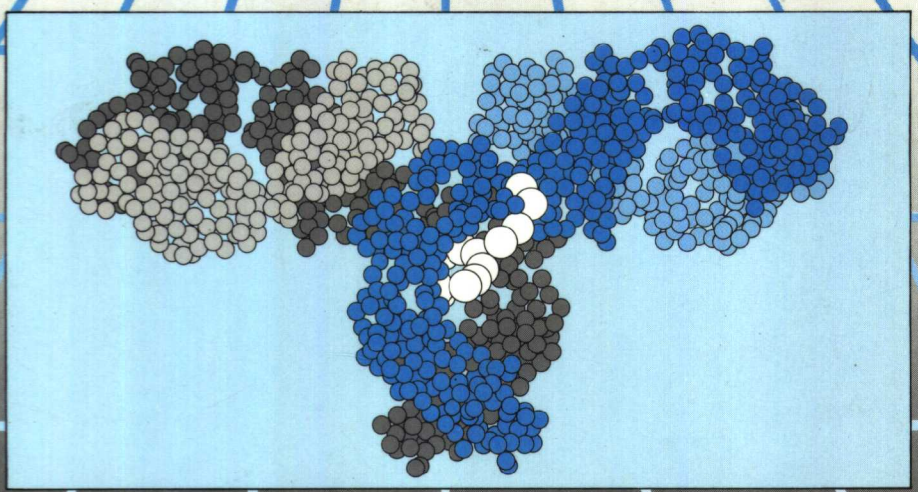
MOLECULAR IMMUNOLOGY

Edited by B D Hames & D M Glover



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Preface

Immunology is a subject area of enormous importance in modern biology and medicine. In recent years there has been an explosion of knowledge in the field due to the widespread application of the techniques of molecular cloning. This work has important implications not only for immunologists but also for our understanding of gene structure and function in general. 'Molecular Immunology' is designed to provide a comprehensive, up-to-date account of the major areas of progress in our understanding of the immune system. Five chapters, written by leading researchers, describe recent developments and review current knowledge of immunoglobulin genes, T cell receptor genes and major histocompatibility complex (MHC) genes as well as the processes of lymphocyte recognition and activation and the complement system. The aim is both to provide an in-depth analysis of each subject and, by bringing these reviews together into a single volume for the first time, to stimulate the cross-fertilization of ideas for future progress.

The first chapter, by Frederick Alt and Keith Blackwell, reviews in detail the structure, rearrangement, expression and function of immunoglobulin genes including a description of both the antigen-independent stages of B cell maturation and the development of the mature B cell repertoire. In the second chapter, Mark Davis describes the structure and function of the T cell receptor α, β, γ and δ polypeptides and the organization and expression of the corresponding genes during T cell differentiation. MHC genes are covered in the third chapter, written jointly by François Guillemot, Charles Auffray, Harry Orr and Jack Strominger. The genomic organization, structure, evolution and expression of both MHC class I and class II genes is considered, including analyses of structure–function relationships. The fourth chapter, by Cox Terhorst, Balbino Alarcon, Jan de Vries and Hergen Spits, is devoted to an in-depth consideration of the molecular mechanisms of lymphocyte recognition and activation. The final chapter, by Ken Reid, concentrates on recent advances in our understanding of the structure, function, biosynthesis and genetics of proteins association with the complement system, including the class III products of the MHC.

We wish to thank all the authors for their hard work and enthusiasm in producing such excellent contributions. The best rewards for their efforts will be if the Molecular Biology community finds this volume useful and if, during use, it spawns new ideas for future progress.

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Abbreviations

A-MuLV	Abelson murine leukemia virus
APC	antigen-presenting cell
BiP	H chain binding protein
CDR	complementarity-determining region
CR	complement receptor
DAF	decay accelerating factor
DAG	diacylglycerol
DEC	dendritic epidermal cells
EBV	Epstein–Barr virus
gbt	xanthine-guanine phosphoribosyltransferase
G-protein	GTP-binding protein
HANE	hereditary angioneurotic edema
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IDDM	insulin-dependent diabetes mellitus
IFN	interferon
Ig	immunoglobulin
Il	interleukin
κde	κ-deleting repeat
KLH	keyhole limpet hemocyanin
LCL	lymphoblastoid cell line
LDL	low density lipoprotein
LFA	lymphocyte function antigen
LHR	long homologous repeating structure
LPS	lipopolysaccharide
LTR	long terminal repeat
Mab	monoclonal antibody
MAC	membrane attack complex
MCP	membrane cofactor protein
MHC	major histocompatibility complex
MS	multiple sclerosis
N-CAM	neural cell adhesion molecule
NEPHGE	non-equilibrium pH gel electrophoresis
NK	natural killer
PFGE	pulsed-field gel electrophoresis

PHA	phytohemagglutinin
PIP	phosphatidylinositol monophosphate
PKC	protein kinase C
PMA	phorbol myristate acetate
RFLP	restriction fragment length polymorphism
RS	recombination sequences
SCID	severe combined immunodeficiency disease
Slp	sex-linked protein
SLE	systemic lupus erythematosus
TAP	T cell activated protein
TAPa	TAP-associated protein
T_c	cytotoxic T cell
TCR	T cell receptor
TdT	terminal deoxynucleotidyltransferase
TEA	tetraethylammonium
T_h	helper T cell
TK	thymidine kinase
TNF	tumor necrosis factor
T_s	suppressor T cell
TT	tetanus toxoid
VSG	variant surface glycoprotein

Contents

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Immunoglobulin genes

T.Keith Blackwell and Frederick W.Alt

1. Introduction

The mammalian humoral immune system can respond to a seemingly unlimited array of antigens by producing specific antibodies. Antibodies are made by B lymphocytes and are composed of immunoglobulin (Ig) heavy (H) and light (L) polypeptide chains. At the amino-terminus of H and L chains is a region of highly variable amino acid sequence (variable region). In an antibody molecule, H and L chain variable (V) regions are paired with each other to form the site which recognizes and binds to an antigen. The vast diversity of the antibody repertoire derives largely from the fact that H and L chain V regions are encoded by a portion of the H or L chain gene that is assembled somatically from component germline segments (1). The T cell antigen receptor (TCR) is composed of Ig-like polypeptides, which are also encoded by genes that are assembled somatically, apparently by the same enzymatic mechanisms (2). To understand development of the immune repertoire of B and T lymphocytes, it is essential to understand the mechanisms that regulate assembly and expression of Ig and TCR genes (3). This chapter focuses on studies of these regulatory mechanisms and describes Ig H and L chain gene organization and assembly in the context of how these genes have evolved and how different species generate antibody diversity.

2. The antibody molecule

2.1 Immunoglobulin structure

A monomeric Ig molecule (*Figure 1*) is composed of two identical H and two identical L chains that are linked together by disulfide bonds (4). Both H and L chains are organized into domains that are defined by homology

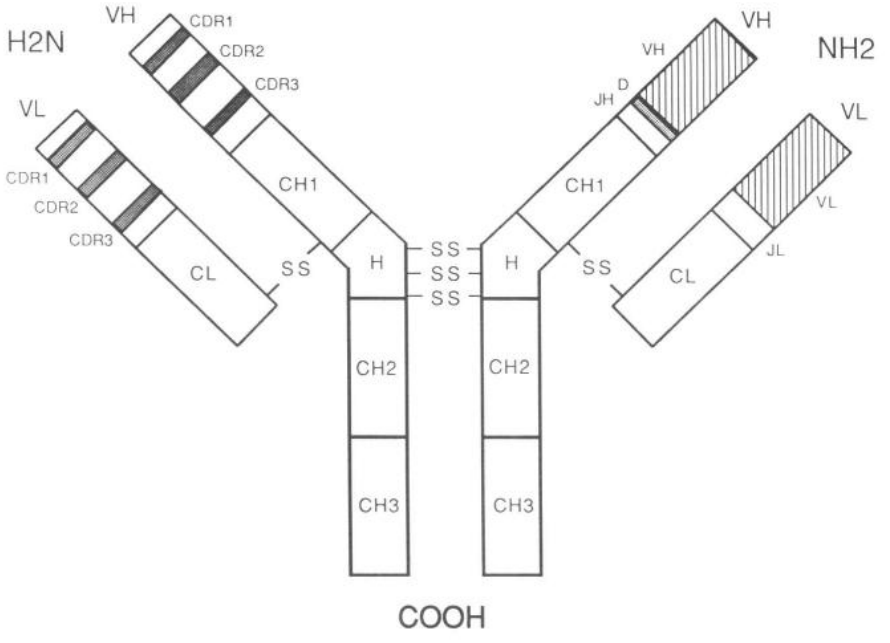

Figure 1. Structure of a mouse IgG molecule. The V_H, C_H, V_L and C_L homology domains are shown as boxes and the hinge region is denoted H. Only the disulfide linkages that join H and L chains are shown. The approximate boundaries of CDR regions and of sequences encoded by V_H, D, J_H, V_L and J_L segments are indicated by different shadings.

and that are approximately 110 amino acids in length (5). Each domain forms a conserved structure known as the 'antibody fold', which is stabilized by an internal disulfide linkage that forms a loop of about 65 amino acids (6). The amino-terminal domain of H and L chains comprises the V region, which is 107 – 118 amino acids in length (*Figure 1*) (1). Within the V region are three areas of greatest sequence variability (hypervariable regions) that are separated by regions of relatively constant amino acid sequence (framework regions) (7). This overall structure has been conserved within H and L chain V regions among species as diverse as humans and sharks. H and L chain hypervariable regions together form the potential antigen-binding site and are therefore referred to as complementarity-determining regions (CDRs) (8).

Amino acid sequences within the remainder of H and L chains (the constant region, *Figure 1*) are relatively constant, but vary among different constant (C) region types (isotypes) that were originally classified according to reactivity to specific antisera (7). H chain C region isotypes define the classes and subclasses of mammalian Igs (4). Thus, in the mouse, the eight Ig classes and subclasses of IgM, IgD, IgG_3, IgG_{2a}, IgG_1, IgG_{2b}, IgE and IgA are defined by H chain isotypes that are denoted by their respective Greek letters (e.g. μ, δ etc.). Mammalian H

chain C regions mediate immunologic effector functions, such as complement fixation, placental transfer and binding to cell surface Fc receptors that are specific to particular isotypes (9). Functional differences have not been identified for the two isotypes of mammalian L chains, kappa (κ) and lambda (λ). H chain C regions contain between two and four domains (CH_1, CH_2 and CH_3 in *Figure 1*) that are distantly homologous to each other and each L chain C region domain (CL; *Figure 1*). Certain H chain isotypes also contain a hinge region (Figure 1) that may facilitate antigen binding by increasing H chain flexibility (10). H chains can be produced in either a membrane-bound or a secreted form (e.g. μ_m or μ_s for μ H chains), which are distinguished by specific sequences at their carboxy-termini (11–13). A membrane-bound Ig molecule is monomeric, but secreted Ig molecules are composed of between one and five monomers, depending upon their class (4).

2.2 Antibody production is triggered by clonal selection

Each B lymphocyte displays on its surface a unique species of membrane-bound Ig that functions as an antigen receptor. The clonal selection theory (*Figure 2*) states that the immune response to an antigen is initiated when that antigen is recognized by a B lymphocyte surface receptor of a pre-existing specificity (14). When a particular B lymphocyte binds antigen, it is induced to proliferate. B lymphocytes within this selected clone can differentiate into plasma cells, which produce large amounts of the selected Ig molecule in secreted form as antibody. This mechanism requires that each Ig molecule produced by a given B lymphocyte display an identical set of antigen-binding specificities. Otherwise, antigenic triggering of a B lymphocyte clone might lead to production of multiple different and potentially harmful antibodies (e.g. autoreactive), and production of the selected antibody might be inefficient. Each B lymphocyte produces Ig molecules with identical antigen-binding specificities because it expresses as Ig the products of only a single H and a single L chain gene, a phenomenon referred to as allelic exclusion (15,16). As is discussed in detail in Section 8, Ig gene assembly appears to be regulated specifically to impose H and L chain allelic exclusion (17).

3. Model systems for studying B cell differentiation

In mammals, B lymphocytes are generated in the liver during fetal life, but shortly after birth the bone marrow becomes the site of lymphopoiesis and remains so throughout adult life (18). In the bone marrow, B lymphocytes arise from a small self-renewing population of stem cells. These lie within a matrix of stromal cells that produce factors which immature B lymphocytes require for growth. Ig genes are assembled during this differentiation process, which generates 'virgin' B lymphocytes

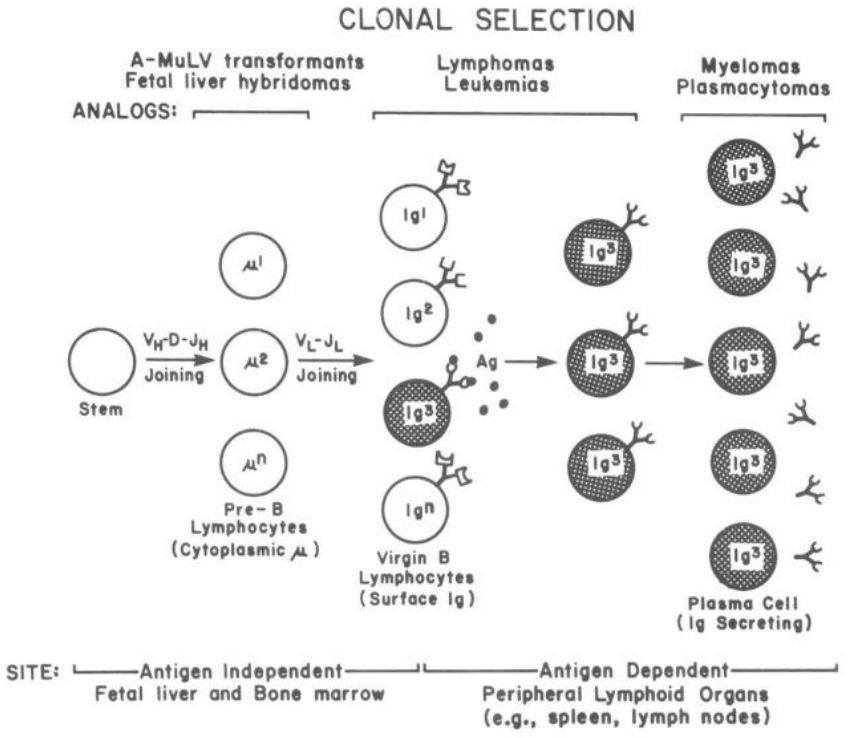

Figure 2. Clonal selection and B lymphocyte differentiation. The antigen-independent stages of differentiation generate a vast number of independent B lymphocyte clones, each of which bears a surface receptor with a unique antigen-recognition site. Binding of antigen by the receptor of a particular clone triggers clonal expansion and differentiation into plasma cells which secrete antibody molecules that display the same antigen-recognition site. Tumor cell analogs of the various stages of B lymphocyte differentiation are indicated. Ag = antigen.

that produce surface Ig and have not yet been stimulated by antigens. Virgin B lymphocytes migrate out of the bone marrow to peripheral lymphoid tissues, such as the lymph nodes and spleen, where antigen contact and further differentiation may occur.

Availability of tumor cell analogs of different stages in B lymphocyte differentiation has made it possible to study the process by which Ig genes are assembled (*Figure 2*). Myelomas and plasmacytomas, which represent terminally differentiated Ig-secreting cells, have been utilized for studies of the structure and expression of assembled Ig genes (1). Fetal liver hybridomas, which are prepared by fusion of immature B lymphocytes from fetal liver with myeloma cells, have provided a representation of Ig gene rearrangements in differentiating B cells within the phenotypic context of a myeloma (19–21). A more complete picture of the various stages in the Ig gene assembly process has been provided by studies of B lineage cell lines isolated by transformation with Abelson murine