Summary

The major histocompatibility system, or HLA (Human Leukocyte Antigens), is encoded by chromosomal region 6p21.3 and includes numerous immune genes that are involved in antigenic peptide binding and presentation to T lymphocytes. HLA-peptide-T cell interaction (via its receptor) is necessary for T cell activation and initiation of the adaptive immune response. The classical HLA genes are characterized by high degree of diversity and are distinguished into class I (HLA-A, -B and –C) and II genes (DPA1, DPB1, DQA1, DQB1, DRA and DRB1). HLA class II molecules consist of two polypeptidic chains, alpha (α) and beta (β), of similar size. The heterodimer HLA molecule forms an extracellular region, a transmembrane region and a cytoplasmic tail. In the extracellular region, the majority of polymorphic sites among HLA class II alleles are observed. Specific binding pockets are formed along the entire length of the peptide-binding cleft of class II molecules, into which anchoring peptides of the antigen can interact with the HLA molecule. These interactions include the formation of hydrogen bonds and/or ion bridges, which stabilize the fitting of the peptide into the groove. The antigenic repertoire of a given molecule is determined by the amino acids found in the groove and their physiochemical properties. Amino acid positions as well as pocket variants have been associated with various diseases, such as autoimmune conditions and infections.

One of the infectious diseases that are associated with HLA alleles is West Nile virus disease. West Nile virus is a zoonotic neurotropic arbovirus of the Flaviviridae family that emerged worldwide as a major cause of viral encephalitis in 2010. In the period 2010-2013, cases of diseases with neuroinvasive symptoms were reported in several countries in Europe. In Greece, 1419 cases of WNV infection have been reported since 2010. The majority (1011 patients) developed neuroinvasive symptoms, while 200 deaths were recorded. The higher proportion of patients with central nervous system involvement, which is a rare manifestation of West Nile disease, reflects the number of unreported subclinical or asymptomatic patients. The majority of WNV infections are asymptomatic while approximately 1/5 of the infected individuals develop WNV fever. An even smaller proportion (<1%) develop neuroinvasive disease which includes meningitis, encephalitis, and acute flaccid paralysis (AFP)/poliomyelitis. The risk for developing a more severe form of WNV disease increases among immunosuppressed individuals, organ transplant recipients and the elderly.

In this thesis, we focused on the pocket variants that are found among all HLA class II alleles and have a determinant role in peptide-cleft interaction. Our goal was to investigate the patterns of selection of the pocket variants and their combinations, found in human

population and determine how nucleotide polymorphism is reflected on the actual peptide binding region. This analysis highlighted the existence of amino acid constraints and preferences in the groove pockets, leading to the establishment of only a fraction of the possible variants in the population. The role of each pocket in the binding of antigenic peptides seems to function as a filter for the "freedom" of maintaining multiple variants, both at pocket and sequence level of the peptide binding region. The role of HLA class II polymorphism in West Nile Virus disease was also studied. More specifically, we made an analysis of HLA class II allelic frequencies in an infected population from Greece compared to control populations, in order to investigate the role of class II locus in infection and disease severity. HLA-DQA1 and -DRB1 alleles associated with the disease were identified, providing some evidence that HLA class II is involved in the response to WNV infection. Furthermore, protein models of HLA-DRB1 alleles that were identified in our population were generated, in order to study the effect of polymorphic sites on the structure of the peptide binding cleft. Finally, we examined the role of the binding pockets of HLA-DPA1, -DQA1 and–DRB1 in the severe form of the disease, in frequency and protein level. Amino acid signatures associated with either susceptibility to or protection from neuroinvasive WNV disease were identified.