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The Immune Protein CD3ζ Is Required for Normal Development of Neural Circuits in the Retina

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SUMMARY

Emerging evidence suggests that immune proteins regulate activity-dependent synapse formation in the central nervous system (CNS). Mice with mutations in class I major histocompatibility complex (MHCI) genes have incomplete eye-specific segregation of retinal ganglion cell (RGC) axon projections to the CNS. This effect has been attributed to causes that are nonretinal in origin. We show that a key component of MHCI receptor, CD3ζ, is expressed in RGCs. CD3ζ-deficient mice have reduced RGC dendritic motility, an increase in RGC dendritic density, and a selective defect of glutamate-receptor-mediated synaptic activity in the retina. Disrupted RGC synaptic activity and dendritic motility is associated with a failure of eye-specific segregation of RGC axon projections to the CNS. These results provide direct evidence of an unrecognized requirement for immune proteins in the developmental regulation of RGC synaptic wiring and indicate a possible retinal origin for the disruption of eye-specific segregation found in immune-deficient mice.

INTRODUCTION

Recent studies suggest that genes typically associated with the immune system, such as those in the major histocompatibility complex, are expressed by neurons in various regions of the central nervous system (CNS) and may play important roles in synapse formation (Corriveau et al., 1998; Huh et al., 2000; Ishii et al., 2003; Syken and Shatz, 2003; Syken et al., 2006; Baudouin et al., 2008). Synaptic circuits in the visual system, particularly eye-specific retinogeniculate projections, have proven to be an excellent model for these studies. Genetic deletion or mutation of a number of MHC class I genes (MHCI), including β2-microglobulin (an MHCI cosubunit) or CD3ζ (a key component of MHCI receptors), results in the failure of eye-specific segregation of retinal ganglion cell (RGC) axon projections to the dorsal lateral geniculate nucleus (dLGN) (Huh et al., 2000). In addition, genetic deletion of MHCI molecules enhances long-term potentiation (LTP) and abolishes long-term depression (LTD) in hippocampus (Huh et al., 2000) and increases the frequency of spontaneous "miniature" synaptic currents (mEPSCs) in hippocampal and cortical neurons (Goddard et al., 2007). Furthermore, spatial learning, memory, and neurogenesis in hippocampus are markedly reduced in immune-deficient mice (Ziv et al., 2006), strongly suggesting that a common immune-associated mechanism might regulate various aspects of activity-dependent synaptic development and plasticity in the CNS.

Activation of immune molecules in neurons could produce similar intracellular signals as those generated in immune cells but with different ultimate effect, such as altering synaptic development, strength, neuronal morphology, or circuit properties downstream of synaptic activity (Boulanger et al., 2001; Fourgeaud and Boulanger, 2007; Syken et al., 2006). For instance, in the immune system activation of CD3ζ regulates immune cell morphology by reorganizing the actin-based cytoskeleton (Baniyash, 2004). Similarly, direct activation of CD3ζ on hippocampal neurons affects cell morphology by promoting dendritic pruning through a tyrosine-based phosphorylation signaling motif common to the immune system (Baudouin et al., 2008). Alternatively, MHCI proteins in neurons may interact with non immune proteins through nonclassical signaling pathways (Ishii et al., 2003; Ishii and Mombaerts, 2008). We sought to shed light on the role of MHCI proteins in the CNS by examining whether and how genetic mutation of CD3ζ affects RGC dendritic pruning, synaptic activity, and eye-specific segregation during development.

Numerous reports show that both the developmental segregation of eye-specific projections of RGC axons to the dLGN and the laminar-specific distribution of RGC dendrites in the retina are regulated by retinal synaptic activity (Akerman et al., 2002; Bansal et al., 2000; Bodnarenko and Chalupa, 1993; Chapman, 2000; Grubb and Thompson, 2004; Huberman et al., 2003; Muir-Robinson et al., 2002; Penn et al., 1998; Rossi et al., 2001; Shatz and Stryker, 1988; Torborg et al., 2005; Tian and Copenhagen, 2003; Wong et al., 2000; Wong and Ghosh, 2002; Xu and Tian, 2007). Recent studies examining immune-deficient mice found that abnormalities in RGC eye-specific segregation are not associated with functional retinal defects during the first postnatal week and therefore concluded that abnormal retinogeniculate projections are due to a loss of

immune-protein-mediated signaling in the dLGN (Huh et al., 2000;). However, it is well documented that retinal activity during early postnatal development is mediated by two major excitatory neurotransmitters, acetylcholine during the first postnatal week and glutamate thereafter (Bansal et al., 2000; Demas et al., 2003; Feller et al., 1996; Zhou, 2001). Pharmacological or genetic blockade of either cholinergic or glutamatergic retinal synaptic activity perturbs the development of eye-specific segregation of RGC axonal projections to the dLGN (Rossi et al., 2001; Chapman, 2000; Grubb and Thompson, 2004; Muir-Robinson et al., 2002; Penn et al., 1998; Torborg et al., 2005). Therefore, it remains unanswered whether retinal activity mediated by glutamate receptors (GluRs) during the second postnatal week is impaired in immune-deficient mice and whether this impairment plays a role in eye-specific segregation defects in these mice. A further goal of the present study is to determine whether genetic mutation of CD3ζ affects the development of retinal synaptic circuitry.

Accordingly, we examined the development of RGC dendritic/axonal structure and synaptic activity in wild-type (WT) mice and mice with genetic mutation of CD3ζ (*CD3ζ*$^{-/-}$ mice). We reveal a mechanism by which CD3ζ regulates the formation of RGC synapses in both retina and dLGN. Our data show that CD3ζ is preferentially expressed by neurons in the RGC layer of the retina. In *CD3ζ*$^{-/-}$ mice, the kinetics of RGC dendritic elimination is markedly reduced, and the number of dendritic protrusions is significantly increased during early postnatal development. Application of GluR antagonists to developing WT retinas mimics the RGC dendritic defects of *CD3ζ*$^{-/-}$ mice, confirming the synaptic origin of the dendritic phenotypes in CD3ζ mutants. In mature CD3ζ mutants, RGCs have increased dendritic density, widespread dendritic ramifications in the inner plexiform layer (IPL), and retarded segregation of RGC dendrites into ON and OFF synaptic pathways. In addition, the strength of spontaneous retinal activity mediated by GluRs during the second postnatal week, but not that mediated by nicotinic acetylcholine receptors (AChRs) during the first postnatal week, is selectively reduced. Consistent with this, the activity-dependent eye-specific segregation of RGC axonal projections in dLGN is normal at the end of the first postnatal week but fails to improve during the second postnatal week in CD3ζ mutants, suggesting that the dominant phenotypes described in the dLGN of *CD3ζ*$^{-/-}$ mice are a downstream effect of the deficiencies in GluR-mediated synaptic activity in the retina. Furthermore, light-evoked GluR-mediated responses of RGCs and amacrine cells (ACs) in immature CD3ζ mutants are reduced without significant change of presynaptic bipolar cell (BC) light response, suggesting that in the visual system the initial site of CD3ζ-mediated effects is at synapses between BCs and RGCs. In total, these results directly demonstrate that CD3ζ regulates synaptic wiring and selectively impairs GluR-mediated synaptic activity in the retina during development.

RESULTS

RGCs of *CD3ζ*$^{-/-}$ Mice Have Altered Dendritic Structure in Developing Retina

We first examined the expression of CD3ζ in the developing mouse retina. We observed strong CD3ζ immunoreactivity mainly in the inner retina, including both cells located in the RGC layer and IPL, in both developing and more mature mice (Figure 1A). Double labeling of CD3ζ with RGC or AC markers revealed that most, if not all, Brn3b-positive RGCs are CD3ζ positive (Figure 1B), as are many displaced ACs located in the RGC layer (labeled by the pan-AC antibody, Pax6). However, most ACs in the inner nuclear layer (INL), labeled with the same antibody, are CD3ζ negative (Figure 1B). In situ hybridization confirmed the expression of CD3ζ mRNA in the RGC layer in both developing (P14) and more mature (P28) mice. To further investigate the synaptic localization of CD3ζ in the retina, we performed double immunostaining using antibodies against CD3ζ and synaptic markers. We observed many CD3ζ-positive puncta that colocalized with the postsynaptic protein PSD95 in the IPL (Figure 1C). We also found that many CD3ζ-positive puncta in the IPL were closely associated with the presynaptic ribbon protein, CtBP2, in both young and more mature retinas (Figure 1D). Taken together, these findings demonstrate that CD3ζ is preferentially expressed by retinal neurons located in the RGC layer in the developing retina and is localized at synapses in the IPL during the period of synaptic formation. In addition, we used RT-PCR to confirm that retinal neurons in *CD3ζ*$^{-/-}$ mice express only a truncated mRNA of the gene (*CD247*) encoding CD3ζ protein. Exons 2 and 3 of the *CD247* gene are not expressed in this truncated mRNA (Figure 1E), which is consistent with previous reports on immune cells in *CD3ζ*$^{-/-}$ mice (Love et al., 1993).

Recent studies reported that immune-deficient mice, including *CD3ζ*$^{-/-}$ mice, have defects in eye-specific segregation of RGC axon projections to the dLGN (Huh et al., 2000). We therefore examined whether genetic mutation of CD3ζ affects the structure of RGC dendrites as well. To visualize the fine dendritic structure of RGCs, we used a line of transgenic mice in which Yellow Fluorescent Protein (YFP) is expressed in a small fraction of RGCs (Feng et al., 2000). At P12, RGC dendrite morphology in YFP^{+}/*CD3ζ*$^{-/-}$ mice was very different than littermate controls, particularly in the number of dendritic protrusions (Figure 2A). We classified YFP^{+} RGCs into 12 morphological subtypes following the approach developed by Sun et al. (2002) and Diao et al. (2004), which is based on the soma size, dendritic field size, dendritic stratification level in the IPL, and branching pattern of RGCs. Although the total branch number is increased, RGC soma and dendritic field size as well as dendritic stratification level in the IPL are well conserved (see below), making RGC subtype classification straightforward for *CD3ζ*$^{-/-}$ mice. We manually traced and quantified the dendritic branches of two RGC morphological subtypes (A1 and A2). On average, the number of dendritic protrusions of both A1 and A2 RGCs in *CD3ζ*$^{-/-}$ mice are 6-fold higher than that of age-matched WT controls (Figure 2B). A similar increase in dendritic protrusions was found in other RGC subtypes (Figure S1). In addition, the density of dendritic protrusions in both A1 and A2 RGCs of *CD3ζ*$^{+/-}$ mice are significantly higher than that of WT mice, but much lower than that of *CD3ζ*$^{-/-}$ mice (Figure 2B), suggesting a gene dosage effect. We also examined the dendritic density of all morphological subtypes of RGCs using Sholl analysis (Figure 2C) (Sholl, 1953) and found that the dendrites of 4 (B2, B3, C1, and C5) out of a total of 12 morphological RGC subtypes were significantly denser than that of age-matched WT controls