VH of DNA-1 were predicted to support the loop conformation and directly contact the ligand, respectively. It was concluded that Tyr H101 likely participates in hydrogen bonding, while Tyr H104 and Tyr H105 may be involved in aromatic-aromatic interactions with the ligand, oligo(dT). It was also suggested that CDR3-VH of DNA-1 interacts with the bases rather than the phosphates. A threefold increase in affinity was observed by mutation of Asp H108 to Ala.

Computer modeling was also applied to recognition of ds B-DNA and Z-DNA by human monoclonal autoantibody B3 [129]. This model provided a hypothesis to explain the ability of a single autoantibody to bind two different antigens. It was suggested that dsDNA is bound in a groove of B3 lined by three Arg residues donated by CDR1-VL, CDR2-VL and CDR2-VH. Whereas B-DNA could be docked into the B3 combining site along the plane of the VH-VL interface, Z-DNA was best docked at an angle of 90° to that orientation.

Molecular modeling proposed a structure of the V regions of anti-ssDNA antibody V-88, a linear epitope map and candidate sequences for the idiotopes of the cross-reactive Id [104]. Two key residues, Tyr L32 and Trp H100a in the V-88 model, were in the same relative orientation as in BV04-01, and the possible interaction with DNA was indicated. FR3-VH, which was highly solvent accessible and in the cross-reactive Id region, lay adjacent to the CDR1-VH and CDR2-VH and could influence antigen-binding specificity.

Computer modeling based on experimental binding data is also helpful in epitope mapping of antigen in the absence of detailed structural information for the antibody. An epitope of dsDNA for the lupus mouse antibody H241 was modeled on the basis of competitive immunoassays with defined synthetic oligonucleotides [128]; the epitope included portions of the bases exposed in the major groove as well as the deoxyribose-phosphate backbone. Two monoclonal anti-Z-DNA antibodies, Z22 and Z44 from a C57BL/6 mouse immunized with Z-DNA, were shown by nuclear magnetic resonance (NMR) spectroscopy to bind to the oligonucleotides, $(dCdG)_2$ and $(dCdG)_3$, and to interact with different parts of the helix [133]. The greatest interaction of Z44 was to the dC5 protons, although all of the base protons interact with this antibody. A Z44 binding site on the convex surface of the Z-helix (analogous to the major groove of B-DNA) and a Z22 binding site on the sugar-phosphate backbone were also explored.

Further Challenges

Many laboratory work has combined to uncover a great deal of information about antibodies to DNA and other nucleic acids. Major challenges remain in clarification of mechanisms by which pathogenic antibodies cause tissue damage. A prominent issue in this regard is that of cross-reactions of these antibodies with non-nucleic acid targets. It will be important to learn whether or not the selection of antibodies for improving reactivity with DNA is accompanied by an increased affinity of binding to cross-reactive antigens. The result may not alter the current view that DNA is the selecting stimulus, but will be important for development of therapeutic approaches based on blocking the combining sites of anti-DNA antibodies. Crystallographic structural analysis of antibodies complexed to helical nucleic acids and to cross-reactive peptides will also provide important information for this approach.

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