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Identification of gene regions regulating inflammatory microglial response in the rat CNS after nerve injury

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ABSTRACT

Local CNS inflammation takes place in many neurological disorders and is important for autoimmune neuroinflammation. Microglial activation is strain-dependent in rats and differential MHC class II expression is influenced by variations in the *Mhc2ta* gene. Despite sharing *Mhc2ta* and MHC class II alleles, BN and LEW.1N rats differ in MHC class II expression after ventral root avulsion (VRA). We studied MHC class II expression and glial activation markers in BN rats after VRA. Our results demonstrate that MHC class II expression originates from a subpopulation of IBA1$^+$, ED1$^-$, and ED2$^-$ microglia. We subsequently performed a genome-wide linkage scan in an F2(BNxLEW.1N) population, to investigate gene regions regulating this inflammatory response. Alongside MHC class II, we studied the expression of MHC class I, co-stimulatory molecules, complement components, microglial markers and Il1b. MHC class II and other transcripts were commonly regulated by gene regions on chromosomes 1 and 7. Furthermore, a common region on chromosome 10 regulated expression of complement and co-stimulatory molecules, while a region on chromosome 11 regulated MHC class I. We also detected epistatic interactions in the regulation of the inflammatory process. These results reveal the complex regulation of CNS inflammation by several gene regions, which may have relevance for disease.

1. Introduction

Inflammatory microglial activation is a feature of many central nervous system (CNS) disorders, including Alzheimer's disease (AD) and Multiple sclerosis (MS), see (Block and Hong, 2005). During disease, as well as after injury, microglia become activated and start to express MHC class II, MHC class I, CD11b, co-stimulatory molecule B7-2, cytokines and reactive oxygen species (ROS), see Kreutzberg (1995, 1996) and Raivich and Banati (2004). There is increasing evidence that microglial inflammation can be both harmful and beneficial, see Ekdahl et al. (2009), Glezer et al. (2007) and Raivich et al. (1999). Thus, microglia clear the CNS from cellular debris and express neurotrophic factors after brain injury (Lalancette-Hebert et al., 2007; Thored et al., 2008). On the other hand, the production of pro-inflammatory cytokines and ROS has been suggested to worsen the disease process in models of CNS disease (Ekdahl et al., 2003; Ponomarev et al., 2005; Shie et al., 2005; Wirths et al., in press). However, it remains unclear what underlying signals lead to the different outcomes of microglial activation, in terms of disease progression. It is therefore of interest to identify pathways that regulate the inflammatory responses in the damaged CNS.

The risk of developing an autoimmune disease such as MS is influenced by genetic factors, with the major histocompatibility complex (MHC) class II region as the main determinant (Hafler et al., 2007; Jersild et al., 1973; Lincoln et al., 2005; Weissert et al., 1998). Under normal conditions, MHC class II expression in the CNS is very low, but increased expression has been reported in several neurological diseases, mainly associated with activated microglia (Neumann et al., 2002).

Previous studies in this laboratory have shown that polymorphisms in the class II transactivator gene, *Mhc2ta,* lead to differential expression of MHC class II after ventral root avulsion (VRA) in the rat, and that expression levels of MHC class II affect the risk for developing autoimmune disease (Harnesk et al., 2008; Lidman et al., 2003; Swanberg et al., 2005). However, the BN rat shows higher early injury-induced expression of MHC class II than the LEW.1N strain after VRA, despite carrying the same *Mhc2ta* haplotype (Sedgwick et al., 1993a; Swanberg et al., 2005). This suggests influences on MHC class II expression from regions outside *Mhc2ta*.

In order to characterize the local early inflammatory response to nerve injury, we measured MHC class II as well as expression of glial markers in the spinal cord of BN rats, 5 days after VRA. Our results

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show a higher expression of MHC class II in a subpopulation of microglia, which are IBA1^{+}, ED1^{-}, and ED2^{-}. We then set out to investigate gene regions, aside from *Mhc2ta*, which regulate this phenotype. A whole genome linkage scan was performed in an F2 (BNxLEW.1N) population, 5 days after VRA. Alongside MHC class II, we investigated the regulation of beta-2-microglobulin, (*B2m*, marker for MHC class I), complement components 1, *C1q*, and 3, *C3*, *Cd11b* (C3 receptor, microglial marker), interleukin 1 beta (*Il1b*) and co-stimulatory molecule B7-2 (*Cd86*). Two new quantitative trait loci (QTLs) regulating MHC class II expression were found, in addition to

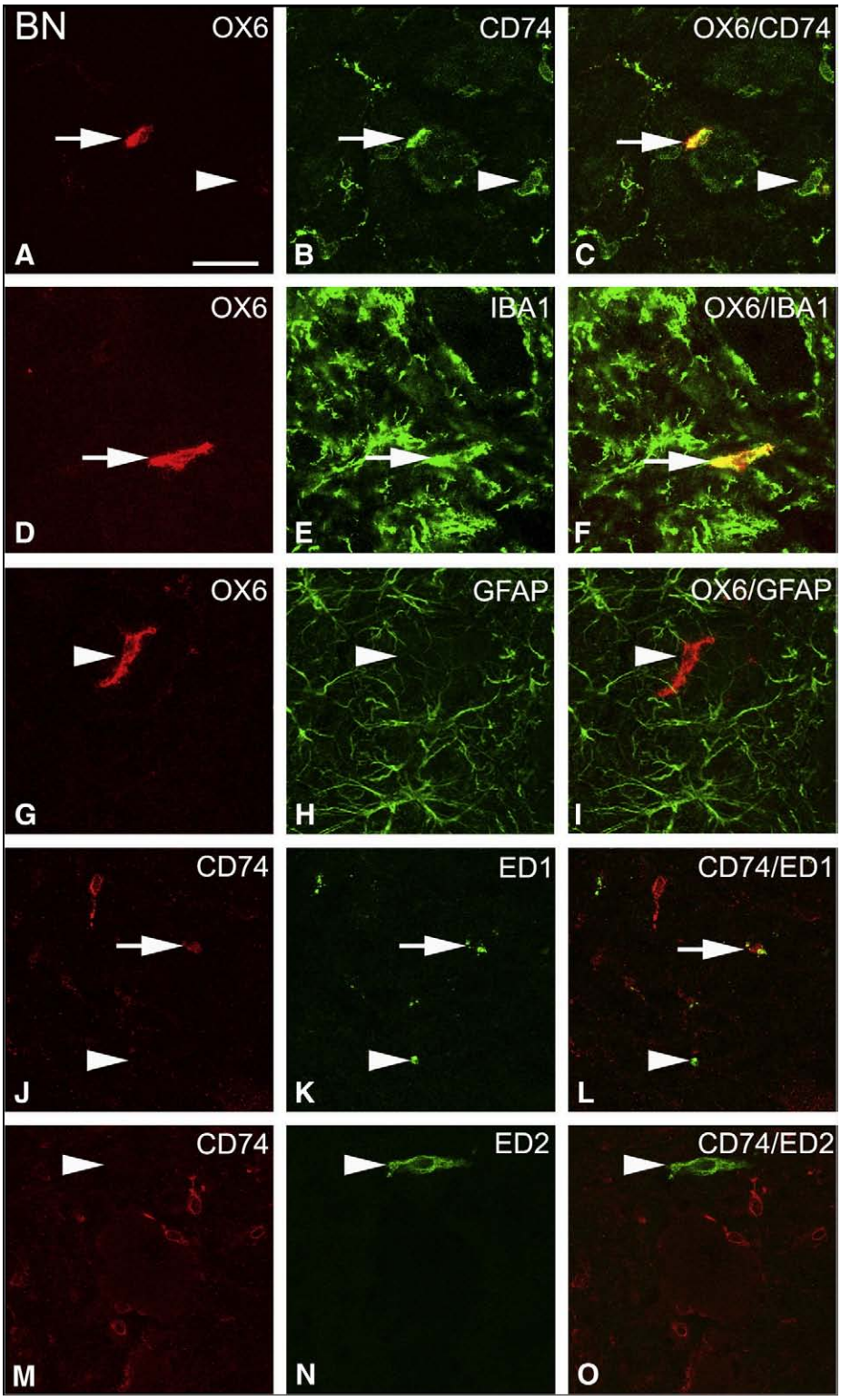

Fig. 1. Characterization of the OX6-immunoreactive cells present at higher numbers in BN rats 5 days after nerve injury. Arrows indicate co-localisation, arrow heads indicate lack of co-localisation. (A–C) Cells that are OX6 positive are also CD74 positive, although there is a population of CD74 positive cells that is OX6-negative. (E–F) Most OX6 positive cells are also IBA1 positive, indicating that MHC class II is primarily expressed by microglia. (G–I) No co-localisation between OX6 and GFAP was observed, excluding astroglial origin of MHC class II. (J–L) Overlap can be detected between CD74 and ED1 immunoreactivity. (M–O) CD74 positive cells are not labeled by perivascular phagocyte marker ED2. Scale bar = 250 µm.