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A New Humanized HLA Transgenic Mouse Model of Multiple Sclerosis Expressing Class II on Mouse CD4 T Cells

Ashutosh Mangalam, Ph.D.[1], **Moses Rodriguez, M.D.**[1,2], and **Chella David, Ph.D.**[1]

1*Department of Immunology, Mayo Clinic College of Medicine, Rochester, MN.*

2*Departmnet of Neurology, Mayo Clinic College of Medicine, Rochester, MN.*

Abstract

Among all the genetic factors associated with MS susceptibility, strongest association has been seen with expression of certain MHC class II molecules, although analysis of their exact function remains complicated. In general expression of class II is restricted to professional antigen presenting cells, however human but not mice CD4+ T cells express class II on their surface. Functional studies of classII+CD4+ T cells have been hampered due to lack of proper animal model. Here we describe development and characterization of a new humanized class II transgenic mice expressing HLA-DR3 on mouse endogenous class II negative background.

Keywords

CD4 T cells; MHC/HLA; MS; transgenic mouse; antigen presenting cells; PLP; EAE

Introduction

MS is a chronic, demyelinating disease of the CNS resulting from T cell driven aberrant immune response to a number of myelin antigens including proteolipid protein (PLP) [1]. Among all the genetic factors associated with Multiple sclerosis (MS) susceptibility, strongest association is seen with expression of certain MHC class II molecules[2]. The most common MHC molecules associated with MS are DR2, DR3 and DR4 alleles. Although HLA-DR2/DQ6 is the most frequently associated HLA haplotype in MS, HLA-DR3 alleles has also been shown to be associated with susceptibility to MS in different part of world[3]. MHC class II molecules present antigenic peptides to CD4+ T cells and play an important role in selection of T cells repertoire in thymus. It is hypothesized that certain HLA class II (susceptible alleles) molecules select self reactive CD4+ T cells in thymus, and these auto reactive T cells escape to periphery. Activation of these auto-reactive T cells in the periphery and subsequent migration in CNS leads to initiation of inflammation and subsequent neurological deficit resulting in MS. Studies using T cells isolated from MS patients as well as adoptive transfer studies in animal model of MS such as EAE, has confirmed the role of CD4+ T cells in disease pathogenesis[1]. Thus identification of class II gene(s) associated with disease and myelin epitope selected by these susceptible alleles will not only help in understanding the pathogenesis of MS but will also help in design of future antigen specific therapy.

However number of factors such as genetic heterogeneity of MHC, role of non-MHC genes, contribution of environment and linkage disequilibrium between MS associated HLA-DR and

Corresponding Author: Ashutosh Mangalam, Mayo Clinic College of Medicine, 200 Ist ST SW, Rochester, MN, 55905, 1-507-284-4562, 1-507-266-0981, mangalam.ashutosh@mayo.edu.

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-DQ genes has been a major obstacle in clarifying the contribution of individual HLA molecules in MS pathogenesis. Therefore, to overcome these obstacles we have generated and characterized a transgenic (tg) mice expressing HLA-DR3 gene on mouse endogenous class II negative background. Expression of human DR3 molecule was detected on all antigen presenting cells, as well as also on a subset of CD4+ T cells just like human T cells. It is well established that mouse T cells differ from humans in their inability to express class II on their cell surface. We have also induced and characterized EAE in these tg mice using $PLP_{91–110}$ peptide.

Material and Methods

Transgenic mice

Generation of transgenic mice expressing HLA-DR3 (DRB1*0301) (gift from Dr. Gunter Hammerling, Germany) has been described previously[4]. Most HLA class II transgenic mice currently used as animal models for human diseases are generated on Ab° background, which have a nonfunctional Aβgene and since this knockout involved the H-2b haplotype (Strain 129), the Eα gene is also nonfunctional. Thus, Abo mice do not generate a functional mouse class II molecule. However, mice do express the Aα and Eβ genes. It is speculated that Aα and Eβ chains might pair with human class II DQβ and DRα chains to form a functional molecule and modulate immune response. To overcome this problem, we mated our HLA transgenic mice with MHC class $II^{\Delta/\Delta}$ mice (AE°) generated by Mathis and Benoist[5]. These knockout mice lack all four conventional MHC class II genes (Aα, Aβ, Eα and Eβ) due to a large (80-kilobase) deletion of the entire class II region and thus do not express any mouse class II. HLA-DR3 transgenic mice were mated to class II-deficient Aβo or AEo mice, backcrossed to B10 and intercrossed to generate the HLA DR3.Aβo and HLA DR3.AEo transgenic mice lacking endogenous class II molecules. Transgene negative littermates were used as controls in these studies. All mice were bred and maintained in the pathogen free Immunogenetics Mouse Colony of Mayo Clinic according to National Institutes of Health and institutional guidelines. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC), at Mayo Clinic, Rochester.

FACS analysis

The expression of various cell surface markers was analyzed by flow cytometry using FACS IV (BD Biosciences, San Jose, California, USA) using specific Abs according to the manufacturer's protocol on peripheral blood leukocytes (PBLs) or splenic cells of naive and immunized mice. Expression of HLA-DR on cell surface was analyzed using FITC-anti-DR (clone 243) antibody from BD Biosciences PharMingen (San Diego, California, USA).

T cell proliferation assay and Disease induction

Synthetic human PLP peptides 91–110 were synthesized at the peptide core facility of Mayo Clinic, Rochester, MN. Mice were immunized subcutaneously with 100 μg of PLP peptide in CFA and mice were sacrificed 10 days after immunization. T cell proliferation was analyzed in draining lymph node cells using standard thymidine incorporation assay. For disease induction 10–14 weeks old tg mice were immunized subcutaneously with 100 μg of $PLP_{91–110}$ emulsified in CFA containing Mycobacterium tuberculosis H37Ra (400 μg/mice). Pertussis toxin (Sigma Chemicals, St. Louis, MO, USA; 100ng) was injected i.v. at day 0 and 48 h post immunization. Mice were observed daily for clinical symptoms. Disease severity was scored using standard scoring system.

Histopathology

Brain and spinal cord of mice with or without disease were removed and preserved in 10% formalin. Thin slices of CNS tissues were prepared and stained with H&E. The tissues were examined under light microscope and extent of inflammation and demyelination was scored as described previously[6].

Results

DR3.AEo tg mice showed normal expression of class II in spleen, lymph node as well in peripheral blood lymphocytes and class II expression in DR3.AEo tg mice were comparable to levels seen in B6 and DR3.Abo mice. All the CD4+ T cells in DR3.AEo mice were selected by human class II molecule and this selection was efficient as evident by selection of a diverse CD4+ T cell Vβ repertoire. Further, CD4+ T cell Vβ repertoire specific for DR3 such as Vβ6, Vβ8, and Vβ10 were selected while wild type (B6) specific Vβ repertoire such as Vβ5, and Vβ11 and were deleted. Thus DR3.AEo tg mice developed normally and showed a normal T cell development restricted to human class II, HLA-DR3.

Human T cells are unique from mice in expression of MHC class II molecules on their cell surface. Interestingly, similar to humans, T cells from AEo.DR3 mice expresses HLA-DR molecules on their cell surface. Thus these mouse CD4+ T cells behave like human in context of class II expression on their cell surface. Since it has been suggested that mouse T cells can acquire class II molecules from neighboring antigen presentation cells, we analyzed expression of class II at mRNA levels by standard RT-PCR assay. Our RT-PCR data suggested that class II expression on CD4+ T cells in DR3.AEo tg mice was de novo and was not acquired from neighboring cells. We also tested ability of these class II^+CD4^+T cells to present antigen and have observed that class II^+CD4^+T cells can present antigen PLP91–110 peptide. This antigen presentation was class II restricted as addition of class II blocking antibody abrogated the T cell response.

Functional role of class II^+CD4^+T cells expression has been controversial as some studies have shown that they play a pathogenic role[7] while others have suggested that expression of class II induce anergy in CD4+ T cells[8]. It has been shown that in MS, CD4+T cells infiltrate the CNS and initiate inflammation. In addition it has also been shown in a RAT-EAE model that class II expressing CD4+ T cells play a very important role in development of neurological injury in CNS [7]. It is hypothesized that class II molecules on T cells may present myelin antigen in CNS and exacerbate the disease. To investigate the functional role of these class II expressing T cells, we induced EAE in DR3.AEo transgenic mice and compared the disease development between DR3.AEo (express class II on T cells) and DR3.Abo tg mice (do not express class II on T cells). We observed that DR3.AEo tg mice developed severe EAE as compared to DR3.Abo mice (table 1). The disease was characterized by an early onset of disease (11±0.8 Vs 14±1.0) as well as high average daily clinical score (3.2±0.7 Vs 2.4±0.5).

The difference in severity of disease was further confirmed by histopathological analysis of CNS tissue. DR3.AEo tg mice with EAE showed a higher inflammation and demyelination in brain as well as spinal cord as compared to DR3.Abo tg mice. Thus our EAE data suggested that class II expressing T cells play an important role in imunopathogenesis of EAE as DR3.AEo tg mice showed more severe from of EAE as compared to DR3.Abo tg mice. Further, to confirm role of class II expressing T cells in our model, we transferred bone marrow cells from DR3.AEo mice into lethally irradiated DR3.Abo tg mice. The rational being that once bone marrow cells from DR3.AEo mice reconstitute immune compartment in DR3.Abo mice, they will have class II expressing CD4 T cells. Administration of $PLP_{91–110}$ antigen in to recipient DR3.Abo mice leads to development of severe EAE as seen in DR3.AEo tg mice. Recipient DR3.Abo showed similar early disease onset as well as higher average daily clinical

score as observed in DR3.AEo tg mice. Thus our study confirmed that class II expressing T cells play very important role in initiation and development of inflammation and subsequent demyelination in MS. Thus our model overcome a major bottleneck in murine model of EAE by expression of class II on T cells and may simulate immuno-pathogenesis of MS in human.

References

1. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol 2005;23:683–747. [PubMed: 15771584]
2. Giordano M, et al. Genetics of multiple sclerosis: linkage and association studies. Am J Pharmacogenomics 2002;2:37–58. [PubMed: 12083953]
3. Mangalam AK, et al. Identification of T cell epitopes on human proteolipid protein and induction of experimental autoimmune encephalomyelitis in HLA class II-transgenic mice. Eur J Immunol 2004;34:280–290. [PubMed: 14971054]
4. Strauss G, et al. Negative and positive selection by HLA-DR3(DRw17) molecules in transgenic mice. Immunogenetics 1994;40:104–108. [PubMed: 8026858]
5. Madsen L, et al. Mice lacking all conventional MHC class II genes. Proc Natl Acad Sci U S A 1999;96:10338–10343. [PubMed: 10468609]
6. Drescher KM, et al. CNS cell populations are protected from virus-induced pathology by distinct arms of the immune system. Brain Pathol 1999;9:21–31. [PubMed: 9989447]
7. Flugel A, et al. Migratory activity and functional changes of green fluorescent effector cells before and during experimental autoimmune encephalomyelitis. Immunity 2001;14:547–560. [PubMed: 11371357]
8. LaSalle JM, et al. Early signaling defects in human T cells anergized by T cell presentation of autoantigen. J Exp Med 1992;176:177–186. [PubMed: 1535366]

Table 1

DR3.AEo tg mice develop more severe EAE as compared to DR3.Abo tg mice

Mouse strain	Disease incidence (%)	Onset of disease	Mean clinical Score ± SD
B6	0/10 (0)	-	-
Abo	0/10 (0)	-	-
AEo	0/10 (0)	-	-
DR3.Abo	6/10 (60)	14.0±1.0	2.4±0.5
DR3.AEo	8/10 (80)	11.0±0.8[a]	3.2±0.7[b]

[a] $p<0.01$ Mann-Whitney rank sum test, DR3.AEo compared to DR3.Abo mice.

[b] $p<0.05$ Mann-Whitney rank sum test, DR3.AEo compared to DR3.Abo mice