Characterization of meningeal type 2 innate lymphocytes and their response to CNS injury

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The meningeal space is occupied by a diverse repertoire of immune cells. Central nervous system (CNS) injury elicits a rapid immune response that affects neuronal survival and recovery, but the role of meningeal inflammation remains poorly understood. Here, we describe type 2 innate lymphocytes (ILC2s) as a novel cell type resident in the healthy meninges that are activated after CNS injury. ILC2s are present throughout the naive mouse meninges, though are concentrated around the dural sinuses, and have a unique transcriptional profile. After spinal cord injury (SCI), meningeal ILC2s are activated in an IL-33-dependent manner, producing type 2 cytokines. Using RNAseq, we characterized the gene programs that underlie the ILC2 activation state. Finally, addition of wild-type lung-derived ILC2s into the meningeal space of IL-33R−/− animals partially improves recovery after SCI. These data characterize ILC2s as a novel meningeal cell type that responds to SCI and could lead to new therapeutic insights for neuroinflammatory conditions.

INTRODUCTION

The healthy central nervous system (CNS) parenchyma is void of resident immune cells besides microglia. However, there is a full repertoire of resident immune cells, including macrophages, T cells, and B cells, in the meningeal spaces. Meningeal leukocytes support normal brain function (Derecki et al., 2010; Filiano et al., 2016), can drain to local lymph nodes (Louveau et al., 2015), and present autoantigens in the context of experimental autoimmune encephalomyelitis (EAE; Kivisäkk et al., 2009), but their activation states on outcome, largely through its effect on second-
RESULTS AND DISCUSSION

The meningeal compartment of the CNS hosts a wide variety of immune cells under homeostatic conditions. ILC2s, known for their important functions in barrier tissues, had previously been implicated in CNS diseases (Besnard et al., 2015; Russi et al., 2015), but their presence in healthy meninges has not been assessed. We asked whether ILC2s are present in healthy meninges and surprisingly identified a population of CD45+/Lineage−/Thy1.2+/ST2+ cells (Fig. 1 A). Furthermore, this population had a surface marker profile characteristic of ILC2s, expressing C-kit, Sca1, CD25, and IL-7Rα (Fig. 1 B). Interestingly, the meninges have more ILC2s relative to ILC3s (CD45+/singlets/lineage−/viable/RORγt cells) in the healthy state, and both ILC populations were concentrated in the brain rather than spinal cord meninges (Fig. 1 C). No ILCs were detected in the healthy spinal cord parenchyma (Fig. 1 C).

We next sought to explore the physical localization of ILC2s in brain and spinal cord meninges. To that end, we crossed the Yet-Cre 13 (Price et al., 2010) and tdTomato loxP lox mice, generating a new model where any cell that expressed IL-13 is permanently labeled with tdTomato (IL-13tdt mice). This strategy labels the majority of ILC2s in healthy brain/spinal cord meninges and lung (Fig. 1, D and E) and also labels populations of T cells and mast cells in these tissues (Fig. 1, F and G). A whole mount of IL-13tdt dura mater revealed a concentration of tdTomato expression in the transverse and sagittal dural sinuses, which drain venous blood from the brain (Fig. S1 A). To differentiate tdTomato+ ILC2s from T cells and mast cells, we costained IL-13tdt meninges with CD3 and toluidine blue to identify T cells and mast cells, respectively. ILC2s were identified as tdTomato+/CD3−/toluidine blue− cells in dural sinuses, dura mater, and spinal cord meninges (Fig. 1, H−J). ILC2s were heavily concentrated in the dural sinus and found more sparsely throughout the dura mater and spinal cord meninges (Fig. 1 K).

It has previously been noted that ILC2s expand in response to IL-33 stimulation (Mološky et al., 2013), and, to further confirm the nature of the newly identified meningeal cells, we sought to repeat this observation in meningeal ILC2s. Indeed, mice treated with IL-33 i.p. for 6 d had up to 3.5-fold expansion of ILC2s in the spinal cord and brain meninges (Fig. 1, L−N).

Given the unique localization of brain meningeal ILC2s, we characterized them transcriptionally to assess their degree of similarity with other ILC2s, choosing to compare them with the well-studied population of ILC2s in the lung. ILC2s were FACs purified from meninges and lung, and >90% pure ILC2s were used for RNAseq analysis. Among the top genes expressed by meningeal ILC2s are numerous canonical markers, including the Il-2α, Il-7r, and IIfilr1 (IL-33R; Fig. 2 A and Table S1). To assess the similarity of highly expressed genes in meningeal and lung ILC2s, we compared the genes expressed at least 1.5 standard deviations above the mean between the cell types. As predicted, the majority of highly expressed genes are common between lung and meningeal ILC2s, but there are also discreet sets of genes delineating them (Fig. 2 B). We identified 472 significantly altered genes between the groups (adjusted p-value <0.05; Fig. 2 C and Table S2). Interestingly, the majority of these genes were up-regulated in lung relative to meningeal ILC2s (Fig. 2 C). To describe functional consequences of this differential expression, we identified gene sets enriched among our differentially expressed genes (Table S2). Among the enriched gene sets were several relating to inflammation, signal transduction, and metabolism, suggesting an increased basal activation state in lung relative to meningeal ILC2s (Fig. 2, D and E). The basis for these differences is somewhat unclear. Lung ILC2s are exposed to far more environmental irritants and stimuli than meningeal ILC2s, likely leading to the observed alterations in transcription. Furthermore, meningeal ILC2s are proximal to the brain, an exceptionally sensitive tissue, and therefore, a relatively quiescent rest state could be optimal for healthy brain function.

IL-33 is a potent stimulus for ILC2s and is released in abundance into the CSF after SCI (Gadani et al., 2015b). We therefore tested whether meningeal ILC2s are acutely activated after SCI. Type 2 cytokine production is a hallmark of ILC2 activation, and using the Yet-cre 13 mouse (which have YFP expressed under the IL-13 promoter), we compared IL-13 induction in uninjured and 1 d post-injury (1DPI) ILC2s, finding increased YFP expression in brain but not spinal cord meningeal ILC2s (Fig. 3 A). We repeated this observation using intracellular antibody staining, finding that brain meningeal but not spinal cord meningeal ILC2s have increased production of IL-13 and IL-5 at 1DPI (Fig. 3, B and C). Finally, we tested whether brain meningeal ILC2 cytokine production is IL-33 dependent using the IL-33−/− mice. IL-33−/− animals show no significant up-regulation of IL-13 or IL-5 relative to uninjured animals (Fig. 3, D and E).

Brain meningeal ILC2s respond to spinal cord-derived signals after injury with increased cytokine production, and we next characterized the transcriptome of SCI-activated meningeal ILC2s by RNAseq. We measured global transcriptomic changes in brain meningeal ILC2s 1 d after SCI, finding numerous up- and down-regulated genes (Fig. 3 F and Table S3). Among genes up-regulated after injury are immune checkpoint genes, such as Pdcd1, receptors, such as Tnfrsf9, Gpr35, and IIfilr1, and those involved in neuroprotection, such as Calca (encoding calcitonin gene-related peptide [CGRP]) and the shuttle for its receptor Ramp3 (Fig. 3 F). CGRP is known to be rapidly up-regulated in the injured CNS (Bulloch et al., 1998; Ackery et al., 2007) and is involved in regeneration of peripheral nerves (Blesch and Tuszynski, 2001; Toth et al., 2009). Notably, expression of IIfilr1 (encoding ST2), Il5, and IIfil were unchanged after injury. The lack of measured difference in IL-5 and IL-13 mRNA could represent the transient nature of their up-regulation in this system. Although the Yet-cre reporter demonstrated increased YFP, and thus IL-13 promoter activity at
1DPI, it is possible that increases in IL-13 mRNA are out-lived by the YFP reporter.

We next identified gene sets enriched in meningeal ILC2s isolated from uninjured versus injured mice. Several inflammatory gene networks were enriched in ILC2s after injury, including TNF signaling, IL-2 signaling, biosynthetic processes, and general cell activation (Fig. 3, G and H; and Table S3). Interestingly, several of the same genes and gene sets were up-regulated in meningeal ILC2s after injury and lung ILC2s. Extracting sufficient RNA for analysis from meningeal ILC2s required pooling five mice per sample, and we therefore chose to validate results using RNAseq replicates instead of individual qPCR. RNAseq replicates were collected on different days, and only genes/gene sets consistently different across samples were accepted.

ILC2s are known to accumulate at injury sites in the periphery (Rak et al., 2016), but the capacity for ILC2 migration into inflamed sites is poorly understood. Given that the healthy CNS parenchyma is void of ILC2s (Fig. 1 C), we reasoned that if ILC2s accumulate at the SCI site, some of them must have migrated there. We used the IL-13 flow mouse to identify ILC2s that have infiltrated the lesion site, finding that they migrate to the injury site by 10DPI (Fig. 4 A). Using flow cytometry, we performed a time course of ILC2 numbers in the SCI site. ILC2s were detectable in the injury site by 3DPI, but numbers maximized at 10DPI and persisted through 30DPI (Fig. 4 B). Notably, these cells were competent to produce IL-13 (Fig. 4 C). This migration was IL-33 independent, as IL-33−/− and WT mice had similar ILC2 infiltration at 10DPI (Fig. 4 D).

Finally, we sought to test the functional impact ILC2s have on recovery from CNS injury. We pursued a global ILC depletion strategy: Rag1−/− animals, lacking T and B cells but having Thy1.2+ ILCs, were reconstituted with Thy1.1 lymphocytes. In this mouse, reconstituted lymphocytes will bear a different isotype Thy1 than endogenous ILCs. We then injected mice with a Thy1.2-depleting antibody, targeting ILC2 and ILC3s (CD45+/singlets/lineage−/toluidine blue− cells). (K) Quantification of ILC2 localization demonstrates greater expansion in brain meninges (M; P = 0.009) and spinal cord meninges (N; P = 0.001; n = 3, representative of three experiments; Student’s t test). Error bars representing mean ±SEM, **, P ≤ 0.01; ***, P ≤ 0.001.

performed in literature (Monticelli et al., 2011; Gorski et al., 2013). There was no significant effect of depleting ILCs in this way on SCI outcome (Fig. S2 A); however, the depletion strategy was actually found to be ineffective for meninges. Anti-Thy1.2 did not deplete meningeal ILCs, but instead only blocked the Thy1 epitope (Fig. S2 B and C). Our observation should also serve as a caution against validating depletion by staining for the antigen used to deplete.

We next pursued the converse experiment: adding back ILC2s into the CSF before injury. Meningeal ILC2s are sparse, and it is not feasible to extract sufficient numbers for reconstitution. Given their relative abundance and increased basal activity profiles, we used lung-derived ILC2s for adoptive transfer experiments. ILC2 cytokine production is dependent on IL-33 and to restrict IL-33’s actions only to our transferred cells, we used ST2−/− recipients. 5,000 FACS-purified, lung-derived ILC2s were injected into the cisterna magna of ST2−/− mice 1 d before SCI. This moderate increase in ILC2 numbers had a significant beneficial effect on functional recovery, as measured by Basso mouse scale (BMS) score, a behavior score widely used to assess functional outcome of SCI in mice (Fig. 4 E). Additionally, we measured the volume size of the lesions, and as expected from BMS scores, ILC2-treated mice had smaller lesion volumes than control mice (Fig. 4, F and G). Lung ILC2s were selected based on their availability and activation profile, which includes elevated immunomodulatory and neuroprotective gene expression. It is important to realize that in using these cells, we may have tilted the balance in favor of recovery and that reconstitution with more meningeal ILC2s would be required to achieve the same benefit. Alternatively, meningeal ILC2s may be specialized to support the injured CNS through unidentified mechanisms, in which case our reconstitution underestimates the true impact of ILC2s on SCI.

The meningeal space represents a largely unstudied venue for neuroimmune interactions in the healthy and diseased CNS conditions. This area is densely populated by a variety of immune cells, some resident (Goldmann et al., 2016).
and other patrolling (Radjavi et al., 2014). The recent discovery of meningeal lymphatic vessels (Aspelund et al., 2015; Louveau et al., 2015) has shed a new light on the importance of the meningeal space in the neuroimmune axis. Meningeal immunity is unique as it exhibits an obvious predominance of type 2 inflammation (Derecki et al., 2010) for reasons that are not fully understood yet. Moreover, after SCI, type 2 immune responses are intensified and required for improved outcome (Walsh et al., 2015). One of the immediate cells responding to injuries in peripheral/barrier tissues are ILC2s. Here, we provide the first evidence for a meningeal population of ILC2s, demonstrating their abundance in the brain meninges and characterizing them transcriptionally. We further studied their response to SCI, demonstrating that after injury, meningeal ILC2s are functionally activated, and they enter the injury site and improve recovery.

Our finding of differential gene expression from brain and lung further suggests that ILC2 phenotype could be determined, at least in part, by tissue of residence. Indeed, diverse tissue-dependent ILC2 functions have already been described in tissues such as adipose (Molofsky et al., 2013; Odegaard et al., 2016). Of note, lung ILC2s had up-regulation of numerous inflammatory gene sets relative to meninges, possibly because of continuous exposure of these cells to environmental irritants and stimuli. The extent of plasticity between and diversity of roles for putative ILC2 phenotypes remains an important topic for future study.

After SCI, meningeal ILC2s are activated, producing type 2 cytokines and up-regulating inflammatory gene sets. Among the most prominently up-regulated genes are Calca, encoding CGRP, and Ramp3, encoding shuttling protein for its receptor. CGRP is up-regulated in the CNS after injury (Bulloch et al., 1998; Ackery et al., 2007) and has been implicated in regeneration of sensory neurons both in and out of the CNS (Blesch and Tuszynski, 2001; Tho et al., 2009), migraine pain (Russo, 2015), and immunomodulation (Bracci-Laudiero et al., 2005). Prior studies have noted CGRP receptor expression in ILC2s, suggesting that they may detect CGRP secreted by nociceptive neurons (Saenz et al., 2013; Talbot et al., 2015). Our results identify CGRP as a novel factor potentially released by activated ILC2s.

This study is an early foray into the biology of meningeal ILC2s and demonstrates a novel role for meningeal immune cells as sentinels for CNS-derived alarms. IL-33 released after CNS injury not only initiates a local response, but also a meningeal one through actions on ILC2s. ILC2s produce IL-13, both in the meninges and at the injury site, which could promote the generation of neuroprotective Th2 cells—or directly boost survival of neurons (Walsh et al., 2015). Many other potentially neuroprotective factors, including Areg, Vegfa, Il1b, and Calca (Blesch and Tuszynski, 2001; Diem et al., 2003; Sun et al., 2003; Zhan et al., 2015) could also contribute to the beneficial effect of lung-derived ILC2 transfer. Further studies are required to fully understand this meningeal ILC2 response and the mechanism by which adding ILC2s is beneficial after injury. Furthermore, meningeal ILC2s, given their perivenous localization, could be first responders in gut–brain communication. This study provides insight into a novel immune cell player after CNS injury, the meningeal ILC2, and further work on this population could lead to therapeutic insights for injury and other neurological disorders.

**MATERIALS AND METHODS**

**Mice**

IL-33−/− mice were generated by the trans-NIH Knock-Out Mouse Project (KOMP) and obtained from the KOMP repository. ST2−/− mice were generated in the laboratory of A. McKenzie (University of Cambridge, Cambridge, England, UK) and were a gift from P. Bryce (Northwestern University, Evanston, IL). Yet-Cre 13 mice were generated by and a gift from R. Locksley (University of California, San Francisco, San Francisco, CA; Price et al., 2010). C57/Bl6 and tdTomato+ mice were obtained from the Jackson Laboratory, stock #004999 and #007905, respectively. All transgenic lines were on the C57/Bl6 genetic background except for Yet-Cre 13, which was BALB/c. The F1 generation of Yet-Cre 13 × tdTomato+ mice was used. All animals were housed in temperature- and humidity-controlled rooms, maintained on a 12-h light/dark cycle (lights on 7:00 a.m.), and age matched in each experiment. All strains were kept in identical housing conditions. For survival surgeries, mice were anesthetized with either 200 µl of ketamine/xylazine (1 ml ketamine HCl [1 mg/ml], 1 ml of 2% xylazine, 8 ml saline) or inhaled isoflurane. All procedures complied with regulations of the Institutional Animal Care and Use Committee (IACUC) at the University of Virginia (UVA).

**Statistics**

Statistical tests were performed in Prism (GraphPad Software) or using R as described in the text and figure legends. In all

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Figure 2. RNAseq analysis of ILC2s purified from uninjured meninges and lung. ILC2s were FACS purified from meninges and lung preparations (DAPI−/CD45+/Lineage−/Thyl 1.2+/ST2+ cells), and purified mRNA was sequenced on an Illumina platform. (A) The top 50 genes by mean normalized read counts expressed in meningeal ILC2s (n = 3, each sample five pooled mice), and purified mRNA was sequenced on an Illumina platform. (B) UpSet plot comparing highly expressed genes (expressed >1.5 standard deviations above mean) of meningeal and lung ILC2s. 1,200 highly expressed genes were common to both groups, whereas 219 were specific to lung and 176 specific to meningeal ILC2s. (C) Heat map of significantly altered genes between groups (472 genes; n = 3, each sample five pooled mice; adjusted p-value <0.05). (D and E) Gene sets enriched among differentially expressed genes in lung versus meningeal ILC2s. Histogram (D) and heat maps (E) of select gene sets and their component genes (n = 3, each sample five pooled mice; adjusted p-value <0.05).
Figure 3. Activation of meningeal ILC2s after SCI. (A) ILC2 IL-13 expression assessed by YFP mean fluorescence intensity (MFI) in YET-cre 13 mice in spinal cord (SC) meninges (i; P = 0.890) and brain meninges (ii; P = 0.007; n = 9, representative of three pooled experiments; Student’s t test). (B) Flow cytometry analysis of IL-13 expression in ILC2s at 1 DPI in spinal cord meninges (i; P > 0.999) and brain meninges (ii; P < 0.001; n = 3, representative of two
Flow cytometry/cell sorting
To prepare single cell suspensions, meninges (brain and spinal cord) were dissected with fine forceps and digested in RPMI + 1.4 U/ml Collagenase VIII ( Worthington) + 1 mg/ml DNase1 (Sigma-Aldrich) for 15 min at 37°C. Lungs were dissected, minced, and digested for 30 min in RPMI + 1.4 U/ml Collagenase VIII + 1 mg/ml DNase1 at 37°C. Digested meninges and lungs were dissociated by pipetting and passed through 70-µm filters. Spinal cords were dissected from PBS-perfused mice, and 1 cm of cord centered on the lesion was isolated, minced, and digested in RPMI + 4 U/ml papain + 1 mg/ml DNase1 for 20 min. Spinal cords were triturated with small- and large-bore fire-polished pipette and passed through a 70-µm filter. Samples were washed in FACS buffer (PBS, 0.05% Na Azide, 1 mM EDTA, 2% FBS) and stained with antibody cocktail and viability dye for 30 min at 4°C in FACS buffer. For lineage staining, cells were labeled with biotinylated lineage antibody cocktail, washed, and then incubated with streptavidin-PeCy7 or -Fitc (eBioscience) for 30 min at 4°C. Zombie aqua fixable viability dye (BioLegend) was used to discriminate live cell populations. To obtain absolute cell counts, counting beads (eBioscience) were added to the samples. After staining, samples were washed in FACS buffer and resuspended in 4% PFA. The following antibodies were used (all from eBioscience unless otherwise noted): CD45-af700, Lineage-biotin (CD11b, B220, CD3, GR1, TER-119, FcRα), Thy1.2-Fitc, ST2-Pe, C-kit-ef780, Sca1-af700, CD25-APC, TCRβ-Fitc or APCCy7, IL-7R–Pe, C-kit–ef780, ICOSL-APC, Gata3-660, Rorγt, IL-13–PeCy7, IL-5–Pe, and CD69-PeCy7.

To stain intranuclear antigens (Gata3, Rorγt, IL-13, IL-5), cells were fixed after extracellular staining with Cytofix/Cytoperm (BD), washed in perm buffer, and stained for 30 min at room temperature. Samples were read on a Gallios cytometer (Beckman Coulter) and analyzed using FlowJo software (Tree Star). ILC2s (selected as CD45+/Lineage−/Thy1.2+/-ST2+ cells) were sorted on an Influx cell sorter (BD) in the FACULTY flow cytometry core facility, achieving a purity of >90%.

RNA sequencing and analysis
ILC2s were FACs sorted, as described above, directly into lysis buffer (PicoPure RNA isolation kit; Applied Biosystems). RNA purification was performed according to manufacturer instructions and stored at −80°C until use. Library preparation, amplification, and RNA sequencing (Illumina) were performed by HudsonAlpha.

In vitro cell stimulation
For in vitro stimulation and cytokine staining of ILC2s, normalized numbers of cells were maintained in RPMI + 10% FBS + Anti-anti at 37°C. Cells were treated with Golgi block (Brefeldin A) and stimulated with PMA/ionomycin for 4 h before being washed and stained as described in the Flow cytometry/cell sorting section.

Image quantification
Images were acquired using an SP8 confocal microscope (Leica; fluorescence images) or a DMI 6000B widefield microscope (Leica; brightfield images). Counting was done in ImageJ (National Institutes of Health) with the “Cell Counter” plugin (Kurt De Vos, University of Sheffield, Sheffield, England, UK). Heat maps were generated with the “HeatMap Histogram” plugin (Samuel Péan).

To quantify spinal cord lesion size, 20-µm coronal sections were stained with GFAP and imaged. Lesion area per slice was quantified using ImageJ, and total volume was calculated using Excel (Microsoft).

Tissue preparation and immunofluorescence
For quantification of ILC2s, mice were perfused with heparinized (5 U/ml) PBS and 4% PFA, followed by meninges and spinal cord dissection. The tissue was postfixed for 48 h in 4% PFA, and spinal cords were cryoprotected in 30% sucrose for 48 h. Spinal cords were cut into 20-µm sections, mounted on gelatin-coated slides, and stored at −20°C until use. To stain, tissue was blocked for 1 h at room temperature in blocking buffer (2% serum [of the secondary’s species]; 1% BSA; 0.1% triton; 0.05% tween; 0.05% Na Azide) followed by overnight incubation in primary antibody at 4°C. The following antibodies were used for immunofluorescence staining: chicken anti–GFAP (AB5541; 1:1,000; EMD Millipore), rat anti-CD3 660 (50-0032; 1:300; eBioscience), rat anti-CD3 biotin (13-0032; 1:300; eBioscience). Slices were washed 3× 10 min and incubated for 2 h at room temperature with the appropriate secondary antibodies (all from Thermo Fisher Scientific; 1:1,000), washed again 3 × 10 min and mounted with AquaMount (Thermo Fisher Scientific) and DAPI.
For staining meningeal mast cells, toluidine blue staining was performed on samples after being stained and imaged for fluorescence markers. Meninges were stained in 0.5% Toluidine blue O (pH 2.5; Sigma-Aldrich) for 15 min at room temperature. Toluidine blue was washed overnight at 4°C, and images were acquired using brightfield microscopy.
Spinal cord contusion
Female mice were first anesthetized with ketamine/xyloazine. The back fur was shaved and underlying skin sterilized with an iodide/betadine solution. An incision was made over the T9–T10 vertebrae, and the skin was held back with retractors. The fascia overlying the spinal cord was removed to expose the vertebrae. The T10 vertebra was removed with fine rongeurs to expose the spinal cord. The IH-0400 Impactor (Precision Systems and Instrumentation) was used to contuse the spinal cord centrally, after which the muscles and skin overlying the spinal cord were sutured closed and the mouse was allowed to recover on warming pads. The force of impact was computer controlled and set to 70 or 90 Kdyn as noted in the text/legends. Mice were maintained on sulfa water, and twice daily we performed manual bladder expulsion. Two blinded observers assessed recovery of hind–limb locomotor activity with the BMS (Basso et al., 2006) after injury.

IL-33 treatment
For IL-33 treatments to expand ILC2s, mice were injected i.p. with 500 ng carrier-free recombinant IL-33 (eBioscience) every other day. After three injections, mice were sacrificed, and ILC2 numbers were analyzed by flow cytometry.

RNAseq analysis
The raw sequencing reads (FASTQ files) went through two stages of preprocessing to remove low–quality reads and bases. First, they were chastity filtered, which removes any clusters that have a higher than expected intensity of the called base compared with other bases. Then they were trimmed with Trimmomatic (Bolger et al., 2014) to remove low–quality bases (minimum read length after trimming = 36). After preprocessing, the quality of the reads was evaluated using FastQC, and after passing quality control (QC), the reads were aligned to the UCSC mm9 genome (Harrow et al., 2012) using the splice-aware read aligner STAR. (Dobin et al., 2013). The quality of the alignments was next assessed by SAMStat (Lassmann et al., 2011), and any low–quality alignments were removed with SAMtools (Li et al., 2009; MAPQ < 10). Next, the number of reads aligning to each gene was quantified with HTSeq (Anders et al., 2015), and then the Bioconductor package (Love et al., 2015) DESeq2 was used to normalize the raw counts and perform exploratory analysis (e.g., PCA) and differential expression analysis. The Benjamini–Hochberg false discovery rate procedure was used to correct the p-values for multiple testing. Heat maps of the differentially expressed genes generated with the R package pheatmap (https://CRAN.R-project.org/package=pheatmap). To compare the highly expressed genes in the uninjured lung versus uninjured brain meninges, the normalized, log2 transformed counts were converted to standard scores (z-score) using the scale function in R. The mean z-score for each gene was then calculated, and those with a z-score >1.5 (i.e., 1.5 standard deviations above the mean) were considered highly expressed. The UpSet plot (Lex et al., 2014) used to visualize the overlap between the uninjured lung and brain samples was created with the R package UpSetR. (Gehlenborg, 2016). The R implementation of Fisher’s exact test, fisher.test, was used to identify enriched gene sets in the differentially expressed genes using two gene set collections: the GO biological process from MSigDB (Subramanian et al., 2005; C5) and the gene families from the Hugo Gene Nomenclature Committee (HGNC; Gray et al., 2015).

Accession number
All sequencing data has been uploaded to the GEO repository under accession no. GSE90908.

Online supplemental material
Fig. S1 contains images of whole-mount IL–13th meninges, demonstrating visually how ILC2s are concentrated in the dural sinus area. Fig. S2 contains data regarding the use of anti-Thy1.2 antibody to deplete ILC2s, demonstrating that, in our hands, it merely blocks the Thy1.2 epitope. Table S1 lists normalized gene expression values for all RNAseq samples (uninjured and injured meninges and uninjured lung ILC2s). Table S2 lists differential expression between uninjured meninges and lung ILC2s, as well as the gene sets enriched in lung ILC2s. Table S3 lists differential expression between uninjured and injured meninges ILC2s, as well as the gene sets enriched in injured meninges ILC2s. Tables S1–S3 are available as Excel files.

ACKNOWLEDGMENTS
We would like to thank all the members of the Kipnis laboratory for their valuable comments during multiple discussions of this work.

This work was supported by a grant from the National Institutes of Health (NS081026 to J. Kipnis).

The authors declare no competing financial interests.

Submitted: 26 November 2016
Revised: 9 December 2016
Accepted: 13 December 2016

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