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MHC class I protein is expressed by neurons and neural progenitors in mid-gestation mouse brain

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ABSTRACT

Proteins of the major histocompatibility complex class I (MHCI) are known for their role in the vertebrate adaptive immune response, and are required for normal postnatal brain development and plasticity. However, it remains unknown if MHCI proteins are present in the mammalian brain before birth. Here, we show that MHCI proteins are widely expressed in the developing mouse central nervous system at mid-gestation (E9.5–10.5). MHCI is strongly expressed in several regions of the prenatal brain, including the neuroepithelium and olfactory placode. MHCI is expressed by neural progenitors at these ages, as identified by co-expression in cells positive for neuron-specific class III β-tubulin (Tuj1) or for Pax6, a marker of neural progenitors in the dorsal neuroepithelium. MHCI is also co-expressed with nestin, a marker of neural stem/progenitor cells, in olfactory placode, but the co-localization is less extensive in other regions. MHCI is detected in the small population of post-mitotic neurons that are present at this early stage of brain development, as identified by co-expression in cells positive for neuronal microtubule-associated protein-2 (MAP2). Thus MHCI protein is expressed during the earliest stages of neuronal differentiation in the mammalian brain. MHCI expression in neurons and neural progenitors at mid-gestation, prior to the maturation of the adaptive immune system, is consistent with MHCI performing non-immune functions in prenatal brain development. These results raise the possibility that disruption of the levels and/or patterns of MHCI expression in the prenatal brain could contribute to the pathogenesis of neurodevelopmental disorders.

Introduction

Proteins of the major histocompatibility complex class I (MHCI) present antigenic peptides for cellular immune surveillance, a key element of the vertebrate adaptive immune response (Neefjes and Momburg, 1993). The central nervous system (CNS) is functionally "immune privileged", meaning that adaptive immune responses in the brain are often blunted or delayed relative to other sites. This was long thought to be due to a lack of expression of MHCI proteins by healthy neurons under basal conditions (Neumann et al., 1995; Rall et al., 1995). However, numerous studies show that MHCI mRNA and protein are expressed by healthy neurons in the postnatal central and peripheral nervous system (e.g., Corriveau et al., 1998; Datwani et al., 2009; Huh et al., 2000; Ishii and Mombaerts, 2008; Ishii et al., 2003; Lidman et al., 1999; Linda et al., 1998; Loconto et al., 2003; McConnell et al., 2009; Miralves et al., 2007; Needleman et al., 2010; Ribic et al., 2011; Thams et al., 2008).

In addition, recent studies show that MHCI has unexpected, non-immune functions in the developing and adult brain. MHCI protein is enriched in synaptic fractions of adult rodent brain (Huh et al., 2000) and co-localizes with the post-synaptic density protein PSD-95 in dendrites of acutely-dissociated postnatal hippocampal neurons (Goddard et al., 2007), consistent with a role for MHCI in regulating synapse function (Boulanger, 2009). Indeed, in the adult mouse hippocampus, genetic reduction of cell surface MHCI is associated with increased NMDAR-mediated synaptic transmission (Fourgeaud et al., 2010) as well as an increase in the magnitude of NMDAR-dependent long-term potentiation (LTP) and loss of NMDAR-dependent long-term depression (LTD) (Huh et al., 2000). In the adult cerebellum, loss of the genes encoding the classical MHCIs H2-K and H2-D is associated with a lower threshold for the induction of LTD at parallel fiber-Purkinje cell synapses (McConnell et al., 2009). Thus endogenous MHCI is essential for normal synaptic transmission and plasticity in multiple regions of the adult mammalian brain.

MHCI is also expressed at earlier postnatal ages. MHCI mRNA is expressed in rodent and cat lateral geniculate nucleus (LGN) during the first two postnatal weeks (Corriveau et al., 1998; Huh et al., 2000), and reduced cell surface expression of MHCI protein in rodent genetic models disrupts postnatal development of projections

Abbreviations: β2m, beta-2 microglobulin; CNS, central nervous system; HES cell, human embryonic stem cell; LGN, lateral geniculate nucleus; LTD, long-term depression; LTP, long-term potentiation; MHCI, major histocompatibility complex class I; NSC, neural stem cell; PNS, peripheral nervous system; VNO, vomeronasal organ.

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from retina to LGN during this time (Datwani et al., 2009; Huh et al., 2000). MHCI mRNA expression has also been detected in prenatal cat LGN (E43 and E52; gestation is 65 days), where it is downregulated by chronic blockade of sodium-based action potentials (Corriveau et al., 1998). However, it is unknown if MHCI plays a role in prenatal brain development *in vivo*. A key first step is to determine if MHCI protein is normally present in the mammalian brain before birth.

In this study, we show that MHCI protein is widely expressed in the developing mouse CNS at mid-gestation. MHCI is expressed by cells positive for markers of neuronal precursors (Pax6, nestin, Tuj1) as well as newly-differentiated neurons (MAP2). Thus MHCI is expressed at the earliest stages of neuronal differentiation in the developing mammalian brain.

Results

To determine if MHCI proteins are expressed prenatally, mouse embryos were stained using a mouse monoclonal antibody (OX-18) that recognizes a monomorphic epitope of rat MHCI (RT-1A; Fukumoto et al., 1982) and reacts with mouse MHCI in Western blots and immunohistochemistry (Corriveau et al., 1998; Datwani et al., 2009; Huh et al., 2000; Needleman et al., 2010; Rolleke et al., 2006). Multiple lines of evidence support the specificity of this antibody in labeling MHCI in neurons (Table 1). First, cell surface immunofluorescence is greatly attenuated in both $\beta2m^{-/-}$ and $\beta2m^{-/-}TAP^{-/-}$ cells (Needleman et al., 2010; Goddard et al., 2007), in which reduced levels of MHCI reach the cell surface (Dorfman et al., 1997; Zijlstra et al., 1989). Second, OX-18 recognizes proteins of the expected molecular weight in western blots of adult mouse brain (Corriveau et al., 1998; Huh et al., 2000; Needleman et al., 2010), and similar banding patterns are seen with a rabbit polyclonal anti-MHCI antibody that recognizes a distinct epitope (Needleman et al., 2010). Third, light- and electron- microscopic localization of OX-18 signals in cortex parallels patterns seen with another anti-MHCI monoclonal antibody raised against a distinct epitope (Needleman et al., 2010). Fourth, OX-18 labeling in marmoset brain is reduced by treatment with an immunosuppressive drug (FK506; Rolleke et al., 2006). We see OX-18 staining in the adult mouse that is identical to published results in all brain regions examined, including cortex, cerebellum, and hippocampus, as well as in acutely-dissociated perinatal mouse hippocampal neurons *in vitro* (Corriveau et al., 1998; Datwani et al., 2009; Glynn and McAllister, 2006; McConnell et al., 2009; Needleman et al., 2010). Our adult mouse brain staining with OX-18 is abolished if the antibody is replaced with an isotype control or applied to MHCI-deficient tissue (not shown). In fetal mouse, we also find that omitting the primary antibody or incubating with an isotype-control antibody both abolish staining (see Fig. 1B–C). Taken together, these studies provide strong support for the specificity of OX-18 in recognizing MHCI in the brain in our hands.

Whole mouse embryos were stained using the anti-MHCI antibody OX-18 at mid-gestation (E9.5–E10.5), based on date of mating and observation of vaginal plug, and confirmed by comparison to anatomical markers of Theiler stages (stages 14–18; http://www.emouseatlas.org/emap/ema/theiler_stages/house_mouse/book.html). These ages correspond roughly to days 22–28 of human embryonic development, or E11-12 in rat, based on Carnegie stage comparisons (http://php.med.unsw.edu.au/embryology/index.php?title=Carnegie_Stage_Comparison).

Table 1
Previous experimental approaches and results obtained supporting the specificity of the OX-18 antibody in detecting MHCI proteins in the brain.

Neuronal technique	Observation	Reference
Immunohistochemistry (OX18)	• Stains neurons in most layers of rat cortex • Stains hippocampal neurons in vitro • Cell surface immunofluorescence attenuated in MHCI-deficient ($\beta2m^{-/-}$ and $\beta2m^{-/-}TAP^{-/-}$) cells • Light-level labeling parallels patterns seen with another anti-MHCI monoclonal antibody raised against a distinct epitope • Labels neurons in marmoset brain • Labeling reduced following treatment with immunosuppressive drug (FK506)	Needleman et al. (2010), Rolleke et al. (2006), Goddard et al. (2007)
Western blot (OX18)	• Bands of expected size detected in rat primary somatosensory cortex membranes • Bands of expected size detected in rat synaptosomal fractions • Bands of expected size detected in rat visual cortex • Similar banding patterns seen with a rabbit polyclonal anti-MHCI antibody that recognizes a distinct epitope	Corriveau et al. (1998), Huh et al., (2000), Needleman et al. (2010)
Electron microscopy (OX18)	• MHCI found in synapses (pre- and post-synaptic), axon terminals and dendrites of layer V, adult rat visual cortex • EM-level labeling parallels results seen with another anti-MHCI monoclonal antibody raised against a distinct epitope	Needleman et al. (2010)

MHC class I is expressed in prenatal brain

Immunofluorescence microscopy of coronal and parasagittal sections revealed specific staining for MHCI throughout the embryo, including the developing somites (not shown), organs, nasal process, and CNS (Fig. 1G). Similar patterns were seen in four rounds of immunostaining, and representative images are shown. Hoechst staining of nuclei (e.g., Fig. 1D–F and J) provides anatomical landmarks. In prenatal brain, MHCI is expressed throughout the developing neuroepithelium, including the mesencephalon, diencephalon and telencephalon (Fig. 1A). Incubation with a mouse isotype control IgG (Fig. 1B) or in the absence of primary antibody (Fig. 1C) did not yield appreciable staining, supporting the specificity of MHCI immunolabeling. In a more lateral section, MHCI expression is detected in the nasal process (Fig. 1G–I). At higher magnification (Fig. 1I), MHCI staining fills the cell soma, consistent with cytosolic and/or membrane localization in these permeabilized sections. Similar somatic labeling is also observed in dorsal neuroepithelial cells in the same section (Fig. 1K and L). Overall, MHCI staining intensity in the embryonic brain was comparable to, or stronger than, staining with the same antibody in adult brain (not shown).

Co-expression of MHCI and early neuronal marker MAP2

To determine if MHCI is expressed by newly-differentiated neurons, double-label immunostaining was performed using an antibody that recognizes microtubule-associated protein 2 (MAP2). MAP2 is a major component of the neuronal cytoskeleton that is critical for neuronal morphology and differentiation (Matus, 1988), and MAP2 is widely used as an early neuronal marker (e.g., Cheyne et al., 2011; Chun and Shatz, 1989; Ferri and Levitt, 1993). In parasagittal sections, MAP2 is expressed mainly by peripheral neuroepithelial cells in both mesencephalon and diencephalon (Fig. 2B). At higher magnification, it is evident that MAP2 is expressed only by cells at the periphery of the neuroepithelium (Fig. 2F), while MHCI is detected more widely throughout the neuroepithelium at this age