Neurobiology of Disease 62 (2014) 260–272

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Mast cells protect from post-traumatic spinal cord damage in mice by degrading inflammation-associated cytokines via *mouse mast cell protease 4*☆

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ARTICLE INFO

Article history:
Received 12 January 2013
Revised 23 August 2013
Accepted 17 September 2013
Available online 26 September 2013

Keywords:
mMCP4
Mast cell
Inflammation
Spinal cord injury
MCP-1
TNF-α
IL-6
IL-10
IL-13

ABSTRACT

Mast cells (MCs) are found abundantly in the central nervous system and play a complex role in neuroinflammatory diseases such as multiple sclerosis and stroke. In the present study, we show that MC-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice display significantly increased astrogliosis and T cell infiltration as well as significantly reduced functional recovery after spinal cord injury compared to wildtype mice. In addition, MC-deficient mice show significantly increased levels of MCP-1, TNF-α, IL-10 and IL-13 protein levels in the spinal cord. Mice deficient in mouse mast cell protease 4 (mMCP4), an MC-specific chymase, also showed increased MCP-1, IL-6 and IL-13 protein levels in spinal cord samples and a decreased functional outcome after spinal cord injury. A degradation assay using supernatant from MCs derived from either mMCP4$^{-/-}$ mice or controls revealed that mMCP4 cleaves MCP-1, IL-6, and IL-13 suggesting a protective role for MC proteases in neuroinflammation. These data show for the first time that MCs may be protective after spinal cord injury and that they may reduce CNS damage by degrading inflammation-associated cytokines via the MC-specific chymase mMCP4.

Introduction

Mast cells (MCs) are abundant at host/environment interfaces, i.e. the skin, airways and gut, where they are known to contribute significantly to the induction of inflammation in the context of allergic reactions and innate immune responses to pathogens (Henz et al., 2001; Marshall, 2004). In the mammalian central nervous system (CNS), MCs are reported as being mainly located in the leptomeninges, the dura mater, the choroid plexus and the parenchyma of the thalamic–hypothalamic region, where they are generally found along the blood vessels (Zappulla et al., 2002). MCs in the healthy brain of rats are found mostly in the thalamus (Brenner et al., 1994), where they are thought to contribute to sensory processing, blood vessel permeability and local hemodynamics (Kil et al., 1999). MC-derived histamine has been reported to potentiate synaptically mediated excitotoxicity in hippocampal neurons of mice in vitro (Skaper et al., 2001) and MC activation reportedly promotes delayed neurodegeneration in murine mixed neuron-glia hippocampal cultures (Skaper et al., 1996).

Substantial progress has been made over the last decade in elucidating the crucial role of MCs in inflammatory CNS disorders such as multiple sclerosis (MS) and stroke [reviewed in Nelissen et al., 2013]. In the animal model of human MS, experimental autoimmune encephalomyelitis (EAE), sites of inflammatory demyelination in the CNS are characterized by MC accumulation and the percentage of degranulated MCs in the CNS correlates with the clinical onset of disease symptoms (Brenner et al., 1994). Furthermore, it has been demonstrated that MC-deficient W/W^{v} mice suffer from a significantly

Abbreviations: MCs, mast cells; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; WT, wildtype; SCI, spinal cord injury; mMCP4, mouse mast cell protease 4; BMS, basso mouse scale; GFAP, glial fibrillary acidic protein; MBP, myelin basic protein; Iba-1, ionized calcium binding adaptor molecule 1; RT, room temperature; SN, supernatants; BMCMCs, bone marrow-derived cultured mast cells; IL, interleukin; TNF-α, tumor necrosis factor α; MCP-1, monocyte chemoattractant protein 1.

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Available online on ScienceDirect (www.sciencedirect.com).

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http://dx.doi.org/10.1016/j.nbd.2013.09.012

less severe myelin-oligodendrocyte-glycoprotein-induced EAE and restoration of the MC population with wildtype (WT) MCs results in disease severity similar to WT mice (Brown et al., 2002; Robbie-Ryan et al., 2003; Secor et al., 2000; Tanzola et al., 2003). In contrast to these previous studies, other data revealed that MCs may be dispensable for EAE development (Bennett et al., 2009; Feyerabend et al., 2011). Conversely, it has been shown that $Kit^{W\text{-}sh/W\text{-}sh}$ mice develop more severe EAE, which is characterized by earlier onset, more severe paralysis, and more extensive demyelination and inflammatory infiltration (Li et al., 2011). MCs are also critically involved in the pathophysiology of ischemic stroke (Lindsberg et al., 2010; Strbian et al., 2009). MCs are one of the first cells to respond to hypoxic–ischemic brain damage in the immature brain. Inhibition of the early MC response resulted in significant neuroprotection (Jin et al., 2009). In the adult rat, MCs are involved in ischemic brain edema early after focal cerebral ischemia onset (Strbian et al., 2006). In the model of intracerebral hemorrhage, MC-deficient rats responded with significantly better neurologic scores than WT animals (Strbian et al., 2007).

In the present study we have analyzed the effects of MC-deficiency in a mouse model of spinal cord injury (SCI). In addition, we propose here a potential new mechanism in which MCs and their secreted protease mMCP4 exert protective effects after CNS trauma by degrading inflammation-associated cytokines.

Materials and methods

Animals

All experiments were performed using 9- to 11-week-old C57BL/6 mice (Harlan, the Netherlands or The Jackson Laboratory, USA), MC-deficient W-sash c-kit mutant knockout mice ($Kit^{W\text{-}sh/W\text{-}sh}$) (Grimbaldeston et al., 2005) (Jackson Laboratory, USA) and mMCP4-deficient ($mMCP4^{-/-}$) mice (Tchougounova et al., 2003) that were housed in a conventional animal facility at Hasselt University under regular conditions, i.e. in a temperature-controlled room (20 ± 3 °C) on a 12 h light–dark schedule and with food and water ad libitum; all experiments were approved by the local ethical committee of Hasselt University and were performed according to the guidelines described in Directive 2010/63/EU on the protection of animals used for scientific purposes.

Spinal cord T-cut hemisection injury

T-cut hemisection injury was performed as described before (Loske et al., 2012; Tuszynski and Steward, 2012). Briefly, 9- to 11-week-old anesthetized female mice underwent a partial laminectomy at thoracic level T8. For the spinal cord bilateral hemisection, iridectomy scissors were used to transect left and right dorsal funiculus, the dorsal horns and additionally the ventral funiculus (T-cut (Loske et al., 2012)). It is important to note that this "T-cut" procedure results in a complete transection of the dorsomedial and ventral corticospinal tract (CST) and impairs several other descending and ascending tracts. The muscles were sutured and the back skin closed with wound clips.

Locomotion tests

Starting 1 day after surgery, functional recovery in SCI mice was measured for three weeks according to the Basso Mouse Scale (BMS) (Basso et al., 2006). The BMS is a 10-point locomotor rating scale (9 = normal locomotion; 0 = complete hind limb paralysis), in which mice are scored by two investigators blinded to the experimental groups, and which is based on hind limb movements made in an open field during a 4-min interval. Data shown represent mean values per experimental group ± SEM.

Immunohistochemical analysis of the spinal cord

Spinal cord cryosections (10 μm) obtained from animals trans cardially perfused 21 days after surgery with ringer solution containing heparin, followed by 4% paraformaldehyde, were preincubated with 10% normal goat serum in PBS containing 5% Triton X-100 for 30 min at room temperature (RT). The following primary antibodies were then incubated for 2 h at RT: rat anti-CD4 (1:500; BD biosciences, Belgium), mouse anti-glial fibrillary acidic protein (GFAP) (1:500; Sigma-Aldrich, Belgium), rabbit anti-myelin basic protein (MBP) (1:100; Millipore, Belgium) and rabbit anti-ionized calcium binding adaptor molecule 1 (Iba-1) (1:350; Wako, Germany).

Following repeated washing steps with PBS, secondary antibodies were applied for 1 h at RT. These were goat anti-rat Alexa Fluor 568, goat anti-mouse Alexa Fluor 568 and goat anti-rabbit Alexa Fluor 488 (dilution 1:250; Invitrogen, Belgium), respectively. After removal of unbound antibodies, DAPI counterstaining was performed for 10 min and sections were mounted. For measurement of lesion size, astrogliosis and inflammatory infiltrate, 5 to 6 sections per animal (8 animals per group) containing the lesion center were analyzed, as described (Loske et al., 2012). Lesion size was evaluated using anti-GFAP immunofluorescence, while the demyelinated area was evaluated using anti-MBP immunofluorescence. The T helper cell infiltration was evaluated by double staining against CD4 and Iba-1 in order to exclude CD4+ microglial cells. Quantification of GFAP and Iba-1 expression was performed by intensity analysis using ImageJ open source software (NIH) within rectangular areas of 100 μm × 100 μm extending from 600 μm cranial to 600 μm caudal from the lesion epicenter. The infiltration of T helper cells was determined by quantifying all T helper cells in the entire perilesional area, 5 mm distal and proximal from the lesion center. For standardization, the analyses were performed on 7 spinal cord sections (per mice) representing the perilesional area, i.e. the lesion epicenter as well as consecutive caudal and cranial sections.

Real-time PCR

Cytokine/chemokine mRNA levels were investigated in different phases after SCI, namely the acute phase (1 h, 6 h, 2 days), the first T cell peak (4 days), the first peak of microglia activation (7 days), the second peak of immune activation (14 and 21 days) and finally at the early stage of the chronic remodeling phase (28 days) (Beck et al., 2010). RNA was isolated from spinal cords of uninjured mice, sham-operated animals and animals with dorsal T-cut hemisection using the RNeasy Mini Plus Kit (Qiagen, the Netherlands), according to the manufacturer's instructions. After reverse transcription (Promega, the Netherlands), cDNAs were amplified by means of specific commercially available primers for interleukin (IL)-1β, IL-4, IL-6, IL-10, IL-13, tumor necrosis factor α (TNF-α) and monocyte chemoattractant protein 1 (MCP-1) (Taqman Gene Expression Assays) on a ABI PRISM 7500 sequence detection system (Applied Biosystems, USA). Briefly, amplification conditions consisted of an initial denaturing/activation step at 95 °C for 20 s, followed by 40 cycles of 3 s at 95 °C and 30 s at 60 °C. A threshold cycle was calculated and relative quantification was obtained by comparison with the threshold cycle obtained by amplifying samples with the reference housekeeping genes β-actin-, hypoxanthine guanine phosphoribosyl transferase 1 and β-glucuronidase. Only statistically significant differences were shown in the figures. If there were no significant differences, findings were reported as "data not shown".

Protein expression in spinal cord and serum samples

Cytokine/chemokine protein levels (systemic and local) were investigated in different phases after SCI (see Real time-PCR). To determine IL-4, IL-6, IL-10, IL-13, MCP-1 and TNF-α protein levels in spinal cord and serum samples from C57BL/6 mice, MC-deficient $Kit^{W\text{-}sh/W\text{-}sh}$ mice and $mMCP4^{-/-}$ mice (3 groups: uninjured mice, sham-operated mice