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Alterations in microglial phenotype and hippocampal neuronal function in transgenic mice with astrocyte-targeted production of interleukin-10

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ABSTRACT

Interleukin-10 (IL-10) is a cytokine classically linked with anti-inflammatory and protective functions in the central nervous system (CNS) in different neurodegenerative and neuroinflammatory conditions. In order to study the specific role of local CNS produced IL-10, we have created a new transgenic mouse line with astrocyte-targeted production of IL-10 (GFAP-IL10Tg). In the present study, the effects of local CNS IL-10 production on microglia, astrocytes and neuronal connectivity under basal conditions were investigated using immunohistochemistry, molecular biology techniques, electrophysiology and behavioural studies. Our results showed that, in GFAP-IL10Tg animals, microglia displayed an increase in density and a specific activated phenotype characterised by morphological changes in specific areas of the brain including the hippocampus, cortex and cerebellum that correlated with the level of transgene expressed IL-10 mRNA. Distinctively, in the hippocampus, microglial cells adopted an elongated morphology following the same direction as the dendrites of pyramidal neurons. Moreover, this IL-10-induced microglial phenotype showed increased expression of certain molecules including Iba1, CD11b, CD16/32 and F4/80 markers, "*de novo*" expression of CD150 and no detectable levels of either CD206 or MHCII. To evaluate whether this specific activated microglial phenotype was associated with changes in neuronal activity, the electrophysiological properties of pyramidal neurons of the hippocampus (CA3-CA1) were analysed *in vivo*. We found a lower excitability of the CA3-CA1 synapses and absence of long-term potentiation (LTP) in GFAP-IL10Tg mice. This study is the first description of a transgenic mouse with astrocyte-targeted production of the cytokine IL-10. The findings indicate that IL-10 induces a specific activated microglial phenotype concomitant with changes in hippocampal LTP responses. This transgenic animal will be a very useful tool to study IL-10 functions in the CNS, not only under basal conditions, but also after different experimental lesions or induced diseases.

1. Introduction

Interleukin 10 (IL-10) is one of the most crucial immunoregulatory cytokines in the periphery where in general it has anti-inflammatory functions (Couper et al., 2008; Moore et al., 2001). In the central nervous system (CNS), IL-10 expression has been reported to be upregulated under a variety of neuroinflammatory and pathological situations including traumatic brain injury (Kamm et al., 2006), excitotoxicity (Gonzalez et al., 2009), middle cerebral artery occlusion (MCAO) (Zhai et al., 1997), Alzheimer's disease (Apelt and Schliebs, 2001), multiple sclerosis (Hulshof et al., 2002) and experimental autoimmune encephalomyelitis (EAE) (Ledeboer

Abbreviations: BBB, blood brain barrier; BSA, bovine serum albumin; CNS, central nervous system; DAPI, 4′,6-diamidino-2-phenylindol; EAE, experimental autoimmune encephalomyelitis; fEPSPs, field excitatory post-synaptic potentials; GFAP, glial fibrillary acidic protein; HFS, high frequency stimulation; hGH, human growth hormone gene; IL-10, interleukin-10; IL-10R, interleukin-10 receptor; LTP, long-term potentiation; MCAO, middle cerebral artery occlusion; MHC, major histocompatibility complex; PBS, phosphate buffer solution; PCR, polymerase chain reaction; pH3, phospho-histone 3; qRT-PCR, quantitative real time-polymerase chain reaction; RPA, ribonuclease protection assay; RT, room temperature; SOCS3, suppressor of cytokine signalling 3; TBS, Tris-buffered saline.

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et al., 2003). Noteworthy, and consistent with the anti-inflammatory role attributed to IL-10 in the periphery (Couper et al., 2008; Moore et al., 2001), upregulation of IL-10 expression after EAE occurs mostly during the recovery phase (Issazadeh et al., 1995; Ledeboer et al., 2003) and in the MCAO model coincides with a decrease of pro-inflammatory mediators (Zhai et al., 1997).

Expression of IL-10 receptor (IL10R) has been described in neurons (Lim et al., 2013), microglia (Ledeboer et al., 2002; Norden et al., 2014), astrocytes (Gonzalez et al., 2009; Ledeboer et al., 2002; Norden et al., 2014) and oligodendrocytes (Cannella and Raine, 2004), in both basal conditions and after injury, indicating a potentially broad range of actions of this cytokine in the CNS. Indeed, administration of IL-10 has been found to reduce astroglial activation (Balasingam and Yong, 1996; Pang et al., 2005), microglial production of LPS-induced inflammatory mediators (Balasingam and Yong, 1996; Kremlev and Palmer, 2005; Lodge and Sriram, 1996; Molina-Holgado et al., 2001b; Norden et al., 2014; Pang et al., 2005; Sawada et al., 1999) and leukocyte infiltration (Ooboshi et al., 2005). Moreover, IL-10 exerts a neuroprotective role in several *in vitro* and *in vivo* models of CNS injury, such as traumatic brain injury, excitotoxicity and MCAO among others (Arimoto et al., 2007; Bachis et al., 2001; Brewer et al., 1999; Knoblach and Faden, 1998; Ledeboer et al., 2000; Molina-Holgado et al., 2001a; Ooboshi et al., 2005; Park et al., 2007; Spera et al., 1998; Xin et al., 2011). Remarkably, the route of IL-10 administration has been reported to be essential for IL-10 protection. Thus, EAE was completely prevented by IL-10 administration only when IL-10 was administered intraparenchymatically, but when administered systemically there was no effect (Cua et al., 2001) or in some cases, worsened the disease (Cannella et al., 1996). Similarly, peripherally administered IL-10 fails to rescue facial motor neurons from the axotomy-induced cell death observed in IL-10 KO mice (Xin et al., 2011). Furthermore, after acute injuries such as spinal cord excitotoxicity or traumatic brain injury, intraspinal or intracerebroventricular IL-10 administration worsens disease (Brewer et al., 1999) or has no effect (Knoblach and Faden, 1998), whereas systemic administration improves the functional outcome of lesions (Brewer et al., 1999; Knoblach and Faden, 1998).

In this context and in order to obtain a better understanding of the role played by intrathecally produced IL-10 specifically in the CNS, we have generated a new transgenic mouse model in which the cDNA encoding for murine IL-10 was placed under the regulatory control of a glial fibrillary acidic protein (GFAP) transgene in astrocytes. The main goal of the present study was to determine the effects that local IL-10 production exerts on the populations of neurons, microglia and astrocytes under basal conditions.

2. Material and methods

2.1. Construction of the GFAP-IL10 fusion gene and production of transgenic mice

The full-length cDNA encoding murine IL-10 was cloned into a construct containing the mouse glial fibrillary acidic protein (GFAP) promoter and the polyadenylation signal sequence from the human growth hormone gene (hGH), as previously described (Stalder et al., 1998) (Fig. 1A).

The GFAP-IL10 construct was microinjected into fertilised eggs from SJL/L mice. Transgenic offspring were identified by PCR on genomic DNA extracted from tail biopsies using primers against the hGH sequence (Fig. 1B). The F1 offspring were backcrossed with the C57BL/6 strain for at least 10 generations to obtain transgenic mice on the C57BL/6 background.

A total of 48 GFAP-IL10 transgenic (GFAP-IL10Tg) animals and their corresponding wild-type (WT) littermates ($n = 49$) of both sexes were used in this study. Animals were maintained with food and water *ad libitum* in a 12 h light/dark cycle during the experiment.

All experimental animal work was conducted according to Spanish regulations (Ley 32/2007, Real Decreto 1201/2005, Ley 9/2003 and Real Decreto 178/2004) in agreement with European Union directives (86/609/CEE, 91/628/CEE and 92/65/CEE) and was approved by the Ethical Committee of the Autonomous University of Barcelona.

2.2. Tissue processing for PCR analysis

DNA was extracted from tail biopsies using the DNA extraction kit (740.952.250, Macherey–Nagel) following the manufacturer's instructions. Briefly, tail samples were incubated for 2 h at 56 °C in 180 µl T1 buffer and 25 µl proteinase-K. The supernatant obtained after centrifugation for 5 min at 12,000 rpm was transferred to a new tube and 200 µl of lysis buffer 3 and 200 µl of 100% ethanol added and mixed gently. DNA was separated using specific columns provided in the kit that were centrifuged at 12,000 rpm for 3 min. After 2 washes and centrifugation rounds (12,000 rpm for 3 min) with washing buffer and 1 with buffer 5, the DNA was eluted from the column and recovered in a new tube by adding buffer BE and centrifuging at 12,000 rpm for 2 min.

2.3. Tissue processing for ribonuclease protection assay (RPA) analysis

For RPA, 4 month-old WT ($n = 3$) and GFAP-IL10Tg animals ($n = 3$) were anaesthetized with ketamine (80 mg/kg) and xylazine (20 mg/kg; 0.015 ml/g) solution and intracardially perfused with phosphate buffer solution (PBS). The brain was quickly removed and the hippocampus, cerebral cortex cerebellum, thalamus, brainstem and spinal cord areas were dissected out and processed separately. RNA was prepared using Tri-reagent (T9424, Sigma Aldrich) performed according to the manufacturer's instructions. RPA was performed as described previously (Campbell et al., 1994). The RNA samples (3 µg of total RNA) were hybridized with $^{[32P]}$UTP-labelled probe sets, containing cRNA probes for IL-10, CD11b, SOCS3, GFAP and L32. For quantification, autoradiographs were scanned and analysed by densitometry using NIH Image J software (Wayne Rasband, National Institutes of Health, USA). The densitometry value for each transcript was expressed as a ratio to the L32 RNA, which served as a control for RNA loading.

2.4. Tissue processing for Bioplex protein microarray

Adult (6 months) GFAP-IL10Tg animals ($n = 8$) and their corresponding WT littermates ($n = 8$) were euthanised under anaesthesia (as described above) and perfused intracardially for 30 s with phosphate buffer solution (PBS), the entire hippocampus was dissected out quickly, snap frozen in liquid nitrogen and stored at −80 °C. Total protein was extracted by solubilisation of samples in Lysis buffer containing 250 mM HEPES (pH 7.4), 0.2% Igepal, 5 mM $MgCl_2$, 1.3 mM EDTA, 1 mM EGTA, 1 mM PMSF and protease and phosphatase inhibitor cocktails (1:100, Sigma Aldrich). Following solubilisation, samples were clarified by centrifugation at 13,000 rpm for 5 min and the supernatant retained. Total protein concentration was determined with a commercial Pierce BCA Protein Assay kit (23225, Thermo Scientific) according to the manufacturer's protocol. Protein lysates were stored aliquoted at −80 °C until used for Bio-plex protein microarray.

2.5. Analysis of IL-10 with Bio-plex protein microarray

The cytokine IL-10 was analysed using a Bio-plex Pro TM Mouse cytokine GrpI panel kit (M60-009RDPD, Bio-rad) according to the manufacturer's instructions. Briefly, 50 µl of hippocampus extracts

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