
Preface

The aim of *MHC Protocols* is to document protocols that can be used for the analysis of genetic variation within the human major histocompatibility complex (MHC; HLA region). The human MHC encompasses approximately 4 million base pairs on the short arm of chromosome 6 at cytogenetic location 6p21.3. The region is divided into three subregions. The telomeric class I region contains the genes that encode the HLA class I molecules HLA-A, -B, and -C. The centromeric class II region contains the genes encoding the HLA class II molecules HLA-DR, -DQ, and -DP. In between is the class III region, originally identified because it contains genes encoding components of the complement pathway. The entire human MHC has recently been sequenced (*1*) and each subregion is now known to contain many other genes, a number of which have immunological functions.

The study of polymorphism within the MHC is well established, because the region contains the highly polymorphic HLA genes. HLA polymorphism has been used extensively in solid organ and bone marrow transplantation to match donors and recipients. As a result, large numbers of HLA alleles have been identified, a process that has been further driven by recent interest in HLA gene diversity in ethnic populations. The extreme genetic variation in HLA genes is believed to have been driven by the evolutionary response to infectious agents, but relatively few studies have analyzed associations between HLA genetic variation and infectious disease, which has been difficult to demonstrate. In distinction, a huge number of studies have described associations between HLA alleles and human diseases, many of which are thought to have an immunological component (such as rheumatoid

arthritis, diabetes mellitus, and multiple sclerosis). This enormous literature was initially collated by Tiwara and Terasaki (2) and has since continued to grow.

The study of MHC disease associations has also stimulated the analysis of genetic variation within other HLA region genes. Because of strong linkage disequilibrium, it has been reasoned that for some diseases the true susceptibility genes may not be the genes encoding the HLA molecules, but other nearby genes. Some of these, such as the *TAP* and *LMP* genes, are obvious candidate susceptibility genes for immunological disorders because of their role in the normal immune response. Similarly, linkage studies of MHC-associated disorders have stimulated the characterization of microsatellite markers and, more recently, single nucleotide polymorphisms (SNPs).

The study of human MHC polymorphism has also contributed to our understanding of many other areas of biology, including cellular immunobiology, virology, and genome evolution.

The first part of *MHC Protocols* describes electronic databases designed to catalog HLA region genes and their alleles. Chapter 1 describes the methods involved in accessing HLA sequence data from these databases and Chapter 2 describes one of these databases, 6ace, in more detail. Inevitably, these databases will continuously change as, for example, the number of identified HLA alleles increases and genomic information is better understood. Similarly, their underlying software and computer interfaces will become more refined. However, the underlying principles governing the use of these databases are likely to remain constant.

The second part describes DNA-based protocols for the study of polymorphism in HLA class I and II genes. The part begins with two chapters describing older methodology based on restriction fragment length polymorphism analysis. Although these techniques have now been superseded, they may still have a place in smaller research projects. Chapters 5 through 7 describe the SSO and SSP typing methodology that is now in use in most tissue typing laboratories. Chapter 8 describes a recently developed technique, reference strand-mediated conformation analysis, and Chapter 9

describes sequencing-based methodology. Because the number of HLA alleles is constantly increasing (as shown in Chapter 1), it is impossible for any of these chapters to comprehensively describe the analysis of all possible alleles. Nevertheless, the methodology is readily adaptable to new alleles.

Chapters 10 and 11 focus on the nonclassical HLA genes, HLA-E, HLA-G, and HLA-DM. Although these genes encode molecules that are structurally similar to class I and II HLA molecules, they differ in a number of key respects: their cell and tissue expression is unique, they exhibit only limited polymorphism, and they have developed specialized biological functions.

The third part of *MHC Protocols* describes methods for the study of polymorphism in non-HLA MHC genes. Although a large number of these genes have now been identified, we have chosen to concentrate on a small number that are of particular interest, either for functional reasons or because they have been associated with human disease. Chapter 12 describes polymorphism in the genes *TAP1* and *TAP2*. The products of these class II region genes bind noncovalently to form the TAP transporter, which pumps peptides from the cytoplasm into the lumen of the endoplasmic reticulum prior to binding by class I molecules. Chapters 13 and 14 concentrate on two class III encoded components of the complement pathway, C2 and C4, and Chapter 15 describes the TNF gene. Chapter 16 describes *MICA*, a recently described gene that has homology to HLA genes and appears to be highly polymorphic.

Finally, Chapter 17 details a number of microsatellites within the human MHC. Clearly, it is possible to identify many more MHC microsatellites, especially now that the entire human MHC genomic sequence is available. Furthermore, the development of MHC SNPs may eventually supersede the use of microsatellites. Nevertheless, the markers described in this chapter represent a well-characterized core set that can be used for medium resolution analysis of the region.

In a fast developing field such as MHC genetics, it is impossible to produce a written text that will remain up to date. However, we hope that the protocols described in *MHC Protocols*

will provide a secure base for those who wish to study genetic variation in this fascinating part of the human genome.

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References

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2. Tiwari J. L., Terasaki P. I. (1985) *HLA and Disease Associations*. Springer Verlag, New York, NY.



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