[1] Modeling of Antibody Combining Sites

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Introduction

Antibodies constitute an extremely large family of closely related serum proteins, termed immunoglobulins, produced in vertebrates by a class of lymphocytes, termed B lymphocytes, which are programmed to respond to contact (immunization) with foreign substances (antigens) or, under certain circumstances, to antigens of an individual's own tissues (autoantibodies). Antibodies may cause the elimination of antigens by phagocytosis, neutralization of toxins or viruses, lysis of tissue cells through the complement system, precipitation with the antigen used for immunization, or clumping of bacteria or red cells containing the antigen or the antigen adsorbed to inert particles (agglutination).

Antibodies may be produced to almost all classes of substances, e.g., proteins, polysaccharides, nucleic acids, and to more complex particles, e.g., pollens, infectious agents, viruses, and tissue cells. Unless there is some structural similarity between two antigens, one will generally not react with antibody to the other; when structural similarity does exist, reactions with both will occur (termed cross-reactions), the antibody reacting more strongly to the antigen used for immunization and to a lesser degree with the other. (For exceptional, poorly understood, anomalous cross-reactions, see Future Prospects and Problems.)

A second class of proteins, termed T cell receptors for antigen, is produced by a different class of lymphocytes, termed T lymphocytes. The T cell receptors for antigen do not circulate in the bloodstream but remain attached to the cells which synthesize them and, in conjunction with proteins of the major histocompatibility complex and cells of the macrophage system, can destroy tissue cells infected with viruses, tumor cells, etc. This is termed the cellular arm of the immune system.

Antibodies and T cell receptors for antigen show certain structural as well as certain genetic similarities, but appear to have arisen at different evolutionary periods and by different pathways. This chapter on modeling will focus exclusively on the antigen-binding sites of antibodies (the antibody combining sites).

The high degree of specificity of an antibody for the antigen used for immunization and the wide range of specificities that the immune system is capable of generating have been the subject of numerous investigations. The specificity of antibody:antigen interactions is accepted as being due to

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the complementarity of the antibody combining site structure and that of the antigenic determinant. The diversity of antigen-binding specificities is then due to variation in the combining site topography brought about by variation in primary and three-dimensional structure.

Recognition of the central importance of the antibody combining site in immune function has prompted intensive study of the primary and three-dimensional structures of antibodies. Considerable three-dimensional information has become available from X-ray crystallography,[1-9] although the number of antibody structures that will be elucidated by X-ray analysis can be only a very small fraction of the total number of different antibodies that higher organisms can produce. Other techniques are needed in the study of antibody combining sites and one that can make a significant contribution is modeling.

Here, we review the various modeling procedures that have been applied to antibodies, evaluate the success of these procedures in predicting combining site structures, and discuss potential improvements and problems.

Structural Background

Antibodies are multimers of a basic unit that consists of four polypeptide chains identical in pairs (Fig. 1[10]). There are two light (L) chains of about 220 amino acids and two heavy (H) chains of 450–575 amino acids. Both L and H chains are made up of regions of sequence homology (domains) of about 100–120 residues. There are two such domains in the L chain and four or five in the H chain. The N-terminal domains of both L and H chains are variable, i.e., they differ in sequence from antibody to antibody; the other domains are constant, i.e., they are the same in antibody chains of the same type except for single amino acid differences at a few positions. The variable domains of the L and H chains, V_L and V_H,

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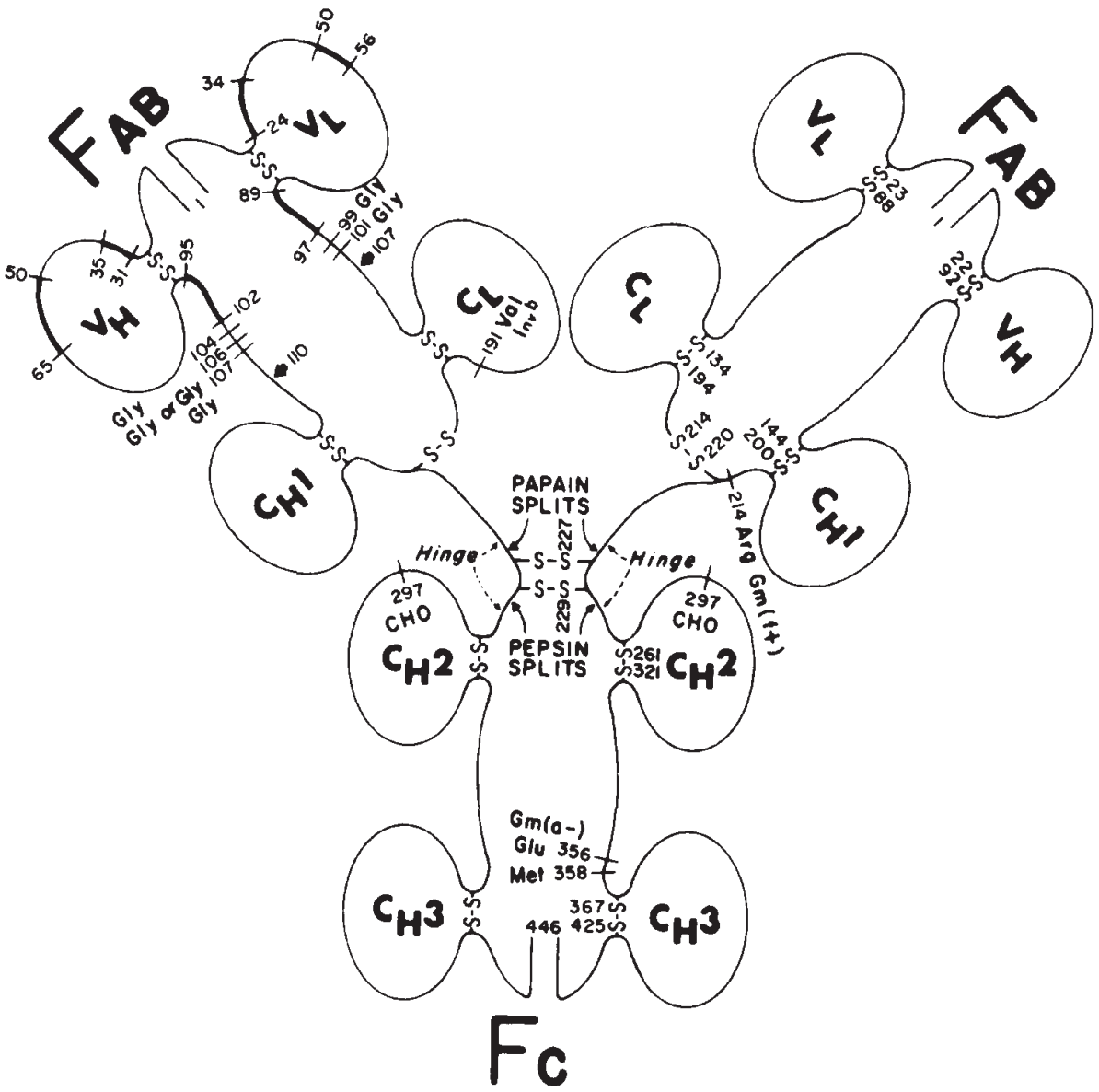

FIG. 1. Schematic representation of the four-chain structure of human IgG_1 molecule. The numbers on the right-hand side denote the actual residues of protein Eu [G. M. Edelman, B. A. Cunningham, W. E. Gall, P. D. Gottlieb, U. Rutishauser, and M. J. Waxdal, *Proc. Natl. Acad. Sci. U.S.A.* **63,** 78 (1969)]. The numbers of the Fab fragments on the left side are aligned for maximum homology: light and heavy chains are numbered according to T. T. Wu and E. A. Kabat, *J. Exp. Med.* **132,** 211 (1970), and E. A. Kabat and T. T. Wu, *Ann. N.Y. Acad. Sci.* **190,** 382 (1971). The heavy chains of Eu have residues 52A and 82A, B, C but lack the residues termed 100A–K and 35A, B. Thus, residue 110 (the end of the heavy-chain variable region) is 114 in the actual sequence. Hypervariable or complementarity-determining regions are shown by heavier lines. V_L and V_H denote the light- and heavy-chain variable regions; C_H1, C_H2, and C_H3 are domains of the constant region of the heavy chain; C_L is the constant region of the light chain. The hinge region, in which the two heavy chains are linked by disulfide bonds, is indicated approximately. The attachment of carbohydrate is at position 297. Arrows at positions 107 (in the light chain) and 110 (in the heavy chain) denote transition from variable to constant region. The sites of cleavage by papain and pepsin and the locations of various allotypic genetic factors [Gm(ft), Gm(a-), Inv^b] are indicated.[10]

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display significant sequence similarity, as do the constant domains[10]; there is no obvious sequence similarity between variable and constant domains. The site on the antibody molecule that binds to antigen is formed by the association of V_L and V_H, the Fv module. The fragment formed by the association of the L chain and the two N-terminal domains of the H chain is called the Fab, or antigen-binding, fragment (Fig. 1). The antigen-binding properties of an antibody are determined entirely by the variable domains; indeed, a chimeric structure, in which the variable domains have been linked to the constant domains of the heterologous chains, was shown to display exactly the same ligand-binding characteristics.[11]

A comparison of the sequences of variable domains from a variety of immunoglobulins revealed the existence of regions of hypervariability, three each in the L and H chains.[12,13] These hypervariable regions were predicted by Wu and Kabat[12] to fold up three-dimensionally to form the walls of the antigen-binding site of the antibody (the antibody combining site) several years before any X-ray structures had been determined.

X-Ray crystallographic studies have allowed the direct visualization of the three-dimensional structure of complete antibodies,[14,15] although only at low resolution. Structures at high resolution, however, have become available for a number of antibody fragments, including several Fabs, which bear the antigen-binding sites. These studies have revealed the high degree of structural similarity of the homologous domains and, as would be expected, the similarity in the three-dimensional structure was found to parallel the similarity in amino acid sequence. Thus, the V_L and V_H domains were found to have very similar structures, as were the constant domains; constant and variable domains, on the other hand, share only a basic tertiary structure and appear to be rotational isomers.[16–18] The hypervariable segments were found to exist mainly as loops that are for the most part exposed and located at one end of the variable domains.

In spectacular confirmation of the prediction of Wu and Kabat,[12] the

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hypervariable regions were indeed found to form a continuous surface at the tip of the Fabs.[16,19] Furthermore, crystallographic studies of complexes of Fabs with specific ligands unequivocably established the essential identity of the hypervariable surface with the antibody combining site. It is the chemical nature of this surface that determines the particular specificity of an antibody and the affinity with which it binds to its specific ligand. Furthermore, the insertions and deletions that are frequently found in the hypervariable regions, together with the variation in the amino acid residues in these regions, result in the variability of the combining site topography, thus providing an obvious structural basis for the wide diversity of antigen-binding specificities. In view of the undisputed importance of the hypervariable regions in the binding interaction with the antigen, these regions are now also called complementarity-determining regions (CDRs).

The comparison of the three-dimensional structures of variable domains from different antibodies reveals that the nonhypervariable or framework regions of these domains are essentially superimposable, so that the structural variation is mainly confined to the hypervariable segments.[20,21] In addition to the close similarity in the tertiary structures of the homologous domains, it had also been found that the mode of association of the paired domains is essentially invariant.[6,22,23] Thus the antibody combining site can be viewed as being formed by a small number of segments of variable structure grafted onto a scaffolding of essentially invariant architecture.

Even the hypervariable regions have been found to display a high degree of structural similarity, so that canonical structures have been advanced for several of the CDRs. For example, it was found that hypervariable regions with the same number of residues, especially those with significant sequence similarity, have very similar backbone conformations.[4,20,21,24] Furthermore, Kabat *et al.*[25] noted that certain positions in the CDRs did not vary but were conserved and they suggested that those residues play a structural role. Indeed, structural comparisons of known CDR structures have shown that there is a small repertoire of main-chain conformations for at least five of the six CDRs and that the particular

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