Astrocyte-derived interleukin-33 promotes microglial synapse engulfment and neural circuit development

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Neuronal synapse formation and remodeling are essential to central nervous system (CNS) development and are dysfunctional in neurodevelopmental diseases. Innate immune signals regulate tissue remodeling in the periphery, but how this affects CNS synapses is largely unknown. Here, we show that the interleukin-1 family cytokine interleukin-33 (IL-33) is produced by developing astrocytes and is developmentally required for normal synapse numbers and circuit function in the spinal cord and thalamus. We find that IL-33 signals primarily to microglia under physiological conditions, that it promotes microglial synapse engulfment, and that it can drive microglial-dependent synapse depletion in vivo. These data reveal a cytokine-mediated mechanism required to maintain synapse homeostasis during CNS development.

Despite the emerging roles of astrocytes and microglia in neuronal synapse formation and remodeling, how they coordinate synaptic homeostasis in vivo remains obscure. Interleukin-33 (IL-33) is an IL-1 family member with well-described roles as a cellular alarmin released from nuclear stores after tissue damage, including in spinal cord injury (11, 12), stroke (13), and Alzheimer’s disease (14). Whereas many cytokines are primarily defined by their roles in inflammation and disease (e.g., IL-1, tumor necrosis factor–α, or IL-6), IL-33 also promotes homeostatic tissue development and remodeling (15). The CNS undergoes extensive synapse remodeling during postnatal brain development, but a role for IL-33 or other stromal-derived cytokines is unknown. Here, we report that IL-33 is produced postnatally by synapse-associated astrocytes, is required for synaptic development in the thalamus and spinal cord, and signals to microglia to promote increased synaptic engulfment. These findings reveal a physiologic requirement for cytokine-mediated immune signaling in brain development.

We previously developed methods to identify functionally heterogeneous astrocytes by expression profiling of distinct CNS regions (16). In an RNA-sequencing screen of developing forebrain astrocytes (P9) (flow sorted using an Aldh1L1GFP reporter), we identified the cytokine IL-33 as a candidate that is both astrocyte-enriched and heterogeneously expressed by astrocytes throughout the CNS (fig. S1). We confirmed astrocyte-specific developmental expression of IL-33 in spinal cord and thalamus using a nuclear-localized IL-33 reporter (R26rtmCherry/eYFP) (Fig. 1A) and validated these findings with flow cytometry and protein immunostaining (fig. S2). By adulthood, a subset of oligodendrocytes also coexpressed IL-33 (fig. S2, C to E), consistent with previous reports (11). Thus, astrocytes are the primary source of IL-33 during postnatal synapse maturation.

Although most IL-33–positive cells were astrocytes, not all developing astrocytes expressed IL-33, and this number increased in the early postnatal period (fig. S3) (17). In fact, IL-33 was detected only in gray matter, where most synapses are located (Fig. 1B and figs. S2H and S3D). In the thalamus, which receives regionally distinct sensory synaptic inputs, IL-33 expression in the visual nucleus (dLGN) increased coincident with eye opening [postnatal days 12 to 14 (P12 to P14)] (Fig. 1, C and D). Removal of afferent sensory synapses by enucleation at birth prevented this developmental increase in IL-33 expression (Fig. 1, E and F), whereas dark rearing, in which synapse maturation is largely preserved (18), had no effect. Molecular profiling of IL-33–positive astrocytes in both thalamus and spinal cord (Fig. 1, G and H) revealed a negative correlation with white-matter astrocyte markers (Gfap and Vimentin), enrichment for genes involved in astrocyte synaptic functions (connexin-30/Gjb6) (19), and enrichment in G protein–coupled and neurotransmitter receptors (e.g., Adora2b and Adra2a) (tables S1 to S3 and data S1). Together, these data demonstrate that IL-33 expression is correlated with synaptic maturation and marks a subset of astrocytes potentially sensitive to synaptic cues, raising the question of whether IL-33 plays a role in synapse development.

To determine whether IL-33 regulates neural circuit development and function, we examined the effect of IL-33 deletion on synapse numbers and circuit activity. In the thalamus, a region with high IL-33 expression, an intrathalamic circuit between the ventrobasal nucleus (VB) and the reticular nucleus of the thalamus (RT) displays spontaneous oscillatory activity that can also be evoked by stimulating the internal capsule that contains cortical afferents (20, 21). We quantified this oscillatory activity in slices from young adult mice (P30 to P40), which revealed enhanced evoked activity in response to stimulation (Fig. 2, A and B, and fig. S4, A to D), as well as elevated spontaneous firing in the absence of IL-33 (Fig. 2C and fig. S4C). This increase could result at least in part from enhanced numbers of glutamatergic synapses. To investigate this hypothesis, we performed whole-cell patch-clamp recordings of VB neurons to quantify miniature excitatory postsynaptic currents (mEPSCs) (Fig. 2D). We found that the frequency of mEPSCs was enhanced in VB neurons from IL-33–deficient mice, whereas the amplitude and the kinetics were unchanged (fig. S4D). Together, these results suggest that IL-33 deficiency leads to excess excitatory synapses and a hyperexcitable intrathalamic circuit.

In the spinal cord, α-motor neurons (α-MN) are the primary outputs of the sensorimotor circuit and receive inputs from excitatory (VGLUT2+) and inhibitory (VGA+) interneurons (Fig. 2E) (22). We conditionally deleted IL-33 from astrocytes (hGFAPCre) (16) (fig. S5A) and found increased numbers of excitatory and inhibitory neurons.
inputs onto α-MN at P30; global deletion of Il1rl1 (ST2) (Fig. 2, F to I) or Il33 (fig. S5, B and C) phenocopied this finding. Neuronal soma size, interneuron numbers, and oligodendrocyte numbers were unchanged (fig. S5, D to F).

However, by adulthood, IL-33 deficiency led to increased gray-matter expression of glial fibrillary acidic protein (GFAP) (fig. S5, G and H), a marker of tissue stress. We also found that Il33−/− animals had deficits in acoustic startle response, a sensorimotor reflex mediated by motor neurons in the brainstem and spinal cord (Fig. 2, J and K) (23). Auditory acuity and gross motor performance were normal (fig. S5, I and J). Taken together, these data demonstrate that IL-33 is required for normal synapse development, as in other CNS regions (7, 8), and found decreased engulfment in microglia from Il33−/− animals (P15) (Fig. 3D). This was further validated by dye labeling of spinal cord motor neurons, which revealed fewer dye-filled microglia in Il33−/− (fig. S7). Conversely, local injection of IL-33 increased PSD-95 within microglia throughout development, as in other CNS regions (7, 8), and found decreased engulfment in microglia from Il33−/− animals (P15) (Fig. 3D). This was further validated by dye labeling of spinal cord motor neurons, which revealed fewer dye-filled microglia in Il33−/− (fig. S7). Conversely, local injection of IL-33 increased PSD-95 within microglia in both spinal cord (Fig. 3E) and thalamus (fig. S8, A and B) and altered markers consistent with increased PSD-95 (Fig. 3F).

To determine the cellular targets of IL-33 signaling, we first quantified expression of its oblique co-receptor IL1RL1 (ST2) (15). We detected Il1rl1 in microglia by RNA sequencing (7.1 ± 2.1 fragments per kilobase of transcript per million mapped reads) and by quantitative polymerase chain reaction (qPCR), in contrast to astrocytes, neurons, or the lineage-negative fraction (Fig. 3A). The transcriptome of acutely isolated microglia from Il33−/− animals revealed 483 significantly altered transcripts, including reduced expression of nuclear factor κB (NF-κB) targets (e.g., Tnf, Nfkbia, Nfkbia, and Tnfaip3) (Fig. 3, B and C; fig. S6A; and data S2), consistent with decreased engulfment in microglia throughout development, raising the question of whether it promotes physiologic microglial functions.

Given the increased synapse numbers in IL-33-deficient animals, we investigated whether IL-33 is required for microglial synapse engulfment. We detected engulfed PSD-95 synaptic puncta within spinal cord microglia throughout development, as in other CNS regions (7, 8), and found decreased engulfment in microglia from Il33−/− animals (P15) (Fig. 3D). This was further validated by dye labeling of spinal cord motor neurons, which revealed fewer dye-filled microglia in Il33−/− (fig. S7). Conversely, local injection of IL-33 increased PSD-95 within microglia in both spinal cord (Fig. 3E) and thalamus (fig. S8, A and B) and altered markers consistent with increased PSD-95 (Fig. 3F).

**Fig. 1.** IL-33 is developmentally induced in synapse-associated astrocytes. (A) Representative image of Il33mCherry with Aldh1l1GFP astrocytes and oligodendrocyte marker CC1 in spinal cord ventral horn (scale bar, 50 μm). (B) Gray matter restricted expression of Il33lacZ in the spinal cord at P30 (scale bar, 0.5 mm). (C) P12 (eyes closed) P14 (eyes open) (D) Il33lacZ increases in the visual thalamus (dLGN) during eye opening, normalized to sensorimotor thalamus (VB) (scale bar, 0.5 mm). (E) Representative images of Il33lacZ in P21 thalamus in littermate controls and after perinatal enucleation (scale bar, 0.5 mm). (F) Il33lacZ mean pixel intensity in dLGN. (G) Representative flow plot of spinal cord from Il33mCherry/Aldh1l1GFP mice at P15 with sorting gates indicated. (H) Heat map of the top 444 differentially expressed genes in Il33mCherry− versus mCherry− astrocytes in spinal cord and thalamus (fold change > 2; adjusted P value < 0.05), select candidates highlighted. One-way analysis of variance (ANOVA) with Tukey's post hoc comparison or Student's t test. All points represent independent biological replicates. *P < 0.05, ****P < 0.0001.
with microglial activation, including IL1RL1-dependent down-regulation of P2Y12 (fig. S9, A to C) (25). In vitro, IL-33 promoted synaptic engulfment by purified microglia, whereas the canonical IL-1 family member IL-1β had no effect (fig. S9, D and E). In vivo, injection of IL-33 into the developing spinal cord led to twofold depletion of excitatory synapses (colocalized VGLUT2/PSD-95), whereas conditional deletion of IL1RL1 from microglia partly reversed this effect (C3_br/Il1rl1/+/−) (Fig. 3, F and G). In comparison, global loss of Il1rl1 completely reversed IL-33-dependent synapse depletion in spinal cord (fig. S10) and thalamus (fig. S8, C and D), suggesting that nonmicroglial sources of ST2 could also contribute. These data indicate that IL-33 regulates synapse numbers in vivo at least in part via IL1RL1 receptor-mediated signaling and microglial communication that is required for synapse homeostasis during CNS development.

Our data reveal a mechanism of astrocyte-microglial communication that is required for synapse homeostasis during CNS development. We propose that astrocyte-derived IL-33 serves as a rheostat, helping to tune microglial synapse...
Fig. 3. IL-33 drives microglial synapse engulfment during development. (A) Expression of Il1rl1 by qPCR of flow-sorted populations (S, spinal cord; T, thalamus). (B) A total of 484 differentially expressed genes in spinal cord microglia at adjusted P value < 0.05. (C) Functionally associated gene clustering (STRING) identifies immune genes enriched in wild-type versus Il33−/− microglia. (D) PSD-95 puncta within microglia (yellow arrows) after IL-33 deletion of Il33−/−). (E) Representative image and quantification of engulfed PSD-95 in vehicle or IL-33 injected spinal cord (scale bar, 20 μm). (F and G) Colocalized pre- and postsynaptic puncta in spinal cord ventral horn at P14 (yellow arrows) after IL-33 injection into control mice or in littermates with conditional deletion of Il1rl1 (Cx3cr1cre) (scale bar, 3 μm). Points in (A) represent mice; in (D) to (G), individual microglia from n = 3 to 5 animals per group; in (G), images from n = 5 mice per group. In (D) and (E), Student’s t test and in (G), a one-way ANOVA with Tukey’s post hoc comparison. *P < 0.05, ***P < 0.001, ****P < 0.0001; all data are mean ± SD.

engulfment during neural circuit maturation and remodeling (fig. S1I). Key unanswered questions include the nature of the cues that induce astrocyte Il33 expression, the mechanism of IL-33 release, and the signals downstream of IL-33 that promote microglial function. These data also raise the broader question of how this process affects neural circuit function. Synapses are the most tightly regulated variable in the developing CNS (26) and are a primary locus of dysregulation in neurodevelopmental diseases. Il33 is one of five genes that molecularly distinguish astrocytes from neural progenitors in the developing human forebrain (27), suggesting possibly conserved roles in the human CNS. Defining whether signals like IL-33 are permissive or instructive, promiscuous or synapse specific, is a first step toward understanding how neural circuits remodel during development and under stress.

REFERENCES AND NOTES


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Data and materials availability: Supplementary materials contain additional data. All data needed to evaluate the conclusions in the paper are present in the paper or the supplementary materials. RNA-sequencing data are available through GEO no. GSE109354.

**SUPPLEMENTARY MATERIALS**

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Call to action
The developing brain initially makes more synapses than it needs. With further development, excess synapses are pruned away, leaving mature circuits. Synapses can be eliminated by microglia, which engulf and destroy them. Vainchtein et al. found that the microglia are called into action by astrocytes, supportive cells on which neurons rely. Astrocytes near a redundant synapse release the cytokine interleukin-33 (IL-33), which recruits microglia to the site. In mice, disruptions in this process, as caused by deficiency in IL-33, led to too many excitatory synapses and overactive brain circuitry.

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