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# HLA DR and DQ interaction in myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis in HLA class II transgenic mice

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**Abstract**

Multiple sclerosis (MS) is shown to be associated with the HLA class II genes. The presence of strong linkage disequilibrium between HLA DR and DQ molecules in humans makes it difficult to identify the individual roles of HLA DR and HLA DQ molecule in MS pathogenesis. To address this problem, we used HLA class II transgenic mice and the experimental autoimmune encephalitis (EAE) model. Administration of recombinant MOG (rMOG) induced severe inflammation and demyelination in the central nervous system (CNS) of HLA DRB1*1502 mice (60%), whereas no disease was observed in HLA DQB1*0601(0%) and mild disease was observed in DQB1*0302 mice (13%). Lymphocyte proliferation was blocked by anti HLA antibodies, confirming that the rMOG was functionally presented by the HLA molecules. Introduction of DQB1*0302 into DRB1*1502 mice resulted in the development of chronic progressive clinical disease characterized by severe inflammation and demyelination (90%) in response to immunization with rMOG, whereas mild disease was observed when DQB1*0601 was introduced in DRB1*1502 mice (30%). This would suggest that the presence of more than one susceptible allele, namely HLA DRB1*1502 and DQB1*0302 resulted in enhanced severity of disease in the DRB1*1502/DQB1*0302 mice, possibly due to the additional selection and expansion of potential autoreactive T cells. The use of defined single and double HLA transgenic mice may reveal the intricate interactions between class II molecules in human disease.


*Keywords:* EAE/MS; HLA class II; Transgenic; Pathogenesis; Myelin oligodendrocyte glycoprotein

## 1. Introduction

Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) characterized by chronic inflammatory demyelination, leading to progressive loss of neurological function (Steinman, 1996). Although the etiology and pathogenesis of MS is poorly understood, both environmental and genetic factors are suggested to play an important role. It is hypothesized that CD4+T cells play an essential role in the pathogenesis of MS by targeting CNS antigens, such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) (Hellings et al., 2002). Experimental autoimmune encephalomyelitis (EAE), a model of MS, can be induced experimentally either by immunization with PLP, MBP or MOG, or by adoptive transfer of myelin antigen-specific activated CD4+T cells in susceptible strains of laboratory animals(Gold et al., 2000).

MOG is a quantitatively minor component of CNS myelin found only in mammals and is highly conserved across species. MOG is the only autoantigen known to induce both an encephalogenetic T cell response as well as a demyelinating antibody response in rodents and non-human primates with EAE (Iglesias et al., 2001; Weissert et al., 1998). This is in contrast to other myelin antigens such as

*Abbreviations:* EAE, experimental autoimmune encephalomyelitis; LNC, lymph node cells; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PBMC, peripheral blood mononuclear cell; PLP, proteolipid protein; rMOG, recombinant MOG; SI, stimulation index.

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PLP and MBP, which are encephalogenic but unable to induce demyelinating antibody response. Large concentric areas of macrophage infiltration, autoantibody deposition, and vesicular demyelination are characteristic of MOG-induced EAE (Genain et al., 1999; Kerlero de Rosbo et al., 1997; Raine et al., 1999). In MOG-induced EAE this combination of immune effector mechanisms reproduces demyelinating pathology seen in a subset of MS patients (Lucchinetti et al., 2000). Recent studies in MS patients have indicated that they have a high frequency of MOG-specific autoreactive T cells in their peripheral blood, serum and cerebrospinal fluid (CSF) (Diaz-Villoslada et al., 1999; Hellings et al., 2001; Kerlero de Rosbo et al., 1997; Kerlero de Rosbo et al., 1993) as well as a high titer of anti-MOG antibody in the serum and CSF (Lindert et al., 1999; Reindl et al., 1999; Sun et al., 1991). Moreover, MOG reactive T and B cells from human and EAE animals share common epitopes (Haase and Schmidt, 2001; Kerlero de Rosbo et al., 1997; Khare et al., 2003).

As with many autoimmune diseases, linkage and association studies have established that the strongest determinant of genetic susceptibility to MS maps to the HLA region in chromosome 6, specifically to the HLA class II haplotype (Ebers and Sadovnick, 1994; Haines et al., 2002). The association of MS with the HLA class II haplotypes is heterogeneous in different populations. The strongest association of MS is with the HLA DR2 haplotype (DRB1*1501,DRB5*0101,DQA1*0102 and DQB1*0602) found in Caucasians, northern European populations, as well as other non-European ethnic populations (Alvarado-de la Barrera et al., 2000; Hauser et al., 1989; Kankonkar et al., 2003; Kwon et al., 1999; Olerup and Hillert, 1991; Saruhan-Direskeneli et al., 1997). However a small proportion of Japanese MS patients possess the DRB1*1502 but not DRB1*1501 allele (Kawamura et al., 2000). MS patients carrying DRB1*1501 in combination with DQB1*0601 (Sejeanston et al., 1992) and DQB1*0603 (Spurkland et al., 1997) alleles had also been reported. A positive association of HLA DRB1*0301 and DRB1*0401 with MS was observed in Sardinian and other Mediterranean populations (Giordano et al., 2002; Marrosu et al., 1997). While it is thought that HLA DR molecules associated with MS present autoantigenic peptides to initiate the disease process (Martin et al., 1992), some studies have suggested that susceptibility to MS may be associated with the HLA-DR and -DQ molecules (Spurkland et al., 1991). Elucidation of the role of individual HLA-DR or -DQ molecule in human MS is difficult due to strong linkage disequilibrium between certain DR and DQ genes and the heterozygosity of affected individuals.

The goal of this study was to determine whether presence of susceptible or resistant HLA-DQ allele with susceptible HLA-DR can modulate the outcome of disease severity in HLA class II transgenic mice. We have generated transgenic mice that express individual HLA-DR and HLA-DQ molecules lacking endogenous mouse class II molecules. Therefore, the only functional class II molecules on APCs are the human class II molecules, mediating the CD4+T cell responses. We have also generated the double transgenic mice carrying both DR as well DQ allele to recreate the co-expression of HLA-DR and -DQ alleles in human MS. EAE, a well-characterized murine model of MS with MOG, was induced in these transgenic mice to explore the contributions of individual as well as dual HLA molecules in disease pathogenesis. We demonstrate here that DRB1*1502 mice were highly susceptible to rMOG induced EAE, where as DQB1*0302 mice showed mild disease and no disease was observed in DQB1*0601 mice. Of interest, introduction of susceptible HLA molecule DQB1*0302 in susceptible DRB1*1502 mice enhances the severity of disease in DRB1*1502 mice, where as introduction of resistant allele DQB1*0601 suppresses the disease severity in DRB1*1502 mice. The result suggests that expression of DR molecule is required for induction of disease. Expression of DQ molecule alone does not have any effect on disease induction, however its presence can modulate disease profile in HLA-DR transgenic mice. This study is the first to correlate the association of disease severity and pathogenesis in MOG-induced EAE with individual human HLA molecules.

## 2. Methods

### 2.1. Transgenic mice

The production and characterization of transgenic mice expressing HLA-DRB1*1502 (DR2), DQA1*0103/DQB1*0601 (DQ6) and DQA1*0301/DQB1*0302 (DQ8) genes have been described previously (Khare et al., 2003). The HLA transgenic mice were then mated to class II-deficient (Abo) mice and the lines generated by a backcross-intercross mating scheme for several generations. Mating of AboDRB1*1502 and AboDQB1*0302 transgenic mice generated double transgenic AboDRB1*1502/DQB1*0302 (DR2/DQ8) mice. The transgene negative littermates were used as controls. Similarly, transgenic mice that expressed Abo.DRB1*1502 and Abo.DQB1*0601 genes were mated to produce Abo.DRB1*1502/DQB1*0601 (DR2/DQ6) mice. Thus, the different transgenic lines have similar background genes and differ from the controls only for the expression of HLA class II genes. All mice used in this study were bred and maintained in the pathogen-free Immunogenetics Mouse Colony at the Mayo Clinic (Rochester, MN). Mice of both sexes were used in this study and were 8–12 weeks old. All procedures were in accordance with the Mayo Institutional Animal Care and Use Committee.

### 2.2. Antigens

Recombinant MOG (rMOG) corresponding to the extracellular domain of rat myelin oligodendrocyte glyco-