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Research Report

Expression of CD200 in alternative activation of microglia following an excitotoxic lesion in the mouse hippocampus

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ABSTRACT

CD200 is a glycoprotein that is expressed on the surfaces of neurons and other cells. It interacts with its receptor, CD200R, which is expressed on cells of the myeloid lineage, including microglia. The interaction of CD200 with its receptor plays a significant role in maintaining microglia in a quiescent state; thus, a decrease in CD200 expression in the brain is associated with evidence of microglial activation. However, their roles in pathological progression remain unclear. We examined the expression of CD200 in kainic acid (KA)-induced neurodegeneration of the mouse hippocampus. Our quantitative analysis revealed that CD200 was constitutively expressed in the normal brain and transiently upregulated by KA treatment. At the cellular level, CD200 was expressed in neurons in control, and was upregulated primarily in the microglia of KA-treated mouse hippocampi. We examined the contribution of CD200 to both the classical and alternative activation of microglia in vitro using an adult microglia culture, which was exposed to interleukin-4 (IL-4) with and without lipopolysaccharide (LPS). CD200 expression was increased after exposure to IL-4, but not to LPS. These in vivo experiments demonstrated that CD200 was transiently expressed in microglia in a process mediated by the inflammatory response. Based on CD200R expression in microglia, it suggests that microglia is maintained in an activated state with autocrine signaling by interactions between microglial CD200 and its CD200R. Moreover, we suggest that CD200 may be expressed in the alternative activation of microglia and play a beneficial role in neuroinflammation.

1. Introduction

CD200 is a 41–47 kDa surface molecule and a member of the immunoglobulin supergene family. This highly conserved molecule, characterized by two immunoglobulin superfamily (IgSF) domains (Barclay et al., 2002), is found in invertebrates and vertebrates. Many glycoproteins containing this arrangement are involved with immune system regulation. In the

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healthy brain, CD200 is expressed in gray matter areas, including the cerebral cortex, hippocampus, striatum, cerebellum, and spinal cord. CD200 is localized to neurons and oligodendrocytes, but not to astrocytes or microglia. In central nervous system (CNS) samples from patients with multiple sclerosis, CD200 expression was also observed on reactive astrocytes in chronic active plaque centers (Koning et al., 2009). The counter receptor to CD200, the CD200 receptor (CD200R), also contains two IgSF domains and is expressed by perivascular macrophages and brain microglia. In CD200-deficient mice, activated microglia and macrophages were more numerous after the induction of experimental autoimmune encephalitis than in wild-type animals, providing evidence that the CD200/CD200R interaction is related to the regulation of microglial activation and local inflammation (Hoek et al., 2000; Lyons et al., 2007).

Although previous research efforts have focused on identifying triggers that lead to glial activation, interaction with other cells is increasingly recognized to play a significant role in modulating activation. For example, reports have described the interaction of T cells, neurons, and endothelial cells with microglia, which leads to the modulation of microglial activation, but the mechanism by which these interactions result in the down-regulation of microglial activation remains to be clarified (Deckert et al., 2006; Ponomarev et al., 2007b). CD200 is known to play a role in modulating inflammatory responses (Lyons et al., 2007). We sought to assess whether CD200/CD200R interaction played a role in modulating microglial activation in rodent models of neurodegeneration. Kainic acid (KA) is an excitatory glutamate receptor agonist that elicits severe status epilepticus with subsequent neuronal cell death in the hippocampal CA3 region (Andersson et al., 1991; Penkowa et al., 2005). Excessive secretion of glutamate, free radicals, and cytokines has been implicated in the mechanisms of excitotoxic neurodegeneration induced by KA (Beal, 1992). Intracerebroventricular (i.c.v.) injections of KA provide a reliable model for studies of glial modification in response to neuronal death. We thus examined temporal patterns and cellular localization of CD200 expression in this model and investigated the anti-inflammatory role of CD200 using lipopolysaccharide (LPS)- and interleukin-4 (IL-4)-treated primary microglia.

2. Results

2.1. CD200 expression in KA-treated mouse hippocampus

Single bands on Western blots corresponding to CD200 at 47 kDa were detected in both the control and KA-injected mouse hippocampi. Significant changes in CD200 were associated with KA treatment (Fig. 1). CD200 levels began to peak at 1 day post-KA injection, decreased at 3 days post-KA injection, and returned to baseline levels at 7 days post-KA injection. Selective lesions were observed in the hippocampal CA3 regions of KA-injected mice (data not shown), as we described previously (Jeon et al., 2004; Kim et al., 2007; Lee et al., 2011). Pyramidal cell degeneration in the CA3 region appeared at 1 day post-KA injection. At 3 and 7 days post-KA injection, pyramidal cell loss and glial reactivity were evident in the CA3 region. These findings confirmed that the i.c.v. injection of KA used in this study produced a specific loss of CA3 pyramidal cells, in agreement with previously reported results (Mitchell et al., 1993; Nadler et al., 1980).

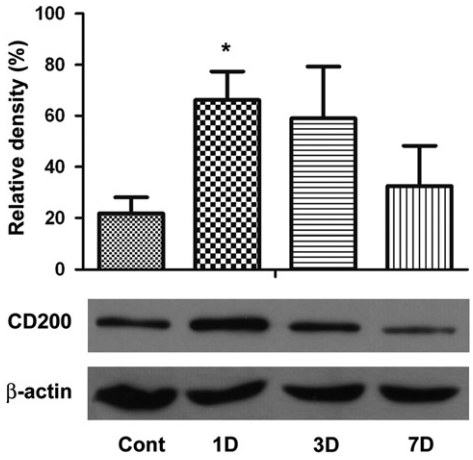

Fig. 1 – Western blot analyses of CD200 in control and kainic acid-treated mouse brains. β-Actin was used as a loading control. Band densities were analyzed with ImageJ and expressed as a percentage of control. Bars indicate mean ± standard errors of the mean of three independent experiments. Differences between means were analyzed with a one-way analysis of variance followed by the Newman–Keuls test. $^{*}P < 0.05$ vs. control.

2.2. CD 200 expression in microglia

Moderate CD200 immunoreactivity (IR) was observed in control mice; it was restricted to the major neuronal layers, i.e., the pyramidal cell layer of the hippocampus and the granular layer of the dentate gyrus (Fig. 2A). In contrast, strong CD200 IR was noted from 1 day post-lesion, when it appeared in scattered glial cells in the hippocampus. Newly emerged CD200 IR cells spread across the whole hippocampus, including the CA3 region (Fig. 2B). At longer survival times, CD200 IR clearly decreased and was gradually confined to the CA3 lesion undergoing neuronal death (Fig. 2C), then returned to control levels at 7 days post-lesion (Fig. 2D). CD200 IR cells showed signs of activation and exhibited mainly pseudopodic/ameboid morphology (Fig. 2F). In contrast, no sign of glial activation or CD200 IR was evident in control hippocampi (Fig. 2E). The CD200-positive cells appeared most likely to be activated microglia, which we confirmed using double-labeling methods for CD200 and ionized calcium binding adaptor molecule 1 (Iba1), a known marker for activated microglia (Ito et al., 1998). As described above, CD200 IR was confined to neuronal cells in the control group. Consequently, the two antigens were differently localized, and no overlap was observed between CD200 and Iba1 IR (Fig. 3A–C). In contrast, CD200 IR was colocalized in Iba1-positive microglia