Clinical Immunohematology: Basic Concepts and Clinical Applications

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Clinical Immunohematology: Basic Concepts and Clinical Applications

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To my whole family, especially Jennifer

Preface

Immunohematology, the study of the effect of the immune system on components of the blood, has a long and glorious history, going back to the origins of immunology. In his experiments on the immune response, Ehrlich used the differences between the red blood cells of sheep and goats to demonstrate that the reaction against foreign cells was specific and that such reactions did not occur against the cells of the animal itself. Landsteiner used these clues to demonstrate antibodies in human serium that were the basis of human disease. Thus, from the very beginning of scientific immunohematology, the relationship between the experimental fact and the clinical entity was established.

The present volume is designed to continue in that tradition. In the first part, the scientific basis on which clinical immunohematology is based is laid out. The nature of antibody and its production, the nature of the antigens of the blood cells, complement, and mechanisms of immune destruction are all delineated.

As this volume progresses, the applications of that knowledge to clinical problems are introduced. The detection of the reaction between antigen and antibody on blood cells and the application of such knowledge to the problems of compatibility of components of the blood in transfusion and in transplacental disease are covered. Finally, disease due to aberrations of the immune system and in the interaction of the components of the immune system is addressed.

This volume was written to comprise a unified whole; there is no other reason to undertake such a project by a single author. Thus, it is best read from beginning; this will enable the reader to see how the first part relates to the latter parts. That is asking a great deal of even the most avid reader so references are made both forward, relating basic science under discussion to clinical states discussed later, and backward, relating the clinical discussion to preceding discussions of the scientific basis. It is hoped that the reader will avail himself or herself of the cross-references so that the component parts of any question can be understood as a whole.

This book is written primarily by a single author, with help in a few areas, and one might ask, in this day of complex and intricate science, whether this is a good idea. In the middle of the project, I was convinced that it was not because of the complexity of the subjects to be covered. Certainly no one, particularly the present author, is equally adept in knowledge of all parts in such a great diversity as covered in the text and some parts will suffer in detail as a result. When the work is all done, however, I feel that the unity given by a single point of view is worth both the effort and lack of detailed knowledge in some areas. It is hoped that, at the very least, the volume will serve as a framework on which more specific knowledge may be placed. As a framework, it strives to place things in their appropriate context.

In the past, "immunohematology" has referred at times to the activities associated primarily with the transfusion of blood. Although that is clearly an important part of the study of immune reaction of blood cells, I have used an expanded outlook for inclusion in the present volume. Even so, it is not as expanded as it should be as the present volume does not cover the large area of allogeneic bone marrow transplantation. This important area is covered by other treatises; perhaps in the next edition, if there is one, it may be covered in this context.

This work would not have been possible without the support of my family, particularly my wife, who has suffered through the process for a period of time longer than either of us anticipated. My co-workers, particularly Dr. Marilyn Telen, Dr. Barton Haynes, and Dr. Dana Devine, have been helpful in supplying ideas and details. Ms. Sharon Hoffman helped greatly in the final editing. But most of all, I wish to acknowledge those anonymous benefactors of mankind who invented the computer and word processing programs; I couldn't have done without them.

—Wendell F. Rosse

Notice

The indications and dosages of all drugs in this book have been recommended in the medical literature and conform to the practices of the general medical community. The medications described do not necessarily have specific approval by the Food and Drug Administration for use in the diseases and dosages for which they are recommended. The package insert for each drug should be consulted for use and dosages as approved by the FDA. Because standards for usage change, it is advisable to keep abreast of revised recommendations, particularly those concerning new drugs.

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Clinical Immunohematology: Basic Concepts and Clinical Applications

PART ONE:
BASIC IMMUNOHEMATOLOGY

Section 1: Antibodies

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1 Structure and Function of Antibodies

Antibodies and structures like them are the recognition units of the immune system. They are designed to recognize both foreign and self-antigens and, according to their instructions, to mediate the appropriate reactions of the immune system. They are best known as the mediators of immune destruction by the humoral arm of the immune system and it is in that context that this discussion is based.

The understanding of the structure, functions, and reactions of antibodies is fundamental to an understanding of immunohematology. Antibodies are the mediators of specificity of the humoral immune system; the vast majority of the reactions that occur in the destruction of blood cells and their precursors involve reactions of the humoral arm of the immune system. In this section, we shall examine the structure of immunoglobulins, how they are formed, and how they interact with antigens of the blood cells.

Structure of Antibodies

Immunoglobulin molecules (and the other immunologically important molecules that resemble them) have developed through evolution to comprise the primary recognition system of the immune system. They are designed so that a portion of the molecule reacts very specifically with one of several hundred thousand different molecular structures (the antigen) while another portion of the molecule subserves various effector functions which are used either in the reactions of antigen recognition and processing or in the reactions of immune destruction. This is brought about by the presence of a highly variable region in one part of the molecule that can recognize the differences in antigen molecules and a highly conserved region in the other part of the molecule that can effect the appropriate responses.

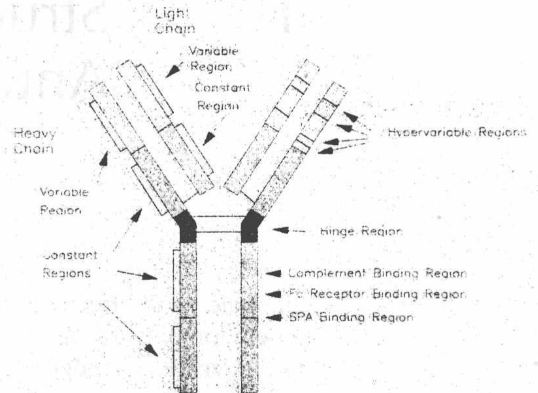
The Fundamental Immunoglobulin Unit

The Multichain Structure

The fundamental unit of antibody structure is a four-chain unit (Fig. 1.1) consisting of two light chains and two heavy chains (1, 2). The two light chains are identical and have a molecular weight of about 25,000 daltons each and the two heavy chains are different from these but identical to each other; they have a molecular weight of about 55,000 daltons each.

The chains of the immunoglobulin unit consist of a series of globular units each of about 110 amino acids (3) (Fig. 1.2). The light chains have two such units each, the heavy chains either four or five. These globular units are similar in many respects to each other; all of about the same size and, even though there are marked differences in the primary structure, they all have a similar secondary and tertiary structure. When examined by x-ray crystallography, all exhibit the characteristic immunoglobulin fold (4) (Fig. 1.3). The polypeptide chains fold back and forth seven times, forming two beta-pleated sheets containing three and four antiparallel strands respectively. Stability is given to this structure as the antiparallel strands react laterally with one another (see Fig. 1.3). The turnings of the strands provide variable areas for specific interaction (see below).

In each immunoglobulin unit, an intrachain disulfide bridge spans about 60 residues (between approximately the 25th and the 85th residue from the amino end of the unit); these residues are on different folds of the chain and the disulfide bond helps to maintain the internal structure of the unit. Because of the characteristics of the amino acids that are found on various parts of the chain, the unit that is thus formed has a hydrophilic exterior and a hydrophilic interior which also help maintain its tertiary structure.



1.1 Schematic generalized structure of the immunoglobulin unit. The component parts of the heavy and light chains are indicated. One heavy and one light chain are held together by a single interchain disulfide bond and the two heavy chains are held together by interchain disulfide bonds. See text for explanation of parts.

This marked similarity among the primary units suggests that they were all derived from a primordial unit that was approximately 100 amino acids in length (5). Through evolution, the unit has presumably been reduplicated to form the complex molecule that is characteristic of mammalian immunoglobulin.

Variable Regions

The globular unit at the amino terminus of both the heavy and the light chain, comprising about 110 to 117 amino acids, varies greatly in primary sequence in antibodies of different specificity; this is called the variable (V) region of the chain. That of the light chain is called V_L and that from the heavy chain V_H. These units contain an extra loop, the V loop, in the folding of the chain (see Fig. 1.3).

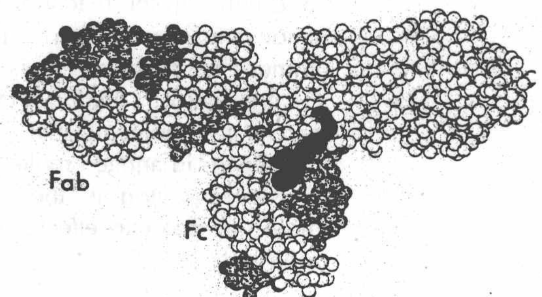
Within this domain which is variable from protein to protein, there are three segments which are *hypervariable,* that is, they differ almost completely in amino acid structure from one protein to the next (6, 7) (Fig. 1.4).

In the V_H unit, these are found in segments 31 to 35, 49 to 55, and 99 to 111; in the V_L unit, the homologous areas are 28 to 38, 50 to 56, and 89 to 97. In the folding of the chain, these hypervariable areas on each chain are all brought into proximity with one another and when the light and heavy chains are assembled in the complete molecule (see below) the three hypervariable regions of the light chain are brought into proximity to the three homologous regions of the corresponding heavy chain. The pocket or area formed by these six hypervariable areas is the antigen binding site (see below) (Fig. 1.5).

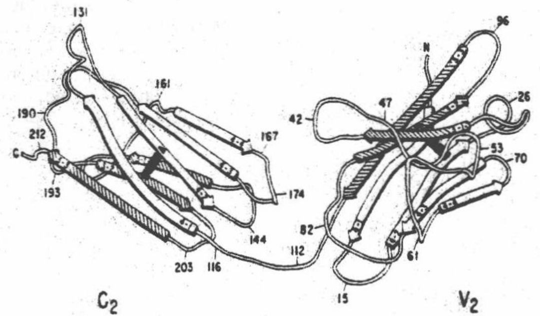
The remainder of the variable domain is called the "framework" and is much more constant from immunoglobulin to immunoglobulin than the hypervariable regions. Nevertheless, there are systematic variations so that these framework regions can be classified into related groups, usually designated by Roman numerals (e.g., V_HIII designates a framework structure, arbitrarily designated III, in the variable portion of the heavy chain). In κ-light chains, there are four distinct classes of framework structure (V_κ I–IV; in λ-light chains, five (V_λ I–V). These framework regions can often be identified by specific antibodies that react with them.

The Constant Regions

The other globular domains of the light and heavy chains show great homology in antibodies of differing specificity; these are called the constant (C) regions and are numbered



1.2 A three-dimensional model of the IgG molecule (reproduced with permission from Jeske DJ, Capra JD. Immunoglobulins: Structure and function. In: Paul WE, ed. *Fundamental immunology*. New York: Raven Press, 1984, p. 132).



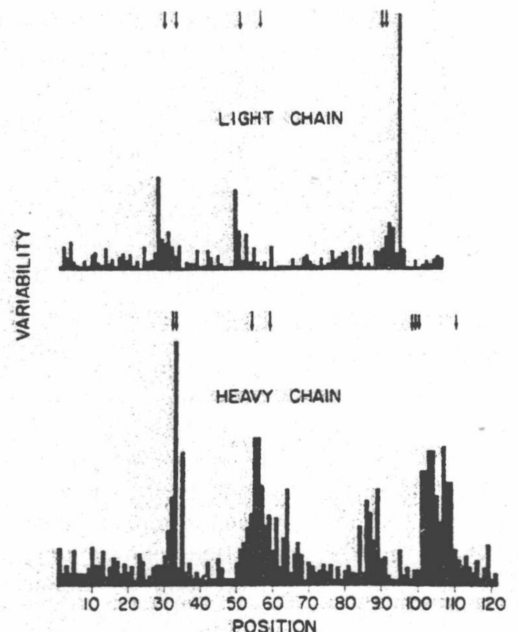
1.3 Schematic diagram of the structure of a light chain, showing two examples of the "immunoglobulin loop." The elements that are part of the β-pleating are indicated by arrows. The intrachain disulfide bonds are indicated by the black bars. The antigen-binding area is on the far right (reproduced with permission from Jeske DJ, Capra JD. Immunoglobulins: Structure and function. In: Paul WE, ed. *Fundamental immunology*. New York: Raven Press, 1984, p. 133).

from the domain nearest the variable domain. Thus, the light chain consists of a variable and a constant region, designated V_L and C_L, and the heavy chain consists of a variable domain and three or four constant domains, designated V_H and C_H1, C_H2, C_H3, and [in the case of immunoglobulin M (IgM) and immunoglobulin E (IgE)] C_H4 respectively.

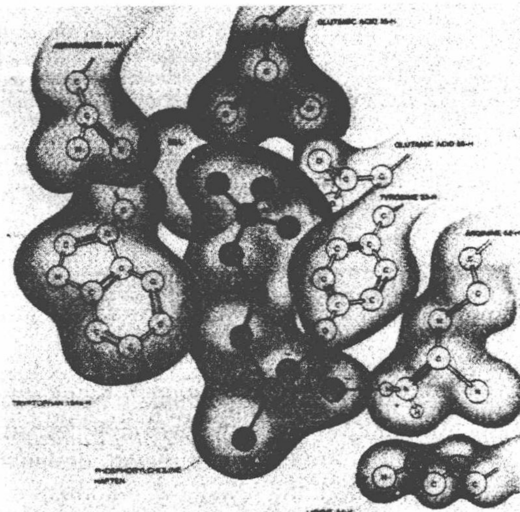
When the immunoglobulin unit is fully assembled, a light chain is attached to each of the heavy chains so that the variable regions of each light chain–heavy chain pair are next to one another. The light chain and heavy chain are held together in place by a number of weak physical bonds (ionic, hydrogen, van der Waals forces, etc.). In the interaction of the constant regions, the four-stranded beta-pleated sheets from each molecule interact hydrophobically to form a solvent-free interface; in the variable domains, it is the three-stranded sheets that do so.

In addition to the physical forces that hold together the light and heavy chains, a single covalent linkage is provided by a disulfide bridge from cysteines in C_L just prior to the carboxy terminus to specific cysteines in the C_H domains. In most immunoglobulins, the cysteine in the C_H domains is at position 131 which lies between the C_H1 and the V_H domains; in immunoglobulin G_1 (IgG_1) molecules (see below) the link is to a cysteine at residue 220 between C_H1 and C_H2 domains. In the three-dimensional model of the assembled molecule, these two residues are equally accessible to the cysteine of the V_L chain.

The two heavy chains are held together by the same hydrophobic forces that hold together the light and the heavy chains in the variable and first constant regions. In this way, homologous C_H2–4 regions of the two chains are held together. The two chains are further bound together by disulfide bridges which are



1.4 The variability of the residues of the variable portions of the light and heavy chains, calculated by the method of Wu and Kabat. The higher the line, the greater the variability of the residues at that position. Antigen binding is indicated by the arrows that demonstrate sides of binding of affinity labels (reproduced with permission from Jeske DJ, Capra JD. Immunoglobulins: Structure and function. In: Paul WE, ed. *Fundamental immunology*. New York: Raven Press, 1984, p. 159).



1.5 The fine structure of the antigen binding site. The hapten, phosphorylcholine, fits into a pocket formed by the six hypervariable regions (reproduced with permission from Jeske DJ, Capra JD. Immunoglobulins: Structure and function. In: Paul WE, ed. *Fundamental immunology*. New York: Raven Press, 1984, p. 161).

present in the so-called "hinge" region (8). The hinge region is a sequence rich in prolines and cysteines which is located between C_H1 and C_H2 in IgG, immunoglobulin A (IgA), and immunoglobulin D (IgD) and between C_H2 and C_H3 in IgM.

The hinge region in all immunoglobulins other than IgM is coded for by a gene or homologous genes which are distinct and separate from the genes coding for the globular domains (see below); a single "dose" of the gene for this region imparts one or two cysteines as well as a structure rich in prolines. In some subclasses of immunoglobulin (IgG_3), the hinge region gene is quadruplicated, resulting in eight interchain disulfide bonds in the area.

In space, the immunoglobulin unit assumes the shape of a Y (9, 10). This again is due to the presence of the hinge region which is rich in prolines. Since proline residues cannot form α-helical structures (since the free hydrogen on the nitrogen atom is removed in forming an imino group with the rest of the molecule), peptide chains rich in them tend not to form rigid structures such as α-helices or β-pleats but to be mobile. The mobility of the hinge region allows the molecule to bend readily to form the Y-structure. Those molecules with longer hinge regions have greater mobility between the $[(V_L\text{-}C_L) - (V_H\text{-}C_H1)]$-containing portions of the molecule and the rest of the molecule; this may have implications with respect to functional capabilities of the molecule (see below).

The regions of contact between light and heavy chains and between heavy chains are markedly conserved in different antibody molecules, indicating the necessity and importance of the primary structure to the formation of the tertiary and quaternary structures.

Variations in the Light and Heavy Chains

Within the constant regions of the light and heavy chains there are distinct and consistent variations which help to identify the chain and, in some cases, give clues to its function. These variations were first identified by differences in reactions with antibodies but in some cases the biochemical basis for the difference is known. These variations are genetically determined and are called either allotypes or isotypes depending on how they occur in individuals of the group.

Isotypes

Isotypic markers are variations coded for by genes that are present in all members of the species. These variations are in the constant regions of the peptides and are often used for marking the molecule when other characteristics of the molecule are not known.

Isotypes of the Light Chains

There are two types of light chains based on isotypic differences in the C_L regions. Although the two types differ by at least 60% of their residues, they are sufficiently homologous that a single identifying antigen is present on each type, called κ or λ. These two classes of light chains are found in all mammals and are derived from separate clusters of genes (see

Table 1.1. Structure and Antigens of Heavy Chains of Different Classes and Subclasses of Immunoglobulin Molecules

Class	Antigen	Constant Regions	Subclasses	Antigens
IgG	γ	3	IgG_1	γ_1
			IgG_2	γ_2
			IgG_3	γ_3
			IgG_4	γ_4
IgM	μ	4	None	
IgA	α	3	IgA_1	α_1
			IgA_2	α_2
IgD	δ	3	None	
IgE	ϵ	3	None	

Table 1.2. Amino Acid Substitutions in Km Allotypes of κ-light chains

	Residue	
Allotype	153	191
Km (1)	Val	Leu
Km (1, 2)	Ala	Leu
Km (3)	Ala	Val

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Abbreviations: Ala, alanine; Leu, leucine; Val, valine.

below). In humans, about 60% of the antibodies produced possess κ-light chains and 40% possess λ-light chains. So far as is known, there is no functional difference between the two types of light chains as the difference between them resides in a portion of the molecule that is not directly associated with the function light chains take part in, antigen binding. Both kinds of light chains bind equally well to heavy chains.

Within the class of λ-light chains, there are four isotypic variations which have been designated $C_{\lambda 1-4}$. These were originally recognized by antibodies against myeloma proteins but the biochemical differences in the four classes are now known. These differences do not seem to impose an alteration in function of the molecule. No such isotypic markers are known for κ-light chain.

Isotypes of Heavy Chains

There are five major varieties of heavy chains based on differences in the constant regions; these define the isotypic classes (11) (Table 1.1). Each of these major varieties is associated with one of the classes of immunoglobulin—IgG, IgM, IgA, IgD, and IgE. Each major variety of heavy chain is identified, as in the case of the κ and λ markers of light chains, by an identifying antigen. This antigen is consistently present on all heavy chain molecules of a given isotype.

Within a given isotype, there may be consistent variations which give rise to antigens identifying subclasses of molecules within the isotype class. For instance, within the class of γ-chains, there are four subclasses denoted γ_1, γ_2, γ_3, and γ_4. The molecules in each of these subclasses possess, in addition to the γ-antigen, an antigen identifying the subclass. These distinctions are important because some of the functions of the molecule can be surmised by the class and subclass of its heavy chains.

The structural differences that give rise to these classes and subclasses occur outside the antigen binding area and antibodies of different isotypes can bind to the same antigen.

Allotypic Markers

Allotypic markers are variations in the proteins that are manifest in all proteins of a given isotype but that may differ in different individuals of the population. Thus, all the κ-light chains of individual A may have one marker and all the κ-light chains of individual B may have another marker.

Among human κ-light chains, there may be allotypic differences at two loci giving rise to three allotypic markers. These were originally designated by Inv but now are frequently called simply Km markers. The Km allotypes arise from substitutions at two residues on the κ chain—153 and 191 (Table 1.2). To date, no allotypes have been found in λ-light chains.

As with the light chains, allotypes of heavy chains also have been identified (11, 12). These are genetic differences that are characteristic of the individual and occur in all heavy chains of a given isotype class or subclass. These differences are antigenic and are identified by specific antibodies produced in persons lacking the antigen.

For IgG heavy chains, there are a number of allotypes called Gm groups. Each antigen characteristic of a Gm group is found on only one subclass of heavy chains. In some cases, the amino acid substitution accounting for the antigenic difference is known (Table 1.3).

Table 1.3. Class and Subclass Identification in Immunoglobulin Molecules

Class	Subclass	Allotype	No.	Residue Substitution
IgG	IgG_1	Gm (1)	355–358	REDL[a]
		Gm −(1)		REEM
		Gm (3)	214	R
		Gm (17)		K
		Gm 2, 18, 20 unknown		
	IgG_2	Gm 23		
		Gm −23		
	IgG_3	Gm 21		
		Gm −21		
		Gm 5		
		Gm −5		
		Gm 11		
		Gm −11		
		Gm 6, 10, 14, 15, 16, 24, 25 all unknown		
	IgG_4	Gm 4a		
		Gm 4b		
IgA	IgA_1	None identified		
	IgA_2	Am 1		
		Am −1		
IgM	None identified			
IgD	None identified			
IgE	None identified			

Abbreviations: IgE, immunoglobulin E.
[a]The single letter code for the amino acids are: R–arginine; E–glutamate; D–aspartate; L–leucine; M–methionine; k-lysine.

Enzymatically Derived Fragments of Immunoglobulin Molecules

When the antibody molecule is acted on by certain enzymes, fractions result which have specific properties. Papain divides the molecule so that three fragments result (13) (Fig. 1.6A).

1. Two fragment antigen binding (Fab) fragments, each containing the light chain and the V_H and C_H1 domains of the heavy chain. This fragment contains the antigen binding site.
2. One fragment crystalline (Fc) fragment which contains the C_H2–3 (4) of both heavy chains. The name "Fc" arose because this fragment was easily crystallized out of solution.

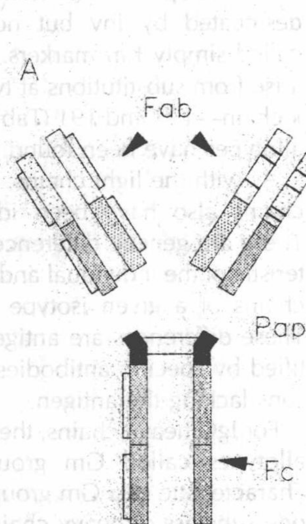
When pepsin is used to digest the protein, the bonds that are broken are within the Fc region so that only two fragments result (Fig. 1.6B)

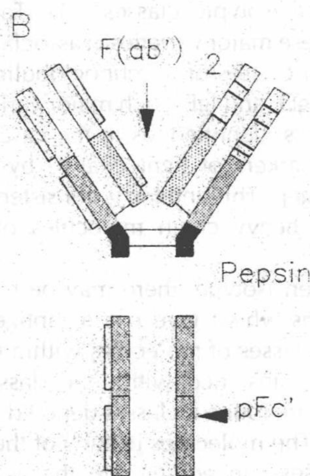
1. The $F(ab)_2$ fragment containing both antigen binding sites consisting of two V_L, two C_L, two V_H, and two C_H1 domains as well as a small portion of two C_H2 domains through which the two halves are connected by one or more disulfide bonds.
2. The remainder of the Fc fragment which is a little shorter than the Fc fragment which results from papain digestion.

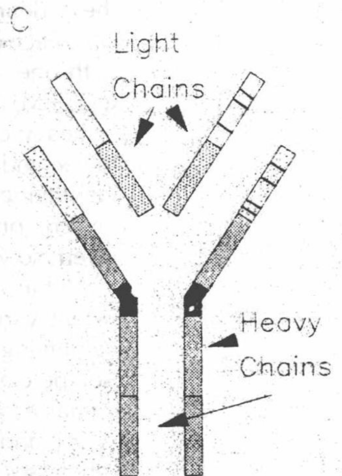
These fragments were important in understanding the structure of the antibody molecule in relation to its structure. The antigen-binding properties of the antibody and the idiotypic markers are resident in the Fab portion of the molecule. The functional capacities of the antibody molecule are mainly carried in the Fc portion as this is the region that carries areas able to react specifically with receptors.

The Antigen Binding Site

The combination of the two variable regions (one from the light chain and one from the







1.6 The fragments resulting from the treatment of the IgG molecule with (A) papain, (B) pepsin, or (C) a reducing agent. The Fc and, particularly, the pFc' fragments may be further digested by the enzymes.

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