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Disparate MHC class II haplotypes in myelin oligodendrocyte glycoprotein- and myelin basic protein-induced experimental autoimmune encephalomyelitis

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Abstract

The major histocompatibility complex (MHC) regulates multiple sclerosis (MS) and its model experimental autoimmune encephalomyelitis (EAE). We created four new intra-MHC recombinant rat strains, between the MHC haplotypes $RT1^n$ (BN) and $RT1^l$ (LEW) on the LEW background, to define disease regulation and localization within the MHC. Immunization with recombinant myelin oligodendrocyte glycoprotein (a.a.1-125; MOG)/IFA induced EAE in strains expressing the MHC class II allele $RT1.B^n$, whereas strains expressing the $RT1.B^l$ were resistant. In myelin basic protein peptide (MBP_{GP}63-88)/CFA-induced EAE, $RT1.B^l$ expressing strains were susceptible whereas strains expressing the $RT1.B^n$ were resistant. High levels of antigen-specific IFN-γ secreting lymphoid cells and antigen-specific serum IgG antibodies were only recorded in rats with an MHC class II allele that permitted MOG- or MBP-EAE, respectively. Genetically, we localized the MHC regulation of the investigated EAE models to the central part of the MHC, containing the MHC class II (RT1.B/D) and the centromeric parts of the MHC class III. No influences were evident from the classical MHC class I (RT1.A), the telomeric parts of the MHC class III or the non-classical MHC class I (RT1.C/E/M) in contrast to previous reports. The MHC class II haplotype-specific regulation of EAE induced with two different CNS antigens demonstrates a strikingly specific MHC-association even within the same target organ.

Keywords: Major histocompatibility complex (MHC); Congenic rat; Myelin oligodendrocyte glycoprotein (MOG); Myelin basic protein (MBP); Experimental autoimmune encephalomyelitis (EAE); Multiple sclerosis

1. Introduction

Multiple sclerosis (MS) is a central nervous system (CNS)-specific inflammatory disease that results from the interaction of multiple genes with environmental factors. The major histocompatibility complex (MHC) is well established as one of the gene regions predisposing to MS (Sawcer et al., 1996). However, the MHC contains a multitude of genes, many with immune functions, and it is still debated which of these that regulates MS. Linkage disequilibrium within the MHC complicates attempts to pinpoint individual genes. We employ an animal model of MS, experimental autoimmune encephalomyelitis (EAE), to better understand the role of different gene regions within the MHC. EAE can be actively induced with different CNS antigens such as myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) in adjuvants (Wekerle et al., 1994), or passively by the transfer of autoreactive $CD4^+$cells (Ben-Nun et al., 1981). We believe that it is informative to perform mechanistic and genetic studies in EAE models that mimic human MS as closely as possible. MOG-induced EAE in the rat fulfills such criteria (Steinman, 1999; Storch et al., 1998). We have previously demonstrated a potent MHC regulatory effect in this model that mapped to the MHC class II region (RT1.B/D) in the $RT1^a$ haplotype. Additional influences from the classical MHC class I region (RT1.A) was suggested by characterization of $RT1^a$ and $RT1^u$ expressing

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intra-MHC recombinant rat strains (Weissert et al., 1998). Modifying influences from the distal part of the non-classical MHC class I region (RT1.M) of the $RT1^n$ haplotype has also been proposed, based on studies of the intra-MHC recombinant strain LEW.1R38 (Stefferl et al., 1999).

MOG-induced EAE in LEW.N ($RT1^n$) rats is characterized by an acute lethal disease course in which there is marked CNS inflammation and demyelination, accompanied by high MOG antibody titers and high T cell proliferation and interferon-γ (IFN-γ) production (Weissert et al., 1998). Interestingly, if immune responses are mapped with 18-meric MOG peptides instead of recombinant MOG(1-125), peripheral T cell immunodominance fail to predict encephalitogenicity (Weissert et al., 2001). If LEW rats, instead of LEW.N rats, are MOG-immunized both peripheral immune responses and clinical disease are abrogated (Weissert et al., 1998). In MBP-induced EAE, LEW rats are susceptible and LEW.N rats resistant (Happ et al., 1988). These contrasting MHC restriction patterns of EAE induced with two different CNS antigens presents an ideal situation to examine the effects of different regions of the MHC, provided that intra-regional recombinant strains are available. We therefore generated four new intra-MHC recombinant rat strains derived from recombination between the $RT1^n$ and the $RT1^l$ haplotypes. These strains were immunized with rMOG(a.a.1-125)/IFA or MBP_{GP}63-88 peptide/CFA and the outcome was studied with regard to clinical EAE, T cell and antibody responses. With these experimental conditions, the MHC regulation maps to the central part of the MHC, containing the MHC class II region (RT1.B/D). The apparent discrepancies with previous studies suggest allele-specific regulation by classical and non-classical MHC I genes, genetic drift, environmental influences and/or specific effects related to adjuvants/induction protocols.

2. Materials and methods

2.1. Antigens

Recombinant rat MOG, corresponding to the N-terminal part of the protein, amino acids 1-125; rMOG(a.a.1-125).rMOG(a.a.1-125) was expressed in *Escherichia coli* and purified to homogeneity by chelate chromatography (Amor et al., 1994). The purified protein in 6 M urea was dialysed against PBS to obtain a preparation that was stored in −20 °C.

The Guinea pig MBP_{GP}63-88 (AARTTHYGSLPQKSQRSQDENPVVHF) sequence was synthesized by F-moc/HBTU (2-(1*H*-bezotrizol-1-yl)-1,1,3,3,-tetramethyluronium hexaflurophosphate) strategy (Dr. A. Engström, Department of Medical and Physiologic Chemistry, Uppsala University, Sweden). The peptide was purified by reverse phase chromatography and, subsequently analyzed by plasma desorption mass spectroscopy. The degree of purity of the used peptide was more than 99%.

2.2. Induction and evaluation of EAE

EAE was actively induced in the rats (for numbers see Table 1) by intradermal or subcutaneous injections at the base of the tail with a total volume of 200 μl of inoculums containing 200 μg of MBP_{GP}63-88 or 50 μg rMOG(a.a.1-125) in saline mixed (1:1) with mineral oil (IFA; Sigma, St. Louis, MO). For MBP_{GP}63-88 immunizations, 1 mg of heat-inactivated *Mycobacterium tuberculosis* (strain H37 RA; Difco Laboratories, Detroit, MI) was added (CFA). Immunizations were performed under inhalation anesthesia with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL).

Clinically scoring and weighing was performed on a daily basis up to day 40 post-immunization (p.i.). Signs were scored as follows: grade 1, tail weakness or tail paralysis; grade 2, hind leg paraparesis; grade 3, hind leg paralysis; grade 4, complete paralysis (tetraplegy), moribund state or death. Since MOG-EAE in the LEW.N rats differs in the expression of clinical signs from that of classical rat EAE, a different scale was used for these rats as follows; 0.5, slow movement and ragged fur; 1, mild balance disturbance; 2, moderate balance disturbance; 3, severe balance disturbance with repetitive circular movements; 4, rolling behavior, moribund state or death.

Table 1
Inbred rat strain designations and MHC (RT1) subregions

Strain	RT1 regions					
	A		B/D			M
	MHC Ia		MHC II	MHC III		MHCIb
	D20Rat32	OX-27	HIS19	D20Rat42	TNFα	RT1.m4
LEW.N	n	n	n	n	n	n
LEW.NR1	n	n	l	l	l	l
LEW.NR2	n	n	n	n	l	l
LEW.NR3	n	n	n	n	n	l
LEW.LR1	l	l	n	n	n	n
LEW	l	l	l	l	l	l

Microsatellite markers: D20Rat32, D20Rat42, TNFα, RT1.m4; polymorphic antibodies: OX-27, HIS19.