

# METHODS IN MOLECULAR BIOLOGY™

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# Immunogenetics

## Methods and Applications in Clinical Practice

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## Preface

The ability of mice to distinguish “self” from “nonself” and recognize foreign organisms or tissues (an immune response) was found to be under genetic control by the seminal experiments of Peter Gorer in 1936 and his description of the H-2 system. This was later extended to the human with the discovery of the HLA system by Jean Dausset in 1958. Since that time the study and knowledge of the genes controlling such immune responses in the human has undergone a rapid expansion. These studies have been facilitated by the development of a range of molecular DNA techniques and a remarkable degree of international collaboration.

The HLA molecules, encoded by a highly polymorphic set of genes located with the major histocompatibility complex (MHC) on Chromosome 6, are important regulators of the immune response through mediating antigen presentation and interaction between key immune mediating cells. They are also the major histocompatibility barriers to transplantation, which is the clinical paradigm of the self vs. nonself concept. Within the MHC, one of the best characterized regions of the human genome, are a number of other gene clusters involved in controlling aspects of the immune response. These encode various accessory molecules such as TNF, components of the classical complement pathway, and heat shock proteins. In addition outside the MHC, many other genes encode components which can act as histocompatibility antigens or are involved in the function of the immune system. These include genes encoding polymorphic tissue-specific peptides presented within the context of HLA molecules which have been shown to give rise to a series of minor histocompatibility antigens which are barriers to allogeneic transplantation but which can also be exploited as targets for immunotherapy. Those polymorphic genes encoding the immunoglobulin and the T cell receptor molecules, important mediators of humoral and cell-mediated adaptive immune responses, also play a role in regulation of immune responses. The immune response is also mediated by a complex of cytokines and cell receptors and polymorphism in the structural and regulatory elements of the genes have been demonstrated and shown to have functional correlates. More recently a cluster of genes encoding receptors on NK cells, including the killer immunoglobulin-like receptors (KIR), which regulate NK cell activation largely through interaction with ligands encoded within the MHC, have been described and been shown to be important determinants in transplantation outcome and in conferring disease susceptibility.

It is now recognized that this diverse range of gene systems involved in the control of the immune response has been shown to be important in many aspects of clinical practice. As a result many new molecular and cellular methods have been developed for identifying these genes and their polymorphisms, and immunogenetic laboratories specializing in these methods have developed to support transplantation and other clinical programs. This volume focuses on such methods. The scope is exclusively for human clinical practice and the emphasis is on those assays which are of established or potential clinical utility and are likely to be included in the repertoire of tests provided by a routine diagnostic and service

laboratory. In the tradition of *Methods in Molecular Biology* series, the methods provide details of the materials and equipment required and a step-by-step description of the laboratory method. Details of the critical factors in the performance and interpretation of these assays and other practical tips are provided through a series of Notes which we trust the reader will find particularly helpful.

The characterization of the HLA and other genes within the MHC is important to the routine diagnostic immunogenetics laboratory, particularly in supporting solid organ and hemopoietic stem cell transplant programs. We have included chapters describing HLA typing by molecular techniques including sequence-specific oligonucleotide hybridization (SSO) and sequence-specific priming (SSP) methods, solid phase bead-based assays and by direct DNA sequencing, and the use of specialist software for HLA allele assignment. An additional chapter focuses on a number of molecular methods adapted for the typing of specific HLA alleles which are of diagnostic utility in disease diagnosis and in drug hypersensitivity reactions. In addition to typing of the classical HLA molecules, we have included chapters describing typing of other MHC genes including the nonclassical HLA molecules HLA-E and HLA-G, the complement component C4, the MICA genes, and a panel of single nucleotide polymorphisms (SNPs) throughout the MHC. Methods for detecting the many non-MHC minor histocompatibility antigens which have been described and for detecting the functionally important polymorphisms in a range of cytokine genes are also included.

The detection and characterization of HLA antibodies directed at epitopes found on donor HLA molecules and the more recently described antibodies directed at MICA epitopes is known to be important in organ transplantation, and the recent development of solid phase bead-based assays to detect these antibodies has led to a new understanding of the role of HLA and MICA antibodies in organ transplant rejection. Chapters describing the methods for the detection of donor-specific antibodies using the traditional, but still gold standard, complement dependent cytotoxicity and the more sensitive flow cytometry-based crossmatches and bead-based antibody detection assays are included. Assays for the detection of T cell-mediated reactivity against alloantigens either directly or indirectly through the cross-reactivity of viral-specific T cells—so-called heterologous immunity—are also described.

The highly polymorphic KIR gene cluster, which encodes a family of NK cell receptors which are important in controlling NK cell function, has generated considerable interest recently. Methods detecting and characterizing the polymorphism of this complex gene system including PCR-SSP and direct sequencing and for detecting NK cell alloreactivity including a flow cytometry-based assay are described. Alloreactive NK cells have the potential to be effective therapeutic agents, and a detailed method for the clinical production of such cells is provided.

In addition to the method chapters we have included a series of overview chapters highlighting aspects of gene function or various methods available for their study. These include reviews of the MHC complex, the KIR complex, the human immunoglobulin allotypes, as well as reviews of the methods for the detection of alloreactive NK cells and the detection of HLA antibodies by solid phase assays. Because of their expertise and understanding of the complexity of the HLA system, many immunogenetics laboratories provide specialist advice to clinicians in their search for suitable unrelated hemopoietic stem cell donors. We have therefore included a chapter which provides a detailed and practical guide to cost-effective strategies for undertaking such searches.

Immunogenetic data is complex and the genes are highly polymorphic and have been extensively studied. These features have meant that extensive databases of such genes and

the polymorphisms are now available. Included are chapters on methods for the establishment and management of immunogenetic databases and which describe the use of specialist HLA, immunoglobulin, and T cell receptor polymorphism databases. In addition two chapters are included which review the analytical methods available for the study and measurement of human population diversity and the identification, quantitation, and mapping of disease susceptibility genes respectively. These chapters also provide a number of worked examples and we trust the readers will find them particularly helpful.

We would like to thank all the contributors to what we believe is an outstanding collection of manuscripts which we are sure will be widely read and used by the immunogenetics community. These contributions represent many hours of work and the sharing of detailed methods and helpful tips gained by many years of experience in the field. We are delighted that they have been prepared to share this information with the wider scientific community.

We would also like to thank John Walker and Humana Press for inviting us to edit this volume of the very successful *Methods in Molecular Biology* series and for his excellent editorial guidance. We trust the final product has rewarded his trust in us.

Finally, we would like to express our special thanks to Natalie Caldwell for her outstanding clerical and editorial assistance. Her enthusiasm and skill has been invaluable as we have worked through the numerous editorial tasks.

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