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Differential nerve injury-induced expression of MHC class II in the mouse correlates to genetic variability in the type I promoter of *C2ta*

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ABSTRACT

Major histocompatibility complex (MHC) class II is of critical importance for the induction of immune responses. Levels of MHC class II in the nervous system are normally low, but expression is up-regulated in many disease conditions. In rat and human, variation in the MHC class II transactivator gene (*C2ta*) is associated with differential expression of MHC class II and susceptibility to autoimmune disease. Here we have characterized the response to facial nerve transection in 7 inbred mouse strains (C57BL/6J, DBA/2J, 129X1/SvJ, BALB/cJ, SJL/J, CBA/J, and NOD). The results demonstrate differences in expression of *C2ta* and markers for MHC class I and II expression, glial activation, and T cell infiltration. Expression levels of *C2ta* and *Cd74* followed similar patterns, in contrast to MHC class I and markers of glial activation. The regulatory region of the *C2ta* gene was subsequently sequenced in the four strains (C57BL/6/J, DBA/2J, SJL/J and 129X1/SvJ) that represented the phenotypical extremes with regard to *C2ta*/*Cd74* expression. We found 3 single nucleotide polymorphisms in the type I (pI) and type III (pIII) promoters of *C2ta*, respectively. Higher expression of pI in 129X1/SvJ correlated with the pI haplotype specific for this strain. Furthermore, congenic strains carrying the 129X1/SvJ *C2ta* allele on B6 background displayed significantly higher *C2ta* and *Cd74* expression compared to parental controls. We conclude that genetic polymorphisms in the type I promoter of *C2ta* regulates differential expression of MHC class II, but not MHC class I, *Cd3* and other markers of glial activation.

1. Introduction

Signs of inflammation are present in many diseases affecting the central nervous system (CNS), including multiple sclerosis (MS), Alzheimer's disease (AD) and stroke. Resident cells of the CNS, in particular microglia, play a key role in the inflammatory process. As part of the initial innate immune reaction, peripheral nerve injury results in a retrograde reaction comprising both astro- and microgliosis, with up-regulation of immune signaling molecules such as MHC class II (Lundberg et al., 2001; Maehlen et al., 1989; Piehl et al., 1999). Inbred rodent strains react differently to mechanical nerve injury, suggesting influence by genetic factors on the nerve injury response (Lidman et al., 2002; Lidman et al., 2003; Lundberg et al., 2001; Piehl et al., 1999). In the WldS mouse, a single mutation in the Wld(s) gene causes delayed Wallerian degeneration after nerve injury (Fujiki et al., 1996; Perry et al., 1990). However, in the majority of cases, phenotypic differences are regulated by multiple gene regions, each with modest effects, making them quite difficult to map. Nevertheless, the identification of such genetic influences may be of importance for the understanding of complex human diseases. Previously, we have identified polymorphisms in the rat class II transactivator, *C2ta*, which affects the axon reaction. This gene was positioned in a quantitative trait locus (QTL) regulating expression of MHC class II on microglia after ventral root avulsion (VRA) (Lidman et al., 2003; Swanberg et al., 2005). *C2ta* was also associated to increased risk of diseases with inflammatory features, including multiple sclerosis (Lindholm et al., 2006; Swanberg et al., 2005). In addition, it was shown to regulate autoimmune neuroinflammation in the rat (Harnesk et al., 2008).

C2ta encodes the CIITA protein, which induces MHC class II expression not by binding directly to the DNA, but by coordinating the assembly of other DNA binding transcription factors such as cAMP responsive element binding protein (CREB) and Regulatory Factor X (RFX) (Zika and Ting, 2005). There are four different *C2ta* isoforms in humans (pI–pIV) and three in mouse and rat (pI, pIII and pIV), which are expressed in a tissue specific manner (LeibundGut-Landmann et al., 2004).

Previous reports suggest that MHC class II expression, as well as microglial response, astrocytosis, T cell infiltration, MHC class I expression and neuronal cell survival, is subject to genetic regulation also in the mouse after nerve injury (Ha et al., 2006; Kigerl et al., 2006; Lidman et al., 2002).

In order to investigate the regulation of *C2ta*, MHC class II and other inflammatory features in the nervous system following mechanical nerve

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injury, we performed facial nerve injury in 7 inbred mouse strains (C57BL/6J, DBA/2J, 129X1/SvJ, BALB/cJ, SJL/J, CBA/J, and NOD) and measured the expression of several immunological markers in the facial nucleus: *C2ta*, *Cd74*/MHC II, *B2m*, *Cd3*, *Aif1* and *Cd11b*. *B2m* (beta-2-microglobulin) is a structural part of the MHC class I complex, while *Cd3* is a component of the T cell receptor complex. *Aif1* (allograft inflammatory factor-1, also called microglia response factor-1) and *Cd11b* (complement 3 receptor) are often used as markers of microglia activation. Alongside these studies, the region upstream of the *C2ta* gene was sequenced in four of these strains in order to identify possible polymorphisms in regulatory regions. The influence of genetic variations in *C2ta* was subsequently tested in C57BL/6J/129X1/SvJ (B6/129) congenic mice. We demonstrate here that single nucleotide polymorphisms in the regulatory region of promoter I (pI) of *C2ta* correlate with differential expression of CIITA and MHC class II. In contrast, the expression of the other studied molecules displayed a different expression pattern, suggesting the presence of additional genetic influences.

2. Materials and methods

2.1. Animals

Six female mice, aged 8 weeks, from each of the following inbred mouse strains were purchased from Jackson Laboratories (Bar Harbor, ME): C57BL/6J ($H2^b$), DBA/2J ($H2^d$), 129X1/SvJ ($H2^b$), BALB/cJ ($H2^d$), SJL/J ($H2^{s2}$) and CBA/J ($H2^k$). Six female NOD ($H2^{g7}$) mice were kindly provided by Petter Höglund, Department of Microbiology, Tumor and Cell biology, Karolinska Institutet. An additional 6 female mice from the strains C57BL/6J, DBA/2J, 129X1/SvJ and SJL/J, respectively, were obtained from Jackson Laboratories and used in a subsequent experiment.

Congenic animals were bred by crossing C57BL/6J and 129X1/SvJ mice. The offspring was genotyped to select for individuals being heterozygous in the *C2ta* locus. Backcrossing onto C57BL/6J for 6 generations, and subsequent brother–sister mating, was performed to create homozygous congenic animals. The maximum size of the introgressed fragment is roughly 15 Mb, spanning from 5.9 Mb (marker D16Mit182) to 21.6 Mb (SNP rs4151975). Ten mice from each respective parental strain, and 10 congenics, were subjected to nerve injury as described below. All animals were housed under pathogen-free and climate-controlled conditions with 12 h light/dark cycles, in polystyrene cages with wood shavings and food and water *ad libitum*.

2.2. Facial nerve injury

Facial nerve injury was performed in deep isoflurane anaesthesia. The left facial nerve was exposed at its exit from the stylomastoid foramen, and a 2–3 mm long segment was resected. The wounds were closed with sutures. The animals were given analgesia at three timepoints following surgery and were allowed to survive 14 days. Animals were sacrificed with CO_2, the brainstem was extracted and the tissue was frozen at −80 °C. All animal experiments in this study were approved by the local ethical committee (Stockholms Norra Djurförsöksetiska Nämnd).

2.3. DNA isolation and genotyping

Genomic DNA was extracted from rat-tail tips using a standard protocol (Laird et al., 1991). Polymorphic microsatellite markers were selected from available Internet databases: Rat Genome Database (http://rgd.mcw.edu), Center for Genomic Research, Whitehead Institute/MIT (http://www-genome.wi.mit.edu/rat/public/) and Ensembl (www.ensembl.org). Flourophore-conjugated primers were purchased from Applied Biosystems Foster City, CA). Genomic DNA was amplified by polymerase chain reaction (PCR) according to a standard protocol. PCR products were separated using a capillary electrophoresis capillary sequencer (ABI3730) and analyzed in GeneMapper v3.7 software (Applied Biosystems).

2.4. Rt-pcr

An ipsilateral portion of the brainstem, containing the facial nucleus, was isolated by micro dissection and was subsequently used for quantitative analysis of immunological and glial markers at the RNA level. The tissue was lysed and homogenised with FastPrep Lysing Matrix D (QBiogene, CA) and RNA was extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany), following manufacturer's instructions. The samples were DNase treated with RNase-Free DNAse Set, Qiagen, following manufacturer's instructions. Reverse transcription was performed with 10 ml of total RNA, random hexamer primers (0.1 μg, Gibco BRL) and Superscript Reverse Transcriptase (200U, Gibco BRL). Amplification was performed using the iQ5 Real Time PCR detection system (Bio-Rad, Hercules, CA). All primers except those for *C2ta* isoforms were designed using the Primer Express software (Applied Biosystems, Foster City, CA). Primers for *C2ta* isoforms were designed using the Beacon Designer software (Bio-Rad). Primer sequences are presented in Table 1. Amplification of genomic DNA was avoided by constructing either one of the primers over and exon/intron boundary. Relative quantitation of mRNA levels was performed using the standard curve method. Standard curves were obtained by using serial dilutions of pooled cDNA. All samples were analyzed in duplicates. In the first study of seven mouse strains, the relative mRNA expression was calculated as the ratio of the target and the corresponding endogenous control, *Gapdh*. In the follow-up studies, *Gapdh* and *Hprt* were used as controls. All expression data was normalized against the median value of C57BL/6J mice and presented as such.

2.5. Immunohistochemistry

Cryosections (12 μm) were collected from the brain stem at the level of the facial nucleus and processed for immunohistochemistry. Sections were fixed in ice cold acetone 80% and methanol 20% (5 min) and incubated overnight at 4 °C with rat anti-mouse I-A/I-E antibody (clone 2G9; BD Pharmingen, NJ), rabbit anti-glial fibrillary acidic protein (GFAP) (Dako), rabbit anti-rat IBA1 (Wako, Neuss, Germany) and goat polyclonal anti-CD3e (Santa Cruz, Wembley, UK). The following day sections were rinsed in PBS, incubated at room temperature for 45 min with the following secondary antibodies: Cy3 conjugated donkey anti-rat (Jackson ImmunoResearch Laboratories, PA), Alexa 594 conjugated goat anti-rat (Molecular Probes, Oregon, USA), Alexa 488 conjugated goat anti-rabbit (Molecular Probes) and Alexa 488 conjugated donkey anti-goat (Molecular Probes) in PBS. Specificity of immunolabeling was tested by omission of the primary antibody and by incubation with unrelated isotype-matched antibody controls. Micrographs were recorded on a Zeiss Axioskop microscope system and processed in Adobe Lightroom 1.4.1 and Adobe Photoshop CS2.

Table 1
Primer sequences for real-time PCR.

	Forward	Reverse
Aif1	5′ GGA GGC CTT CAA GAC GAA GTAC	5′ AGC ATT CGG TTC AAG GAC ATA ATA
B2m	5′ CCT GTA TGC TAT CCA GAA AAC CC	5′ CTG TGT TAC GTA GCA GTT CAG TAT
Cd3	5′ CCC AGA CTA TGA GCC CAR CC	5′ GTC CAC AGA AGG CGA TGT CTC T
Cd11b	5′ CCC AGA GGC TCT CAG AGA ATG TC	5′ CTT CAT CTT CTG AAA GTC AAT GTT
Cd74	5′ ATG GCG TGA ACT GGA AGA TCT	5′ TCT TCC GGG AAG CGT CTC TT
Gapdh	5′ TCA ACT ACA TGG TCT ACA TGT TCC AG	5′ TCC CAT TCT CAG CCT TGA CTG
Hprt	5′CTCATGGACTGATTATGGACAGGAC	5′GCAGGTCAGCAAAGAACTTATAGCC
pI	5′ GGACTTAGACTTGACTTTCTTGAG	5′ GGTGGCACACAGACTATGG
pIII	5′ CATCACTCTGCTCTCTAAATC	5′ CGGCATCACTGTTAAGGAG
pIV	5′ GCAGGCAGCACTCAGAAG	5′ TGTTAAGGAGTTCCAGGTAGC