Essential Immunogenetics

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The production of this book has been more time-consuming than we could have imagined at the outset. The exponential increase in knowledge of immunogenetics that has occurred during the gestation period of this volume has necessitated much reappraisal along the way and many interim chapters became obsolete. The forbearance of the publishers has astonished us. We especially thank Per Saugman (who started the project), Peter Saugman (who revived it) and John Robson who saw it through to a conclusion. The hard work of transforming our text into print was undertaken, initially by Alison Walsh and latterly by Deborah Thompson and Jane Andrew. Oxford Illustrators skilfully converted our rough artwork into attractive figures and were most patient as we requested modification after modification. Many of our scientific colleagues have kindly offered advice and corrected some of our worst errors, although we must, of course, be held responsible for the finished product. We are particularly grateful to Roland Levinsky, James Mowbray, Marcus Pembrey, Sue Malcolm, Christine Kinnon, Ken Reid, Bob Sim and Duncan Campbell. Other colleagues have supplied illustrative material and are acknowledged in the text.

Contents

Part 3 Cells of the Immune System

Part 4 Genetic Aspects of Immunological Diseases

Chapter 1
Introduction

Immunogenetics can appear formidable to the newcomer. This book is intended to make the subject more approachable. We hope that *Essential Immunogenetics* will provide an entry to the field for those who may become specialists in the future, and for those already trained in many aspects of biology, for whom the discipline of immunogenetics has much to offer. For the student of basic science or of medicine, *Essential Immunogenetics* provides a genetic description of immunology and an illustration of the application of immunogenetic methods to the immune system and points the way to broader applications for immunogenetics.

Immunogenetics is much more than a collection of antisera defining antigenic variation or a list of genes. The immunogenetic method has provided a revealing view of the immune system. It also offers an approach to the description and understanding of many other biological systems. With this in mind we have tried to indicate the importance of methodology as a way of asking questions, but without dwelling on technical details.

We have chosen to limit the book to an immunogenetic introduction to immune systems because of the importance of those systems both in medicine and in the immunogenetic method. All aspects of the immune system are of course controlled by genes but the revelation of the working of the immune system has occurred at various levels. The division of this book into four parts reflects the way in which immunogenetics developed, some areas having been extensively studied at the level of the phenotype, either at the protein level or at the cellular level before any genetic input occurred. Other areas were studied at the level of whole animal genetics before molecular definition of phenotypes. Latterly, application of the recombinant DNA techniques of molecular genetics has revolutionized all molecular biology so that many areas of immunogenetics are now being revealed directly at the DNA sequence level.

Recombinant DNA technology was born of bacterial genetics and it is clearly a major revolution in the scientific approach to genes and gene expression. The molecular genetic method as applied to immunoglobulins is introduced in Chapter 7.

Monoclonal antibody technology is a product of the study of immunogenetics, and applications of monoclonal antibodies are making a major impact on the study of the genetics of the immune response and also on the immunogenetic method. Essentially, if a protein molecule can be defined by a monoclonal antibody then its expression and function can be studied; enough protein can be isolated by immune precipitation for a partial amino acid sequence to be determined at the micro level; from an amino acid sequence a DNA probe (albeit redundant) can be defined and chemically synthesized and the gene encoding the protein can be isolated, usually starting from complementary DNA (see Chapter 7).

Clearly the monoclonal antibody approach is a powerful way to enter any biological system. This is especially so since there is no need to have the original protein molecule in pure form at the outset.

These recent methods using recombinant DNA and monoclonal antibodies are moving our knowledge of immunogenetics forward at a rapid pace. In *Essential Immunogenetics* we have tried to cover the historical milestones so that the important findings and hypotheses illustrate how immunogenetics has progressed. Inevitably parts of the book will be out of date by the time it reaches the press. Our intention is that the book will remain valid as a frame of reference into which future findings can be slotted either as new pieces in the jigsaw or occasionally changing a piece here or there.

The book is divided into four parts that focus respectively on *proteins*, *genes*, *cells* and *diseases*. This somewhat artificial division is meant, as explained above, to reflect the development of the field.

In Part 1, 'Proteins of the immune system', we have chosen to cover immunoglobulins, major histocompatibility complex (MHC) proteins and the proteins of the complement system as being those molecules that are basic to the understanding of immunogenetics and immune responses. Also these are molecules where a biochemical description of the proteins involved preceded or paralleled an understanding of their genetics. By

contrast, differentiation antigens or the T cell antigen receptor are molecules for which our understanding developed from cellular immunology and in some cases progressed directly to molecular genetics with little basic knowledge of the proteins involved. Therefore, differentiation antigens are dealt with in a separate chapter (12) later in Part 3, and the T cell antigen receptor is dealt with in Chapter 13 on the genetics of cell–cell interactions.

The second part has chapters on the classical genetics of each of the major molecules of the immune system considered in Part 1, i.e. the immunoglobulins, MHC antigens and proteins of the complement system. This is intended to give the reader a basic understanding of the approaches of classical immunogenetics to the immune system and by extrapolation to any other system. In addition to the presentation of the classical genetics of immunoglobulins in Chapter 5, a separate chapter (6) is devoted to the nature of antibody diversity, a special and fascinating aspect of immunoglobulin genetics. Following on the classical genetics of each of the major molecules is a discussion of the molecular genetics of that particular system. The immunoglobulins come first, and included in Chapter 7 on molecular genetics is a discussion of the methods of molecular genetics and the philosophy. Thereafter, the expression of immunoglobulin genes is discussed (Chapter 8), particularly because of the special controls involved in immunoglobulin gene expression and also as a vehicle for introducing the subject of gene expression in general as it will be of increasing relevance in immunogenetics. Chapter 9 on the classical genetics of the MHC, as revealed initially by transplantation of tumours and skin grafts, is followed by a chapter (10) on the molecular genetics of the MHC. A single chapter (11) covers the classical and molecular genetics of complement.

In Part 3, differentiation antigens are dealt with in Chapter 12 in the context of cells of the immune system. This is an essential base to the next chapter which deals with cell–cell interactions. The latter chapter offers a genetic description of cellular immunology. The complexities of cellular immunology have been more clearly described by the application of immunogenetics than by any other approach. The use of such simple but original immunogenetic methods as the introduction of inbred strains and the use of polyvalent alloantisera made possible important strides in our

understanding of cellular immunology. The more recent use of monoclonal antibodies and the cloning of relevant genes is revealing a control system of apparent complexity, but one which may have many parallels in other differentiation systems.

Finally, Part 4 deals with genetic aspects of immunological disease. This section introduces the use of immunogenetics in the clinical situation. Immune deficiency diseases, paraimmunoglobulinopathies and autoimmune diseases are dealt with in the three separate chapters.

These areas have been selected because they highlight three different ways in which immunogenetics interacts with clinical immunology. The chapter (14) on primary immunodeficiency disorders, whilst revealing the paucity of information on the nature of the underlying defect for most of these diseases, also attempts to show how the situation may soon be transformed by the application of DNA hybridization technology. Some of the rare defects of complement proteins considered in the same chapter have given unexpected insights into the normal physiological control mechanisms regulating this complex system of proteins. The paraimmunoglobulinopathies, discussed in a separate chapter (15) are of particular historic interest because it was such studies of aberrant proteins that yielded early evidence supporting the concept of multiple genes controlling the production of a single immunoglobulin peptide chain.

The third chapter in this section (Chapter 16) discusses various autoimmune disorders, many of which show association, and in some cases linkage, with particular alleles of MHC antigens. Although the significance of these findings remains an enigma, enough is already known in some instances to guide physicians towards a more accurate assessment of a particular patient's prognosis and in other cases the choice between alternative modes of therapy may be decided by such information.

Although we have divided this book into parts and chapters for the reasons stated we would not wish the reader to miss the underlying theme of the immunogenetic method and the more fundamental genetic relationships within the immunogenetics of the immune system. It has been known for some time that immunoglobulin genes constitute a multigene family, i.e. a group

of homologous genes with similar functions. The homology link within this multigene family is a prototype gene encoding a single immunoglobulin domain. This single domain gene encodes about 110 amino acids; these 110 encoded amino acids fold in a characteristic pattern of β-pleated sheets to give the so-called immunoglobulin- or antibody-fold that characteristically is fixed by a covalent disulphide bond closing a loop about 60 amino acids long, centrally placed in the domain. Historically it was the sequencing of human β_2-microglobulin, a small protein of then (1973) unknown function, that revealed it to be homologous to the immunoglobulin domain. Later it was found that β_2-microglobulin is the light chain of class I MHC antigens and that it interacts with the one domain (α_3) of the heavy (α) chain of class I antigens, that is also an immunoglobulin homologue. This extended the evidence deriving from the immunoglobulin molecules that immunoglobulin domains readily pair with themselves or other immunoglobulin domains. The family of proteins that are made up entirely or in part of homologous immunoglobulin-like domains is now known to include, in addition to all immunoglobulins, β_2-microglobulins, MHC class I α chains, MHC class II α and β chains, the antigen-specific T cell receptor chains α, β and γ, the poly immunoglobulin receptor (originally identified as secretory component of immunoglobulin A antibodies

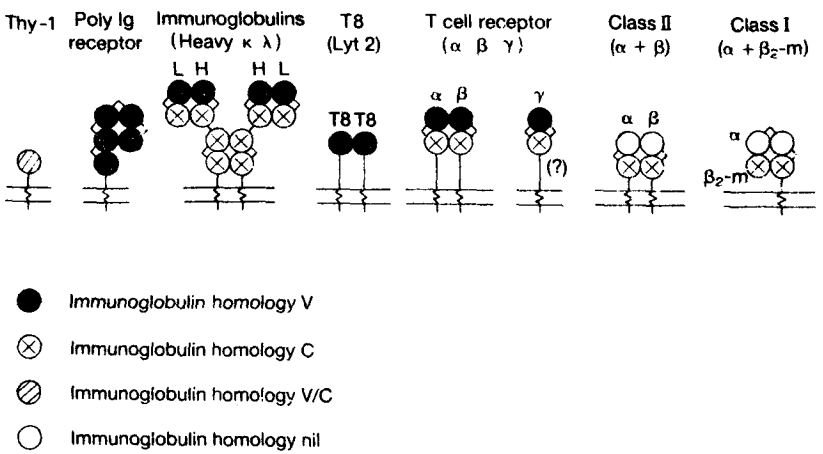

Fig. 1.1 The immunoglobulin supergene family of proteins. Each is shown as a membrane protein with a transmembrane hydrophobic peptide (∿). The domains are coded as indicated in the key. Other members of this supergene family are known to exist.

in external secretions), Thy-1 (an alloantigenic marker on murine T cells) and T-8 (a differentiation antigen on human T cells) (Figure 1.1). Further sequences may well reveal other members of this supergene family. Determination of the exon-intron structures of genes in the immunoglobulin supergene family has shown that each immunoglobulin domain is encoded by a separate exon (Figure 1.2). This immunoglobulin domain exon has apparently been duplicated, mutated, moved to new locations and the products subjected to selective pressures to yield the supergene family as we now know it (see Table 1.1). In the special cases of immunoglobulin and T cell receptor V genes a somatic rearrangement mechanism has evolved that allows V genes to be expressed with C genes using a DNA joining mechanism. A site-specific recombination process is involved that depends on highly conserved recognition sequences

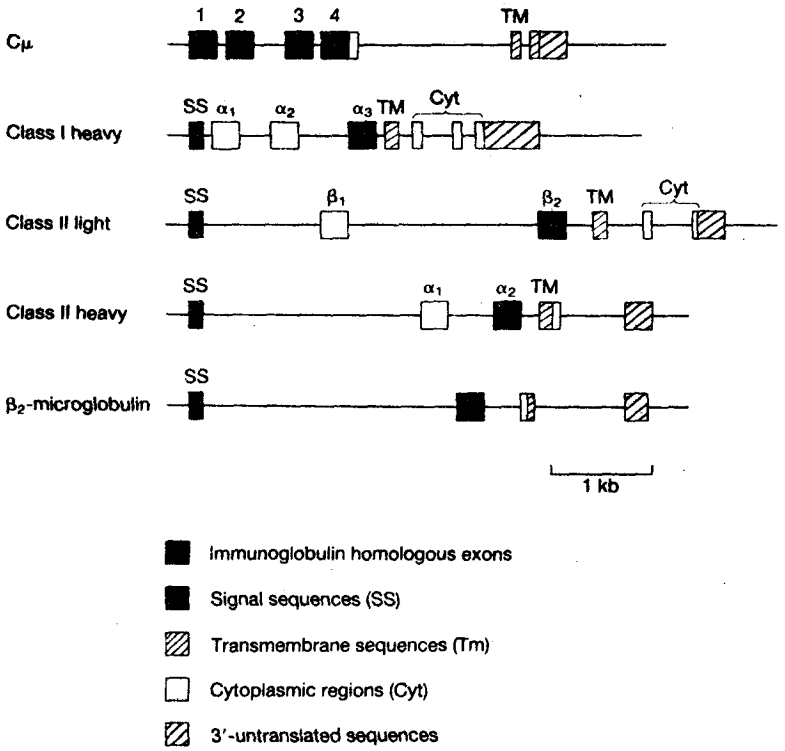

Fig. 1.2 The exon-intron structures of five genes of the immunoglobulin supergene family. Exons are designated as shown in the key.

Table 1.1 Chromosomal location of some loci of the immunoglobulin supergene family

Locus	Phenotype	Chromosome number	
		Mouse	Human
Igl	λ chain	16	22
Igk	κ chain	6	2
Igh	H chain	12	14
MHC	class I α	17	6
	class II α + β	17	6
β_2M	β_2 microglobulin	2	15
Tcra	T cell receptor α chain	14	14
Tcrb	T cell receptor β chain	6	7

common to the flanking regions of all rearrangeable elements in the immunoglobulin and T cell receptor multigene families. This mechanism allows the use of extensive repertoires of V genes and the creation of extra diversity by a lack of precision in the joining mechanism. Thus, the nature and origin of antibody diversity, one of the most fascinating challenges of immunogenetics, can now be explained in molecular genetic terms (see Chapters 7 and 8).

MHC antigens were originally recognized and defined as the major transplantation antigens (H-2), involved in rejection of tissue grafts between mice; a homologous set of MHC antigens (HLA) were identified in man. In both mice and men the extent of polymorphism of MHC antigens was unprecedentedly high. It is what are now called class I MHC antigens that are mainly responsible for rapid graft rejection and that are extremely polymorphic. Immune associated (Ia) antigen genes, now termed class II MHC antigens, were also mapped to the MHC region. The relationship between class II (Ia) and class I MHC antigens was shown by a series of experiments that demonstrated that T cells see foreign antigen presented in association with MHC antigens. Thus class I and II MHC antigens are key molecules in immune recognition. Other differentiation antigens originally identified as serological markers of T cell types are now known to function in the cell–cell interactions necessary for immune responsiveness.

A link between the MHC and complement exists in that certain complement genes map in the MHC. The complement system is an enzyme cascade mechanism activated by immune complexes and capable of lytic

action on bacteria or foreign cells. In the complement cascade enzymic activity is generated by proteolytic cleavage of proenzymes but these same cleavage processes also generate many mediators of inflammation, in particular the multiple products of C3 cleavage. The twenty or so proteins constituting the complement system include at least three minifamilies of proteins showing structural and functional relationships (e.g. Clr-Cls, C2-Factor B, C3-C4-C5) and these are discussed in Chapter 4.

A hint of another multigene family has recently emerged with the publication of sequence homologies between: (1) the macrophage cell-surface glycoprotein (MAC-1 = CR3 receptor) which interacts with particles coated with C3bi; (2) the T and NK cell-surface protein LFA-1; and (3) the α-interferons. It is suggested that such molecules may constitute a very ancient group of genes encoding proteins involved in cell interactions of an antigen non-specific nature.

A new immunopharmacology is now rapidly emerging which encompasses the active peptides of the complement and kallikrein systems, polypeptide transmitters such as the lymphokines, and also low molecular weight molecules such as the prostanoids. Immunogenetics has a key part to play in the study of these lymphokines and receptor proteins for various transmitter molecules.

When the subject was in its infancy, Landsteiner's observations on blood group antigens were no doubt considered by many to be esoteric but they were, of course, destined to be of enormous practical importance. Now that the powerful resolution of the new technologies is slowly revealing more and more detail of the genome it is safe to predict that applications of immunogenetics in the clinic will continue to increase over the next few years. Much effort will be directed towards both prenatal diagnosis for a greater range of lethal conditions and reliable heterozygote identification. These tangible practical advantages for mankind may be attained much sooner than could possibly have been envisaged ten years ago and it is appropriate that the last part of this book should deal briefly with some of the clinical areas most likely to be affected.