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Molecular Genetics of Immunoglobulin

edited by
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MOLECULAR GENETICS OF IMMUNOGLOBULIN

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Volume 17

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Preface

Immunoglobulin genes are not just of interest to immunologists. An understanding of the way in which DNA rearrangement and somatic mutation contribute to antibody diversity is of importance to a wide range of biologists. The cell-type specificity of immunoglobulin gene expression is of concern to many who are interested in gene expression in mammals. Furthermore, the immunoglobulin superfamily itself presents important questions to those interested in evolution.

The analysis of immunoglobulins and of their genetics has advanced rapidly since the mid-1970s, mainly as a result of the application of recombinant DNA and monoclonal antibody technologies. The essential features of the molecular anatomy of both antibodies and their genes have been largely identified; this has resulted in significant insights into the way antibody diversity is generated. Clearly, much still remains to be elucidated in these areas, whilst studies both of regulation and of phylogeny are still in their infancy. We felt nevertheless that it was a good time to draw together what we do know about the molecular genetics of immunoglobulin.

We wish to thank the authors for contributing to this volume and the publisher for prompt publication.

Cambridge

Franco Calabi
Michael S. Neuberger

List of abbreviations

Ars	*p*-azophenylarsonate
BiP	immunoglobulin heavy chain binding protein
C region	constant region
CDR	complementarity determining region
D segment	diversity segment
Fab	antigen binding fragment from papain digestion of immunoglobulin
$F(ab')_2$	antigen binding fragment from pepsin digestion of immunoglobulin
Fc	crystallizable fragment from papain digestion of immunoglobulin
FcR	immunoglobulin Fc receptor
FR	framework region
H	immunoglobulin heavy chain
H_m	membrane form of immunoglobulin heavy chain
H_s	membrane form of immunoglobulin heavy chain
HAT	hypoxanthine, aminopterin, thymidine medium
HIV	human immunodeficiency virus
Ig	immunoglobulin
IL-4	interleukin 4
J segment	joining segment
J chain	joining chain
L	immunoglobulin light chain
LPS	bacterial lipopolysaccharide
LTR	long terminal repeat
MAG	myelin associated glycoprotein
MHC	major histocompatibility complex
N region	nucleotide region
NCAM	neuronal cell adhesion molecule
NK cell	natural killer cell

NP	4-hydroxy-3-nitrophenylacetyl
Ox	2-phenyl-5-oxazolone
PC	phosphorylcholine
Poly-IgR	poly-immunoglobulin receptor
RFLP	restriction fragment length polymorphism
RS	rearranging sequence (κ locus)
S region	switch region
TcR	T cell receptor
tk	thymidine kinase
V region/segment	variable region/segment

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CHAPTER 1

Structure and function of antibodies

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1. Introduction

Antibody molecules are essentially required to carry out two principal roles in immune defence:

(i) to recognise and bind to foreign material (antigen). In molecular terms this generally means binding to structures on the surface of the foreign material (antigenic determinants) which differ from those of the host. Such antigenic determinants are usually expressed in multiple copies on the foreign material, e.g. proteins on a bacterial cell surface. The host needs to be able to recognise a wide variety of different structures – it has been estimated that a human being is capable of producing antibodies against more than 10^6 different molecular structures. This is described as antibody diversity.

(ii) to trigger the elimination of foreign material. In molecular terms this involves the binding of certain molecules (effector molecules) to antibody-coated foreign material to trigger complex elimination mechanisms, e.g. the complement system of proteins, phagocytosis by cells such as neutrophils and macrophages. The effector systems are generally triggered only by antibody molecules clustered together as on a foreign cell surface and not by free unliganded antibody. This is crucial considering the high serum concentration of some antibodies.

The requirements imposed on the antibody molecule by the functions (i) and (ii) are in a sense quite opposite. Function (i) requires great antibody diversity. Function (ii) requires commonality, i.e. it is not practical for Nature to devise a different molecular solution for the problem of elimination for each different antibody molecule. In fact the conflicting requirements are elegantly met by the antibody structure represented in Fig. 1. The structure consists of three units. Two of the units are identical and involved in binding to antigen – the Fab (fragment antigen binding) arms of the molecule. These units contain regions of sequence which vary greatly from one antibody to another and confer on a given antibody its unique binding specificity. The existence of two Fab arms greatly enhances the affinity of antibody for antigen in the normal situation where multiple copies of antigenic determinants are presented to the host. The third unit – Fc (fragment crystalline) – is involved in binding to effector molecules. As shown in Fig. 1, the antibody molecule has a four-chain structure consisting of two identical heavy chains spanning Fab and Fc and two identical light chains associated only with Fab.

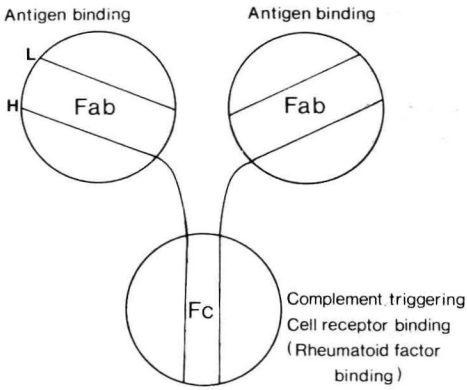

Fig. 1. A schematic representation of antibody structure emphasising the relationship between structure and function. The antibody molecule can be thought of in terms of three structural units. Two Fab arms bind antigen and are therefore crucial for antigen recognition. The third unit (Fc) binds effector molecules triggering antigen elimination. The antibody molecule thus links antigen recognition and antigen elimination. The structure is composed of four chains. Two identical heavy (H) chains span Fab and Fc regions and two identical light (L) chains are associated with Fab alone.

The five classes of antibodies or immunoglobulins termed immunoglobulin G (IgG), IgM, IgA, IgD and IgE differ in their heavy chains termed γ, μ, α, δ and ϵ, respectively. The differences are most pronounced in the Fc regions of the antibody classes and this leads to the triggering of different effector functions on binding to antigen, e.g. IgM recognition of antigen might lead to complement activation whereas IgE recognition (possibly of the same antigen) might lead to mast cell degranulation and anaphylaxis (increased vascular permeability and smooth muscle contraction). Structural differences also lead to differences in the polymerisation state of the monomer unit shown in Fig. 1. Thus, IgG and IgE are generally monomeric whereas IgM occurs as a pentamer. IgA occurs predominantly as a monomer in serum and as a dimer in seromucous secretions.

The major antibody in the serum is IgG and as this is the best-understood antibody in terms of structure and function we shall consider it shortly. The other antibody classes will then be considered in relation to IgG. First, however, a very brief overview of the structure and function of the different immunoglobulins will be presented [1].

IgG is the major antibody class in normal human serum forming about 70% of the total immunoglobulin. It is evenly distributed between intra- and extravascular pools. IgG is a monomeric protein and can be divided into four subclasses in humans. It is the major antibody of secondary immune responses.

IgM represents about 10% of total serum immunoglobulin and is largely confined to the intravascular pool. It forms a pentameric structure and is the predominant antibody produced early in an immune response, serving as the first line of defence against bacteraemia. As a membrane-bound molecule on the surface of B lymphocytes it is important as an antigen receptor in mediating the response of these cells to antigenic stimulation.

IgA forms about 15–20% of total serum immunoglobulin where it occurs largely as a monomer. In a dimeric complex known as secretory IgA (sIgA) it is the major antibody in seromucous secretions such as saliva, tracheobronchial secretions, colostrum, milk and genitourinary secretions.

IgD represents less than 1% of serum immunoglobulin but is widely found on the cell surfaces of B lymphocytes where it probably acts as an antigen receptor analogously to IgM.

IgE though a trace immunoglobulin in serum, is found bound through specific receptors on the cell surface of mast cells and basophils in all individuals. It is involved in protection against helminthic parasites but is most commonly associated with atopic allergies.

2. *Structure of IgG*

2.1. General considerations

In IgG the Fab arms are linked to the Fc via a region of polypeptide chain known as the hinge. This region tends to be sensitive to proteolytic attack generating the basic units of the molecule as distinct fragments. The discovery of this action by Porter in 1959 [2] provided the first great insight into antibody structure. In 1962 Porter proposed a four-chain structure for the IgG molecule [3]. Since then, chemical and sequence analyses, notably by Edelman [4], have confirmed a four-chain structure consisting of two identical heavy (H) chains of molecular weight approximately 50 000 and two identical light (L) chains of molecular weight approximately 25 000. The molecular weight of IgG is thus typically approximately 150 000. The light chains are solely associated with the Fab arms of the molecule whereas the heavy chains span Fab and Fc parts as shown in Fig. 2. A single disulphide bond connects light and heavy chains and a variable number, depending on IgG subclass (see below), connects the two heavy chains. The latter connection is made in the hinge region of the molecule. Papain cleaves heavy chains to the amino-terminal side of these hinge disulphides producing two Fab and one Fc fragment. Pepsin cleaves to the carboxy-terminal side producing a single $F(ab')_2$ fragment and smaller fragments of Fc including a carboxy-terminal pFc′ fragment.

The light chains exist in two forms known as kappa (κ) and lambda (λ); the forms are distinguished by their reaction with specific antisera. In humans, κ chains are somewhat more prevalent than λ, in mice, λ chains are rare [5]. The heavy chains can also be grouped into different subclasses, the number depending upon the species under consideration. In humans there are four subclasses having heavy chains labelled $\gamma 1$, $\gamma 2$, $\gamma 3$ and $\gamma 4$ which give rise to the IgG1, IgG2, IgG3 and IgG4 subclasses. In mouse there are again four subclasses denoted IgG1, IgG2a, IgG2b and IgG3. The subclasses – particularly in humans – have very similar primary sequences, the greatest differences being observed in the hinge region. The existence of subclasses is an important feature as they show marked differences in their ability to trigger effector functions. In a single molecule, the two heavy chains are identical as are the two light chains; hybrid molecules are not found.