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IL-33 Signaling Regulates Innate and Adaptive Immunity to *Cryptococcus neoformans*

Adam Flaczyk,* Claudia U. Duerr,† Mitra Shourian,* Erin I. Lafferty,* Jörg H. Fritz,†,‡,1 and Salman T. Qureshi*,§,1

Susceptibility to progressive infection with the fungus *Cryptococcus neoformans* is associated with an allergic pattern of lung inflammation, yet the factors that govern this host response are not clearly understood. Using a clinically relevant mouse model of inhalational infection with virulent *C. neoformans* H99, we demonstrate a role for IL-33–dependent signaling in host immune defense. Infection of BALB/c mice with 10^4 CFU of *C. neoformans* H99 caused a time-dependent induction of IL-33 with accumulation of type 2 pulmonary innate lymphoid cells and alternatively activated macrophages in the lungs as well as Th2-polarized CD4+ T cells in draining lymph nodes. IL-33R subunit T1/ST2-deficient (T1/ST2$^{-/-}$) mice infected with *C. neoformans* H99 had improved survival with a decreased fungal burden in the lungs, spleen, and brain, compared with wild-type mice. Signaling through T1/ST2 was required for the accumulation and early production of IL-5 and IL-13 by lung type 2 pulmonary innate lymphoid cells. Further analysis of T1/ST2$^{-/-}$ mice revealed increased fungicidal exudate macrophages in the lungs and decreased *C. neoformans*–specific Th2 cells in the mediastinal lymph nodes. T1/ST2 deficiency also diminished goblet cell hyperplasia, mucus hypersecretion, bronchoalveolar lavage eosinophilia, alternative activation of macrophages, and serum IgE. These observations demonstrate that IL-33–dependent signaling contributes to the expansion of innate type 2 immunity and subsequent Th2-biased lung immunopathology that facilitates *C. neoformans* growth and dissemination. *The Journal of Immunology*, 2013, 191: 2503–2513.

Each year, the opportunistic fungal pathogen *Cryptococcus neoformans* is estimated to cause 624,700 deaths throughout the world (1–3). In the absence of robust immunity, infectious propagules in the environment that are inhaled to the lower respiratory tract have the potential to germinate and cause pneumonia and/or meningoencephalitis following dissemination to the CNS. In other cases, cryptococci may rapidly be cleared by the host or establish an asymptomatic latent state with potential for subsequent reactivation in the presence of declining immunity (4, 5). The precise molecular mechanisms that control the development of protective or permissive immunity against cryptococci have not been identified and represent a significant knowledge gap in the field of host–fungal interactions.

Cryptococcal cells that localize to the distal airways and alveoli may replicate and survive extracellularly in association with the respiratory epithelium or intracellularly following recognition and phagocytosis by effector cells such as alveolar macrophages, dendritic cells, and neutrophils (5). In the absence of a rapid and sterilizing innate immune response, the nature and severity of *C. neoformans* infection are determined to a large extent by the pattern of adaptive CD4+ T lymphocyte polarization (6). Specifically, the development of a Th1 cell response that is characterized by classical activation of macrophages and induction of the proinflammatory cytokines IFN-γ and TNF-α is crucial for definitive cryptococcal elimination in humans and experimental mouse models (7, 8). In contrast, defective or absent expression of these proinflammatory mediators facilitates the development of an allergic Th2 response that is characterized by alternatively activated macrophages (AAMΦ), production of IL-4, IL-5, and IL-13, lung eosinophilia, and high serum IgE and is associated with progressive *C. neoformans* growth and dissemination (9). Of interest, the relative balance of Th1/Th2 immunity during experimental *C. neoformans* lung infection is influenced by the combination of fungal strain and mouse genetic background (6, 8).

In addition to its primary barrier function, the respiratory epithelium mediates an initial interaction with *C. neoformans* (6, 10) that triggers the release of soluble inflammatory mediators, including CXCL1 and IL-8 (11). More recently, the epithelial-derived cytokines thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 have been shown to regulate aspects of innate and adaptive immunity in disorders characterized by a type 2 response, such as asthma and atopic dermatitis (12, 13). Extracellular IL-33 targets multiple cell types, including naive and Th2 lymphocytes, B1 cells, macrophages, dendritic cells, and mast cells through a unique heterodimeric receptor composed of T1/ST2 and IL-1R accessory protein (14–19). Detailed characterization of genetically engineered cytokine reporter mice has also revealed a novel family of cells named nuocytes, innate type 2 helper cells, or natural helper cells that have a lymphoid morphology but lack classical lineage markers for B, T, or myeloid cells (20–22). These cells express T1/ST2 and orchestrate type 2 immunity at barrier

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Abbreviations used in this article: AAMΦ, alternatively activated macrophage; BAL, bronchoalveolar lavage; dpi, day post infection; ILC2, type 2 innate lymphoid cell; PAS, periodic acid–Schiff; qRT-PCR, quantitative RT-PCR; TSLP, thymic stromal lymphopoietin; WT, wild-type.

surfaces by secreting large amounts of the cytokines IL-5 and IL-13 in response to IL-33 stimulation and are commonly referred to as type 2 innate lymphoid cells (ILC2s) (20, 22, 23). In animal models of asthma induced by OVA or the fungal allergen *Alternaria alternata*, ILC2s were found to be necessary and sufficient for eosinophilic inflammation and pulmonary IL-5 and IL-13 production even in the absence of adaptive immunity (24, 25).

On the basis of the observation that a Th2 pattern of host response is permissive for *C. neoformans* growth, we hypothesized that IL-33 is a crucial regulator of susceptibility to progressive cryptococcosis. To test this possibility, we infected BALB/c mice with highly virulent serotype A strain (H99) or moderately virulent serotype D strain (52D) of *C. neoformans*, both of which have been shown to provoke a Th2- or a Th1-biased response, respectively (8, 9). A time-dependent increase in *Il33* mRNA expression and differential protein expression in the lungs was observed in response to infection with *C. neoformans* H99. Furthermore, H99 infection of mice that are deficient for the unique IL-33R T1/ST2 resulted in a significantly lower lung fungal burden with less dissemination to the spleen and brain, which conferred a modest survival advantage. At an early time point post infection, T1/ST2 knockout mice also had decreased expansion and reduced production of the type 2 cytokines IL-5 and IL-13 by lung ILC2s. Finally, infected T1/ST2-deficient mice had reduced alternative macrophage activation and diminished Th2 polarization of lung-associated lymph node cells. Collectively, these results reveal the multifaceted contribution of IL-33–mediated signaling to the development of innate and adaptive immune responses against pulmonary *C. neoformans* infection.

Materials and Methods
Mice

Inbred BALB/c mice were purchased from Charles River and maintained in our facility, provided with sterile food and water ad libitum, and cared for according to the Canadian Council on Animal Care guidelines. T1/ST2$^{-/-}$ mice (26) on the BALB/c background were bred on site. All experiments were performed with 6- to 7- wk-old T1/ST2$^{-/-}$ mice, using age- and sex-matched BALB/c mice as controls. Mice were humanely euthanized with CO_2 upon completion of experiments, and every effort was made to minimize suffering. All experimental protocols were reviewed and approved by the McGill University Animal Care Committee.

Cultures of C. neoformans

C. neoformans H99 (ATCC no. 208821) and 52D (ATCC no. 24067) were thawed from glycerol stocks and maintained on Sabouraud dextrose agar (Becton Dickinson). A single colony of *C. neoformans* was resuspended in Sabouraud dextrose broth (Becton Dickson) at a starting concentration of 5×10^5 CFU/ml, and then grown further in a rotating culture until stationary phase (48 h) at room temperature for in vivo infections or at 37°C for in vitro stimulations. Subsequently, the culture was spun, washed twice with PBS, and resuspended to a desired concentration in PBS or DMEM (Life Technologies, Invitrogen) supplemented with 2% FBS (Wisent). Concentrations of *C. neoformans* were verified by plating on Sabouraud dextrose agar at 37°C for 72 h followed by determination of CFU.

Intratracheal infection with C. neoformans

For intratracheal instillation of *C. neoformans,* mice were anesthetized with 10 mg/kg ketamine (Ayerst Veterinary Laboratories) and 125 mg/kg xylazine (Bayer) i.p. A vertical 1-cm incision was made below the jaw along the trachea, and the underlying glands and smooth muscle were pushed aside. A 22-gauge catheter (Becton Dickson) was inserted into the trachea, and in a volume of 50 μl sterile PBS, 2×10^5 CFU/ml *C. neoformans* was instilled, followed quickly by 50 μl volume of air. The incision was closed using a 9 mm EZ Clip Wound Closing Kit (Stoelting). Mice were monitored daily following surgery.

Organ isolation and CFU assay

Following euthanasia by CO_2, mice were immersed in 70% ethanol, subjected to terminal cardiac puncture, and dissected along the midline, using sterile technique. Lungs, spleen, and brain were excised and placed into separate tubes containing sterile PBS on ice. Subsequently, the organs were homogenized using a sterilized glass tube and pestle attached to a mechanical tissue homogenizer (Glas-Col) and plated at various dilutions on Sabouraud dextrose agar. Plates were incubated at 37°C for 72 h, and CFU were counted.

Bronchoalveolar lavage harvest

At the designated time points, an incision was made below the jaw to expose the trachea. A 22-gauge catheter was inserted into the airway and secured in place by a string. A total of 4 volumes of 500 μl ice-cold PBS was instilled via the catheter and subsequently aspirated. The bronchoalveolar lavage (BAL) fluid was then spun at 1200 rpm for 10 min, and the supernatants were stored at −80°C for subsequent analysis. The cell pellet was counted using a Beckman Coulter cell counter (Beckman Coulter), and spun onto slides at a concentration of 5×10^5 cells per slide using a Cytospin (Shandon). Following DIFF-QUIK staining (Dade Behring), differential cell counts of macrophages, lymphocytes, neutrophils, and eosinophils were obtained by counting 300 total cells twice on each slide. These average percentages were multiplied by the total cell count to obtain the total cell numbers of each leukocyte population.

In vitro infections

MLE-12 cells (ATCC no. CRL-2110) were grown to 80% confluence in DMEM (Wisent) supplemented with 2% FBS, 1% L-glutamine (Life Technologies, Invitrogen), and 1% penicillin and streptomycin (Life Technologies, Invitrogen) at 37°C in a 5% CO_2 atmosphere per American Type Culture Collection recommendations. For passaging, cells were washed with sterile PBS, subjected to incubation with 0.25% trypsin (Life Technologies, Invitrogen), and loosened from the bottom of the flask using a cell scraper (Becton Dickson). Cells were counted, plated at 170.000 cells per well in a six-well plate, and grown for 48 h to 100% confluence. Subsequently, cells were stimulated with increasing multiplicities of infection of *C. neoformans* H99 for 6 h at 37°C in a 5% CO_2 atmosphere.

Flow cytometry

Lungs were excised using sterile technique and placed in RPMI 1640 (Life Technologies, Invitrogen) supplemented with 5% FBS (Wisent). Subsequently, lungs were minced using surgical blades, and incubated with 1 mg/ml collagenase (Sigma-Aldrich) at 37°C for 1 h. Following incubation, lung pieces were passed through a 16-gauge needle and filtered through a 100-μm cell strainer (BD Biosciences). RBCs were removed using 1× ACK lysis buffer. Cells were counted with a Beckman Coulter cell counter. Fc receptors were blocked with the addition of unlabeled anti-CD16/32 Abs (93; eBioscience), and single-cell suspensions were stained with the following fluorescence-conjugated mAbs, purchased from eBioscience unless otherwise stated: α-CD45–V500 (30-F11; BD), α-CD11b–APC (M1/70; BD), α-CD11c–PE (N418), α-B220–FITC (RA3-6B2), α-MHCII–PE-Cy7 (M5/114.15.2), α-CD4–V450 (GK1.5), α-CD8–PerCP (53-6.7; BD), α-CD3–PE–Cy7 (145-2C11; BD), and α-Gr-1–V450 (RB6-8C5). Lung ILC2s were stained with α-Sca-1–Alexa Fluor 488 (E13-161.7; BioLegend), α-CD25–PerCP–Cy5.5 (PC61.5), α-CD127–PE–Cy7 (A7R34), α-CD45–APC (30-F11), α-CD117–APC–eFluor 780 (2B8), and lineage markers conjugated to PE: TCR-β (H57-597), TCR-γδ (eBioGL3), CD3e (145-2C11), Gr-1 (RB6-8C5), CD11b (M1/70), Ter119 (TER-119), B220 (RA3-6B2), NK1.1 (PK136), CD5 (UCHT2; BD), CD11c (N418), and CD27 (LG7F9). Nonviable cells were excluded using fixable viability dye eFluor 780 reagent (eBioscience). Data were acquired using a FACS LSR II flow cytometer (BD Biosciences) and analyzed using FlowJo version X software (TreeStar).

Intracellular flow cytometry

For intracellular cytokine staining of ILC2s, lungs were processed as above and lymphocytes were isolated using a 40/70 Percoll gradient (Sigma-Aldrich). Cells were plated in vitro and stimulated for 4 h with 0.1 μl/ml PMA and 1 μl/ml calcium ionophore (Ionomycin; BD Pharmingen) in the presence of Brefeldin A (GolgiPlug; BD Biosciences) for the final 3 h. Cells were then washed and blocked with α-CD16/32 Abs (93; eBioscience) and stained with a surface Ab mixture consisting of α-CD25–PerCP–Cy5.5 (PC61.5), α-CD127–PE–Cy7 (A7R34), α-CD45–APC (30-F11), and lineage markers, as described above. The cells were then fixed and permeabilized using a Cytofix/Cytoperm solution (BD Pharmingen) and stained with Alexa Fluor 488 conjugated Abs to α–IL-13–Alexa 488 (eBio13A; eBioscience) and α–IL-5–APC (TRFK5; BD). For intracellular cytokine staining of lymphocytes, lung-associated lymph nodes were harvested, processed using glass slides, cultured in RPMI 1640 (Life Technologies, Invitrogen) supplemented with FBS (Wisent), and stimulated for 24 h with 0.1 μl/ml PMA and 1 μl/ml Ionomycin. Following stimulation, cytokine secretion was blocked

with Brefeldin A for 6 h, after which the cells were washed, blocked, and stained with a surface Ab mixture consisting of α-CD3–PE–Cy7 (145-2C11; BD), α-CD4–V450 (GK1.5; eBioscience), PerCP conjugated α-CD8–PerCP (53-6.7; BD), and α-CD45–V500 (30-F11; BD). The cells were then fixed, permeabilized, and stained with α–IL-13–Alexa 488 and IFN-γ (XMG1.2; BD) or α–IL-17A–Alexa 647 (eBio17B7; eBioscience). Data were acquired using a FACS LSR II flow cytometer (BD Biosciences) and analyzed using FlowJo software (TreeStar), with gating determined by fluorescence-minus-one controls.

Quantitative PCR

For in vitro studies, MLE-12 cells were scraped from the plate, disrupted using RNeasy Lysis Buffer, and homogenized using a QIAshredder column. Total RNA was extracted using the RNeasy Plus Kit (QIAGEN). For lung RNA extraction, 4-mm-diameter lung pieces were collected in RNAlater solution (Invitrogen), homogenized in lysis buffer using a tissue homogenizer (Fisher Scientific), and processed using an RNeasy Kit. With 0.4 μg RNA, a reverse transcription reaction was performed using an ABI High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Quantitative real-time PCR (qRT-PCR) was performed with an ABI Prism 7500 Real-Time PCR System (Applied Biosystems). Each reaction well contained 10 μL Taq-Man Genotyping Master Mix (Applied Biosystems), 5 μL RNase-free water (Wisent), 1 μL TaqMan probes (Applied Biosystems), and 40 ng cDNA template in 4 μL water. The probes used were $Il33$ (Mm00505403_m1), $Il25$ (Mm00499822_m1), $Tslp$ (Mm01157588_m1), $Arg1$ (Mm00475988_m1), $Retnla$ (Mm00445109_m1), $Chi3l3$ (Mm00657889_mH), $Nos2$ (Mm00440502_m1), $Ccl11$ (Mm00441238_m1), and $β$-$actin$ (Part no. 401846) as a housekeeping gene. Results were calculated using the change-in-cycling-threshold method ($2^{-\Delta\Delta Ct}$) relative to the expression of $β$-$actin$ and presented as fold induction relative to unstimulated samples.

Total lung protein isolation

Mice were euthanized and lungs flushed with 10 ml ice-cold PBS. Whole lungs were homogenized in 2 ml PBS with Halt Protease and Phosphatase Inhibitor Cocktail (Fisher Scientific), using a sterilized glass tube and pestle attached to a mechanical tissue homogenizer (Glas-Col), and spun at 12,000 rpm for 20 min. Supernatants were collected, and aliquots were stored at −80°C for further analysis.

Histopathological analysis

Following euthanasia, lungs were perfused with ice-cold PBS via the right ventricle of the heart. With 10% buffered formalin acetate (Fisher Scientific), the lungs were inflated to a pressure of 25 cm H_2O and fixed overnight. Subsequently, lungs were embedded in paraffin; sectioned at 5 μm; and stained with H&E, periodic acid–Schiff (PAS), or mucicarmine reagents. Images of the slides were acquired using an Olympus BX51 light microscope (Olympus Canada) equipped with a CoolSNAP-Pro cf Digital Capture Kit (Media Cybernetics).

Cytokine production

Cytokine and chemokine contents of cell culture supernatants, BAL fluid, or whole-lung protein samples were analyzed using DuoSet ELISA kits (R&D Systems): IL-33 (DY3626), IL-4 (DY404), IL-5 (DY405), IL-13 (DY413), IL-17A (DY421), CXCL1/KC (DY453), CCL3/MIP-1α (DY450), and IFN-γ (DY485).

Statistical analysis

For all experiments, the mean and SEM are shown, unless otherwise stated. To test the significance of single comparisons, an unpaired Student t test was applied with a threshold $p < 0.05$. Multiple comparisons were tested using a one-way ANOVA with either a Dunnett posttest for comparison of individual means to a control or a Bonferroni posttest for comparison of all individual means within a figure. All statistical analysis was performed with GraphPad Prism version 5 software (GraphPad Software).

Results

IL-33 is differentially induced in mouse lungs by highly virulent C. neoformans

Intratracheal infection of BALB/c mice with 10^4 CFU of the highly virulent *C. neoformans* strain H99 results in the development of a permissive Th2-biased bronchopulmonary response, whereas infection with the moderately virulent *C. neoformans* strain 52D

results in a protective Th1-biased response (9). A role for each of the Th2-associated cytokines IL-4, IL-5, IL-10, and IL-13 in susceptibility to *C. neoformans* infection has been reported (27–30); however, it is not known whether induction of TSLP, IL-25, or IL-33 is associated with this phenotype. To investigate this possibility, a qRT-PCR analysis of whole-lung tissue was performed following *C. neoformans* infection. Interestingly, significant induction of lung $Il33$ mRNA was observed at 7 and 14 d post infection (dpi) with *C. neoformans* H99, whereas no increase in $Il33$ mRNA was evident in mice infected with strain 52D (Fig. 1A). Analysis of whole-lung IL-33 protein showed an equivalent increase at 7 dpi with either 52D or H99 relative to uninfected controls, with a progressive and significant rise in IL-33 lung protein in mice infected with H99 compared with 52D at day 14 (Fig. 1B). In agreement with previously published data (9), infection of BALB/c mice with *C. neoformans* H99 also led to a progressive rise in lung CFU resulting in death, whereas infection with strain 52D led to a gradual clearance of lung fungal burden (Fig. 1C). Thus, the expression of both $Il33$ mRNA and protein rose in parallel with lung fungal burden during the course of *C. neoformans* H99 infection. Further investigations in lungs of mice challenged with *C. neoformans* H99 revealed no significant mRNA induction of $Tslp$ (0.68- ± 0.08-fold, 0.54- ± 0.09-fold, and 0.27- ± 0.03-fold) or $Il25$ (0.82- ± 0.04-fold, 0.70- ± 0.17-fold, and 0.41- ± 0.08-fold) at 3, 7, or 14 dpi. To determine a potential cellular source of IL-33 in mouse lungs, the MLE-12 mouse lung epithelial cell line was stimulated with increasing multiplicities of *C. neoformans* H99. Relative to media-stimulated cells, a dose-dependent increase in $Il33$ mRNA was observed following exposure to *C. neoformans* H99, but not to *C. neoformans* 52D (Fig. 1D). Thus, the highly virulent *C. neoformans* H99 strain, but not the moderately virulent 52D strain, induced $Il33$ expression in immortalized mouse lung epithelial cells.

T1/ST2-dependent signaling controls growth, dissemination, and survival of C. neoformans H99 infection in mice

To investigate the physiological relevance of IL-33–mediated signaling following *C. neoformans* H99 infection, we infected mice deficient for the gene $Il1rl1$ that encodes T1/ST2, the unique and essential transmembrane subunit of the heterodimeric IL-33R (31). At 3 wk post infection with 10^4 CFU of *C. neoformans* H99, T1/ST2$^{-/-}$ mice, compared with wild-type (WT) mice, had a 5-fold reduction in lung fungal burden that was associated with a 16-fold reduction in dissemination to the spleen and an 8-fold reduction in dissemination to the brain (Fig. 2A–C). The decreased fungal growth and dissemination resulted in a statistically significant survival advantage (median survival of 25 d for T1/ST2$^{-/-}$ mice versus 23 d for WT mice) (Fig. 2D); however, the absence of T1/ST2 mediated signaling did not prevent death caused by highly virulent *C. neoformans* H99 infection. Taken together these data demonstrate a role for IL-33– and T1/ST2-mediated signaling in the control of *C. neoformans* H99 pulmonary growth and organ dissemination.

T1/ST2-deficient mice exhibit an altered pulmonary inflammatory response following C. neoformans H99 infection

To investigate the consequences of T1/ST2-dependent signaling in the respiratory mucosa, we performed a pathological analysis of fixed lung sections from BALB/c and T1/ST2$^{-/-}$ mice following *C. neoformans* H99 infection. H&E, mucicarmine, and PAS staining of uninfected sections demonstrated no differences between the groups (data not shown). At 7 dpi, examination of lung tissue stained with H&E revealed markedly increased inflammatory infiltrates surrounding the airways of T1/ST2$^{-/-}$ mice compared with BALB/c
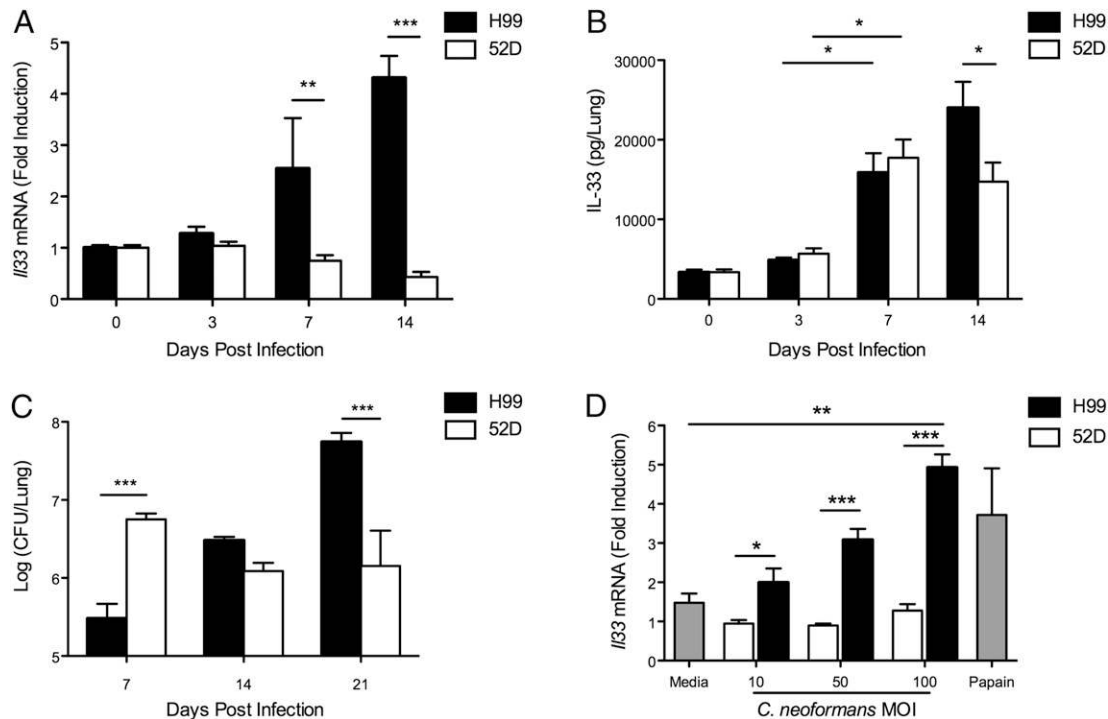
FIGURE 1. *C. neoformans* H99 and 52D differentially induce IL-33 expression. WT BALB/c mice were infected with 10^4 CFU of highly virulent *C. neoformans* H99 or the moderately virulent strain 52D. (**A**) Lung RNA was extracted at 3, 7, and 14 dpi, reverse transcribed, and *Il33* gene expression was normalized to the *β-actin* gene, using the $2^{-\Delta\Delta Ct}$ method. Total mRNA expression is given as *n*-fold induction over PBS-infected animals. Data are shown as mean ± SEM with *n* = 3–6 mice per group. (**B**) The IL-33 concentration in total lung homogenates was determined by ELISA. Data are shown as mean ± SEM with *n* = 4–5 mice per time point per group. (**C**) Total lung CFU were enumerated at 7, 14, and 21 dpi. Data are shown as mean ± SEM with *n* = 6–10 mice per group. (**D**) MLE-12 cells were grown to confluence and were stimulated for 6 h with media, *C. neoformans* strain H99 or strain 52D at increasing multiplicities of infection (MOI), or papain (0.25 mg/ml). Cells were harvested, and total RNA was extracted and reverse transcribed. *Il33* expression was analyzed and normalized to the *β-actin* gene and is shown as *n*-fold induction over media-stimulated cells. Data are expressed as mean ± SEM for each time point, with two technical and three biological replicates per group. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, using two-way ANOVA with a Bonferroni posttest correction. Results shown are representative of two independent experiments.

mice (Fig. 3A). Furthermore, in accordance with whole-lung CFU analysis, mucicarmine staining demonstrated a reduction of *C. neoformans* in T1/ST2$^{-/-}$ mice (Fig. 3A). At 14 dpi, PAS staining demonstrated airway goblet cell metaplasia in BALB/c mice that was nearly absent in T1/ST2$^{-/-}$ mice (Fig. 3B).

Differential staining of the BAL cells was done at 14 dpi to determine the pattern of airway inflammation during *C. neoformans* H99 infection. Compared with airways in BALB/c strain, those of T1/ST2$^{-/-}$ mice demonstrated greater neutrophilia with reduced eosinophilia (Fig. 3C). Consistent with these observations, airway

FIGURE 2. T1/ST2 deficiency restricts pulmonary *C. neoformans* H99 growth and dissemination and prolongs mouse survival time. WT and T1/ST2$^{-/-}$ mice were infected intratracheally with 10^4 CFU of *C. neoformans* H99. (**A**) Lung, (**B**) spleen, and (**C**) brain CFU were enumerated 21 dpi by plating organ homogenates on Sabouraud dextrose agar. Data are shown as mean ± SEM with *n* = 6–8 mice per group and are representative of two independent experiments. $*p <$ 0.05, $**p < 0.01$, $***p < 0.001$, using an unpaired Student *t* test. (**D**) WT (*n* = 18) and T1/ST2$^{-/-}$ (*n* = 15) mice were monitored daily for survival following intratracheal infection with 10^4 CFU *C. neoformans* H99. $*p < 0.05$, using a log-rank test. Data shown are pooled from two independent experiments.
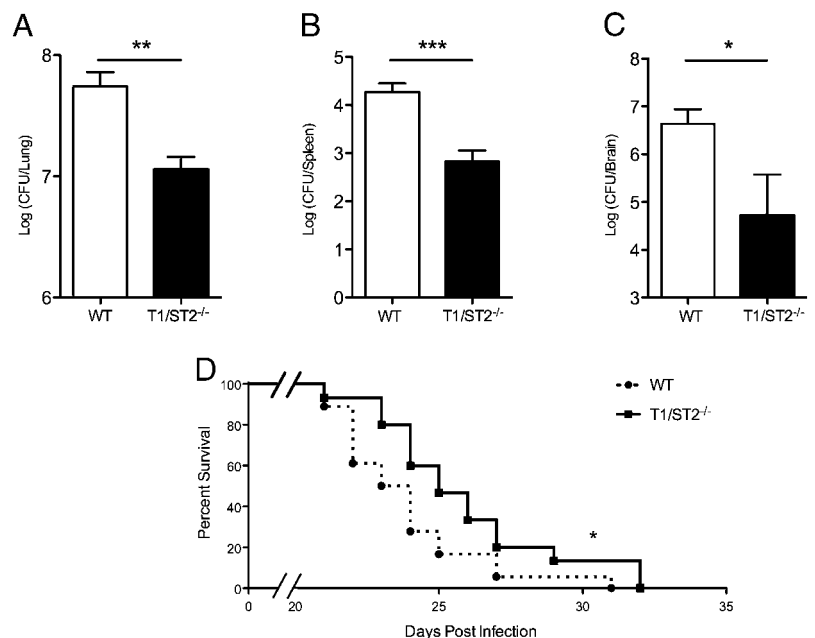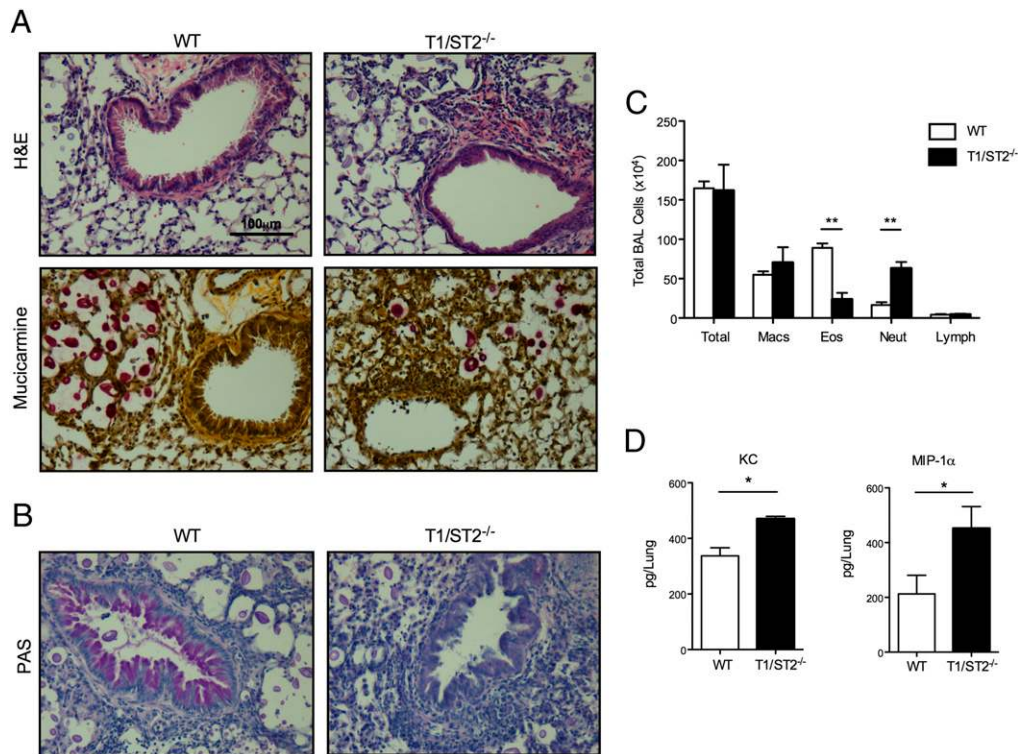
FIGURE 3. Increased airway neutrophilia in the lungs of T1/ST2$^{-/-}$ mice following cryptococcal infection. WT and T1/ST2$^{-/-}$ mice were infected intratracheally with 10^4 CFU of *C. neoformans* H99. (**A**) Lungs were perfused with PBS and embedded in paraffin at 7 dpi and stained with H&E or mucicarmine. Representative H&E images show mild peribronchial inflammation, whereas mucicarmine identifies *C. neoformans* as a red-stained cell surrounded by a clear capsular halo. (**B**) Representative images of lungs stained with PAS at 14 dpi show goblet cell hyperplasia and red airway epithelial mucus in WT mice. (**C**) Total airway cells from BAL fluid of BALB/c (*n* = 3) and T1/ST2$^{-/-}$ (*n* = 6) mice at 14 dpi were isolated, spun onto slides, and stained with DIFF-QUIK to enumerate the leukocyte populations. **$p < 0.01$, using a one-way ANOVA analysis with Bonferroni posttest correction. (**D**) The concentrations of murine KC and MIP-1α in total lung homogenates at 14 dpi were determined by ELISA. Data are expressed as mean ± SEM with *n* = 3–4 mice per group. *$p < 0.05$, using a Student *t* test. Results shown are representative of two independent experiments.

inflammation in T1/ST2$^{-/-}$ mice was associated with significant upregulation of the neutrophil chemokines CXCL1/KC and CCL3/MIP-1α (Fig. 3D). Thus, in response to *C. neoformans* H99 infection, mice deficient in T1/ST2-mediated signaling have increased neutrophil recruitment, reduced eosinophil recruitment, and less mucus hypersecretion in the airways.

C. neoformans *H99 elicits greater macrophage recruitment to the lungs in T1/ST2-deficient mice*

To characterize the cellular immune response during *C. neoformans* H99 infection, flow cytometry analysis of whole-lung digests was performed on BALB/c and T1/ST2$^{-/-}$ mice. Effective control of *C. neoformans* growth in mouse lungs requires the activation of professional phagocytes, such as macrophages that kill *C. neoformans* via inducible NO synthase–dependent mechanisms (32). Of note, T1/ST2$^{-/-}$ mice, compared with WT BALB/c mice, had a 2.5-fold increase in the absolute number of CD11c$^+$/MHCII^{intermediate} macrophages (Fig. 4A, 4B). Further analysis of this population revealed a distinct CD11b$^+$ subset that was previously identified as exudate macrophages with strong fungicidal activity (33) (Fig. 4A, 4C). In T1/ST2$^{-/-}$ mice, compared with WT BALB/c mice, exudate macrophages were 2-fold greater in both absolute number and as a proportion of CD45$^+$ lung cells (Fig 4A, 4C). Fungicidal lung exudate macrophages were recently shown to be classically activated (33), whereas an AAMΦ phenotype is known to be detrimental during *C. neoformans* H99 infection (34). To further characterize the macrophage phenotype in the lungs of BALB/c and T1/ST2$^{-/-}$ mice, a comparative analysis of alternative and classical activation markers was performed by qRT-PCR. A higher induction

of the AAMΦ markers *Arg1*, *Retnla,* and *Chi3l3* was observed in BALB/c relative to T1/ST2$^{-/-}$ mice at day 14 dpi (Fig. 4D), and an equivalent expression of the classical macrophage activation marker *Nos2* was also observed at the same time point (Fig. 4D). Finally, expression of *Ccl11* (eotaxin1), an eosinophil chemotactic factor produced by AAMΦs, was decreased in T1/ST2$^{-/-}$ mice relative to BALB/c mice (Fig. 4D). Taken together, these results indicate that T1/ST2-mediated signaling during *C. neoformans* H99 infection diminishes the recruitment of lung exudate macrophages and promotes the development of an alternative activation phenotype.

T1/ST2-deficient mice have reduced pulmonary ILC2 expansion following C. neoformans *H99 infection*

Type 2 cytokines have been associated with a permissive bronchopulmonary environment for *C. neoformans* growth, whereas Th1- and Th17-associated cytokines have been associated with fungal killing and elimination (30). To determine whether IL-33–dependent signaling influences cytokine expression during the innate host response to *C. neoformans* H99 infection, ELISA analysis of lung tissue homogenates was performed. At 7 dpi, T1/ST2$^{-/-}$ mice had a 50% reduction of the classical Th2 cytokine IL-5 and a 35% reduction in IL-13 expression compared with their WT counterparts (Fig. 5A) Conversely, no statistically significant differences in lung expression of IL-4, IL-17A, or IFN-γ were observed between BALB/c and T1/ST2$^{-/-}$ mice at this time point (data not shown). The expression of IL-5 and IL-13 is typically associated with Th2 differentiation of CD4$^+$ T lymphocytes; however, recent studies have shown that activation of ILC2s also leads to rapid production of these two cytokines (24, 25). To
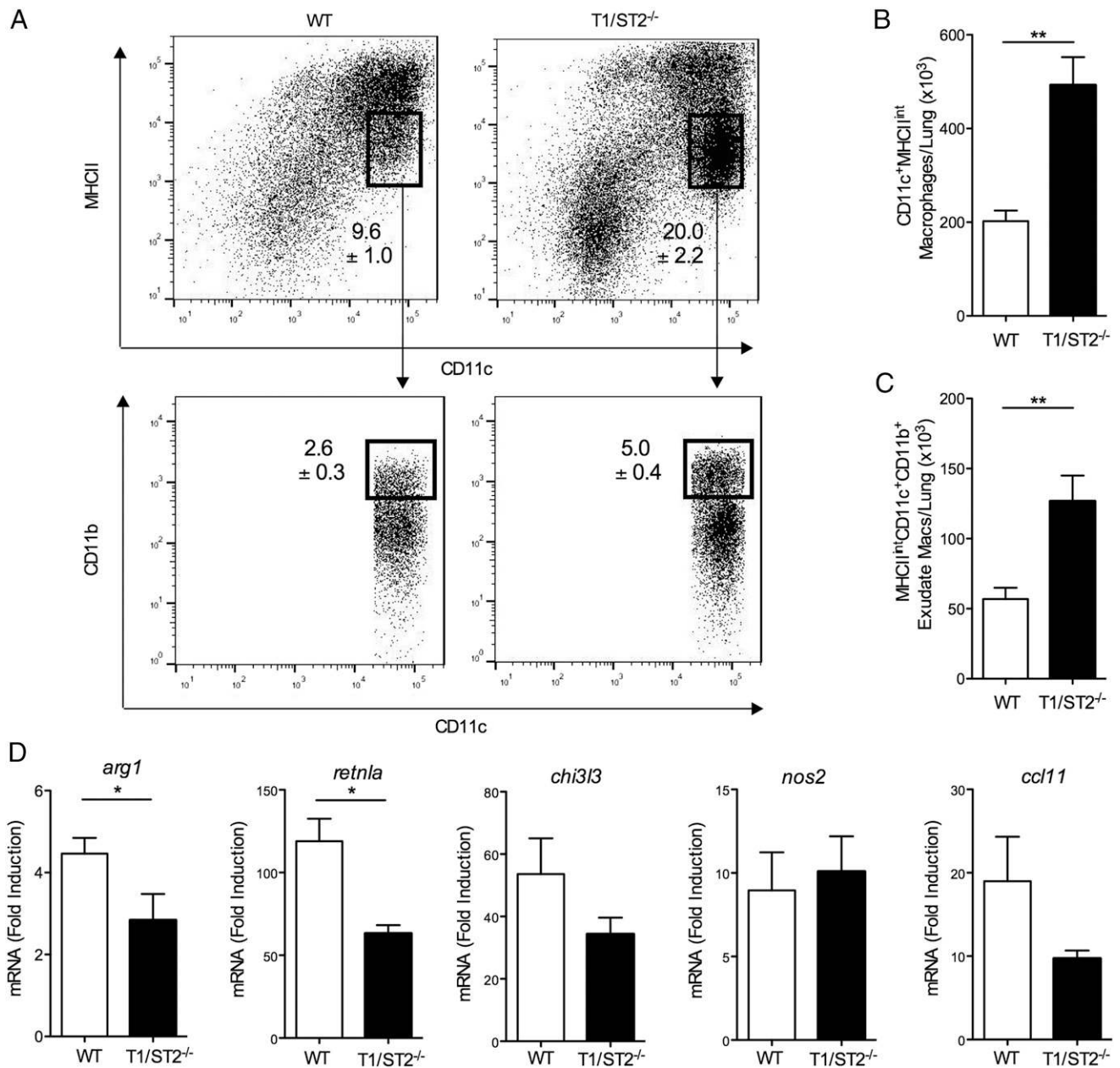
FIGURE 4. T1/ST2$^{-/-}$ mice have increased fungicidal pulmonary exudate macrophage numbers following cryptococcal infection. WT and T1/ST2$^{-/-}$ mice were infected intratracheally with 10^4 CFU of *C. neoformans* H99. Total lung cells were isolated at 14 dpi and analyzed by flow cytometry. (**A**) Representative plots of gating strategies for identification of CD11c$^+$MHCII^{intermediate} macrophages and CD11c$^+$CD11b$^+$ exudate macrophages are shown. Numbers in the plots are representative percentages of the total CD45$^+$ cells in each sample and are expressed as mean ± SEM with $n = 5$ mice per group. (**B**) Total numbers of CD11c$^+$MHCII^{intermediate} macrophages per lung and (**C**) CD11c$^+$CD11b$^+$ exudate macrophages per lung are shown as mean ± SEM with $n = 5$ mice per group. (**D**) Lung mRNA expression levels of the alternative activation markers *Arg1*, *Retnla*, and *Chi3l3*, the classical activation marker *Nos2*, and the macrophage chemokine *Ccl11* are shown. Transcript expression is normalized to the *β-actin* gene, using the 2$^{-\Delta\Delta Ct}$ method, and is presented as n-fold induction over uninfected animals. Data are expressed as mean ± SEM with $n = 3$–6 mice per group. *$p < 0.05$ and **$p < 0.01$ using a Student t test. Results shown are representative of two independent experiments.

evaluate the number of ILC2s during *C. neoformans* H99 infection, a detailed flow cytometric analysis was conducted on whole-lung digests. ILC2s were identified by gating on live CD45$^+$ lineage–negative cell populations that expressed IL-2Rα (CD25) and IL-7Rα (CD127) on the cell surface (24, 25) (Fig. 5B). Further examination of Lin$^-$CD25$^+$CD127$^+$ cells revealed high expression of Sca-1 and low expression of c-Kit (Fig. 5B), resulting in a composite surface marker phenotype that is consistent with previous descriptions of ILC2s. Prior to infection no significant difference was noted in the number of lung ILC2s between BALB/c and T1/ST2$^{-/-}$ mice (Fig. 5C, 5D). Of interest,

at 7 dpi with *C. neoformans* H99 a significantly greater absolute number and proportion of CD25$^+$CD127$^+$ ILC2s had accumulated in the lungs of WT BALB/c mice compared with T1/ST2$^{-/-}$ mice (Fig. 5C, 5D). To characterize the functional significance of lung CD25$^+$CD127$^+$ ILC2s, intracellular IL-5 and IL-13 cytokine production was determined following PMA and Ionomycin stimulation. At 7 dpi, the frequency of IL-5$^+$ and IL-13$^+$ ILC2s in WT BALB/c mice increased 0.8–8.6% and 2.1–6.6%, respectively, whereas IL-5$^+$ and IL-13$^+$ ILC2s from T1/ST2$^{-/-}$ mice showed a smaller increase of 1.3–4.4% and 1.1–1.4%, respectively (Fig. 5E). These results demonstrate that expression of IL-5 and IL-13
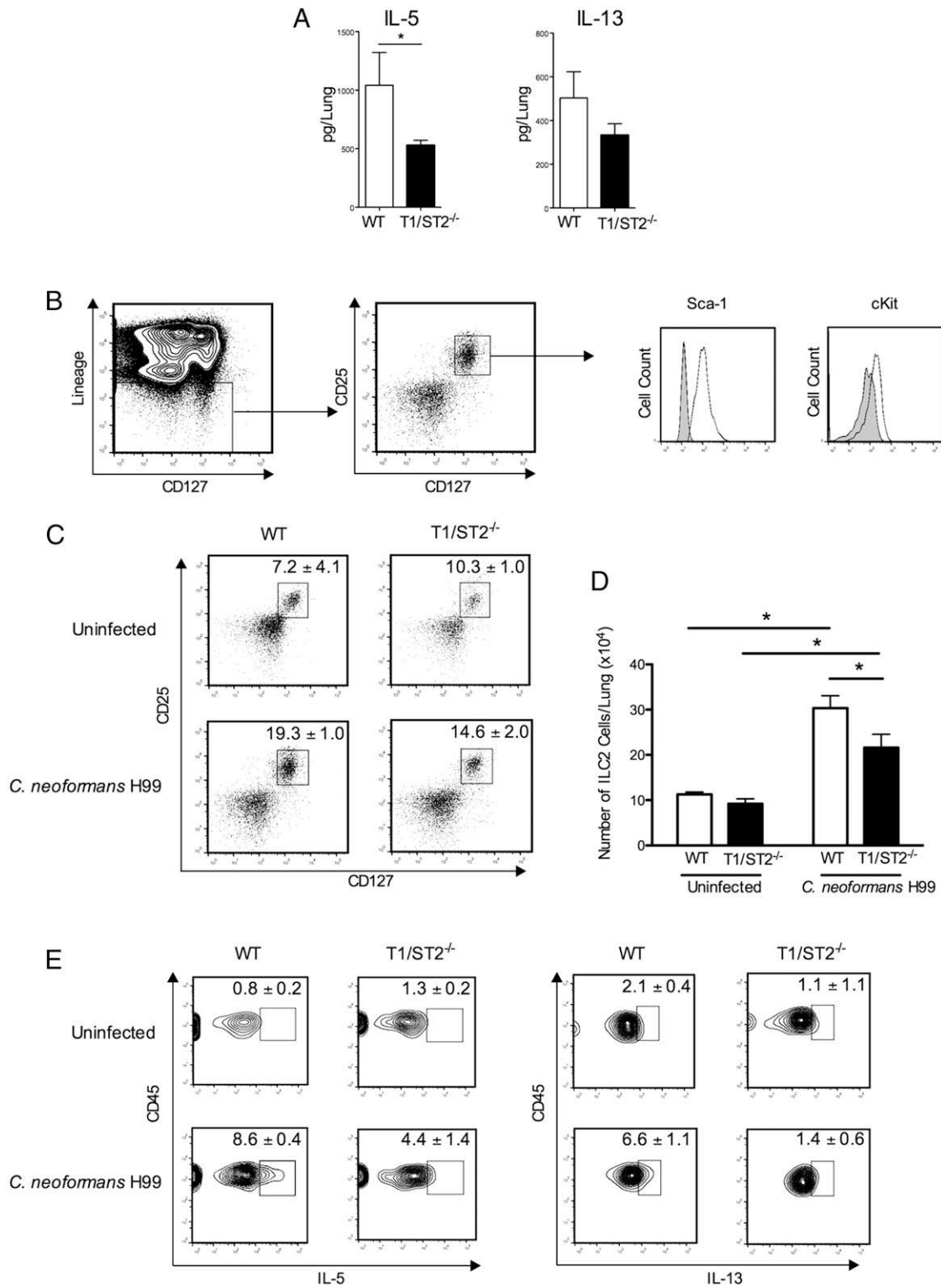
FIGURE 5. T1/ST2$^{-/-}$ mice have reduced pulmonary ILC2 accumulation in the lungs following cryptococcal infection. WT and T1/ST2$^{-/-}$ mice were infected intratracheally with 10^4 CFU *C. neoformans* H99, and lungs were harvested 7 dpi. (**A**) IL-5 and IL-13 concentrations in total lung homogenates were determined by ELISA, with $n = 5$ mice per group. $*p < 0.05$, using a Student t test. (**B**) Gating strategy for identification of lung ILC2s. CD25$^+$ CD127$^+$ cells were also positive for Sca-1 and c-Kit^{low}, with gray shaded areas representing fluorescence-minus-one controls. (**C**) Representative flow cytometry plots from individual mice showing the frequency of CD25$^+$CD127$^+$ ILC2s in infected and uninfected mice. Numbers shown are the percentage of cells in each gate relative to total cells in each plot and are expressed as mean $\pm$ SEM with $n = 3$ mice per group. (**D**) Total numbers of CD25$^+$CD127$^+$ lung ILC2s per mouse are expressed as mean $\pm$ SEM with $n = 3$ mice per group. $*p < 0.05$, using a Student t test. (**E**) Representative intracellular cytokine staining plots for IL-5 and IL-13 in CD25$^+$CD127$^+$ ILC2s following restimulation with PMA/Ionomycin. Numbers shown are the percentage of cells in each gate relative to total cells in each plot and are expressed as mean $\pm$ SEM with two pooled mice per replicate, with $n = 3–6$ replicates per group. Results shown are representative of two independent experiments.

in mouse lungs at an early time point after *C. neoformans* H99 infection is associated with an expansion of ILC2s that is dependent on IL-33 signaling.

T1/ST2-deficient mice have a diminished Th2 immune bias in response to *C. neoformans* H99 infection

Previous studies have shown that a Th1 pattern of adaptive immunity is crucial for the clearance of *C. neoformans* in both mice and humans (35, 36). To study the involvement of IL-33–mediated signaling during the adaptive phase of host immunity to *C. neoformans* H99 infection, lung tissue and draining lymph nodes were harvested at 14 dpi for determination of their cytokine expression and cell polarization pattern, respectively. A significantly decreased expression of IL-5 and IL-13, with a slight increase in IFN-γ and no difference in IL-17A, was observed in the lung homogenates of T1/ST2$^{-/-}$ mice compared with BALB/c mice (Fig. 6A). Single-cell suspensions from lung-associated lymph nodes were restimulated with PMA and Ionomycin and stained for intracellular production of IL-13, IL-17A, and IFN-γ. Compared with the BALB/c strain, T1/ST2$^{-/-}$ mice had a significant decrease in IL-13$^+$CD4$^+$ cells and also exhibited a small, though non–statistically significant, increase

in IFN-γ$^+$CD4$^+$ cells at 14 dpi (Fig. 6B, 6C), whereas no difference in IL-17A$^+$CD4$^+$ and IFN-γ$^+$CD8$^+$ lymph node cells was observed between the two mouse strains (data not shown). No significant difference was seen in the number of CD4$^+$ (2.03 × 10^6 ± 0.34 × 10^6 versus 2.55 × 10^6 ± 0.07 × 10^6) or CD8$^+$ (7.48 × 10^5 ± 1.45 × 10^5 versus 9.27 × 10^5 ± 0.17 × 10^5) T cells in the lung-associated lymph nodes of T1/ST2$^{-/-}$ and WT mice. Finally, T1/ST2$^{-/-}$ mice, compared with WT BALB/c mice, had a 40% reduction in serum IgE level at 21 dpi (Fig. 6D). Taken together, these findings demonstrate that IL-33–dependent signaling during *C. neoformans* infection promotes a Th2 pattern of cellular and humoral adaptive immunity.

Discussion

Both Th1 and Th2 elements contribute to host defense against *C. neoformans* infection in WT mice, and the relative expression of the corresponding host immune factors is a central determinant of the outcome of infection (28). Susceptibility to progressive cryptococcal disease is facilitated by type 2 polarized immunity; however, the precise mechanisms that underlie this association are not fully known (6). In this article we have used a clinically rel-
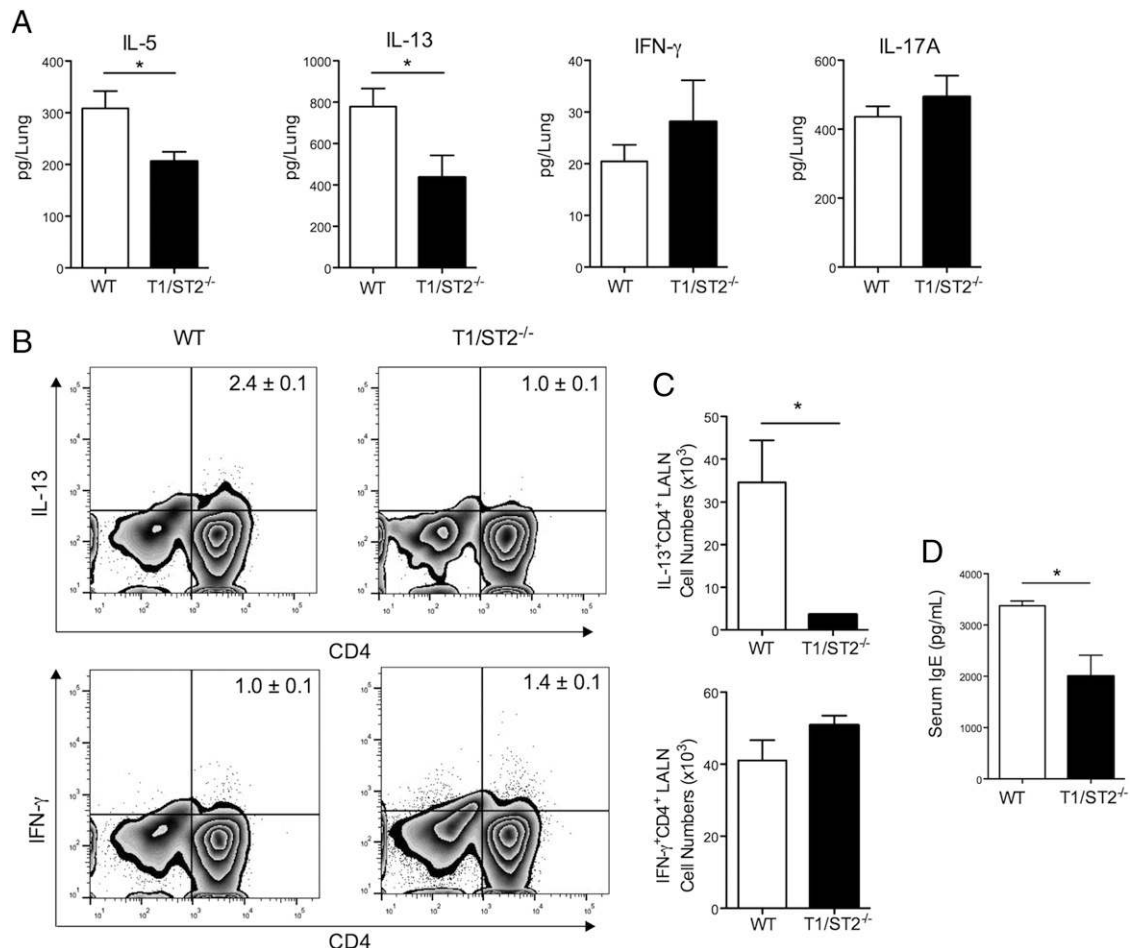

FIGURE 6. T1/ST2 deficiency diminishes Th2 lymphocyte polarization in lung-associated lymph nodes during *C. neoformans* infection. WT and T1/ST2$^{-/-}$ mice were infected intratracheally with 10^4 CFU *C. neoformans* H99. (**A**) Lungs were harvested at 14 dpi, and IL-13, IL-5, IFN-γ, and IL-17A protein levels in whole-lung homogenates were determined by ELISA. Data are expressed as mean ± SEM with $n = 3$–8 mice per group. *$p < 0.05$, using a Student t test. (**B**) Representative flow cytometry plots of mediastinal lymph nodes cells harvested at 14 dpi that have undergone PMA/Ionomycin restimulation, followed by intracellular staining for IL-13 and IFN-γ expression, are depicted. Plots are representative of a single experiment, with percentages expressed as mean ± SEM with $n = 3$ per group. (**C**) Total IL-13$^+$ or IFN-γ$^+$ CD4$^+$ lymph node cells per mouse analyzed in (B) are given as mean ± SEM. *$p < 0.05$, using a Student t test. (**D**) At 3 wk post infection with *C. neoformans* H99, blood was harvested via cardiac puncture and spun down in a gel tube to obtain serum. Serum IgE concentrations were determined by ELISA. Data from one experiment are expressed as mean ± SEM with $n = 3$ mice per group. *$p < 0.05$, using a Student t test. Results shown are representative of two or three independent experiments.

evant mouse model of *C. neoformans* pneumonia to identify a complex series of innate and adaptive immune responses that are regulated by the epithelial-derived cytokine IL-33 through its unique receptor T1/ST2. Our data demonstrate for the first time, to our knowledge, that *C. neoformans* induces IL-33 expression in the lung during the course of infection. Furthermore, we have established a role for IL-33–dependent signaling as an activator of ILC2s as well as several associated molecular and cellular mediators that mitigate host defense and survival by creating a permissive environment for pulmonary cryptococcal replication and organ dissemination. These observations identify a mechanism of potential relevance to the understanding and therapy of human allergic bronchopulmonary mycosis and fungal pneumonia.

The cytokine IL-33 is expressed by epithelial barrier tissues of the lung, brain, and gut (37). On the basis of its spatial expression pattern, IL-33 is believed to function as a first line of defense against several pathogenic microbes, and many studies now suggest that it also functions as an alarmin that is released during cell damage (13, 38). For example, IL-33 has been implicated in host defense to the intestinal nematode *Tricuris muris* through promotion of Th2 immunity (39). Similarly, the yeastlike pathogen *Pneumocystis murina* triggers IL-33 expression by lung epithelial cells, and mice that have been challenged with this organism were able to clear the infection following administration of exogenous IL-33 (40). Conversely, blockade of T1/ST2 by mAbs concomitantly with TLR9 ligand CpG administration attenuated airway hyperresponsiveness, mucus cell metaplasia, and lung fungal growth in a mouse model of allergic bronchopulmonary mycosis caused by *Aspergillus fumigatus* (41). In the current study, we have demonstrated induction of *Il33* mRNA in a mouse lung epithelial cell line (MLE-12) and a progressive increase in lung IL-33 protein expression during infection of susceptible BALB/c mice with the opportunistic yeast pathogen *C. neoformans* H99. In contrast, IL-33 protein expression reached a plateau at day 7 following experimental pulmonary infection with *C. neoformans* 52D, a fungal strain to which BALB/c mice are relatively resistant, suggesting that IL-33 plays a role in modulating the host immune response to *C. neoformans*. The physiological relevance of IL-33–dependent signaling was confirmed by demonstrating a significant decrease in lung, spleen, and brain fungal burdens post infection of T1/ST2-deficient mice with 10^4 CFU of *C. neoformans* H99. As a consequence of diminished *C. neoformans* H99 brain dissemination, T1/ST2$^{-/-}$ mice had a modest, although statistically significant, survival advantage over WT animals, even though all mice eventually succumbed to the infection. Notably, despite a significant reduction in lung fungal burden following *C. neoformans* H99 infection of IL-4/IL-13 double knockout mice, Zhang et al. (30) were also unable to demonstrate significant differences in brain CFU or long-term survival. Taken together, these observations highlight the complexity and distinct nature of the host response to *C. neoformans* in different organs and demonstrate that regulation of lung fungal growth and dissemination by Th2 immunity have limited effects on replication in the brain.

Several recent studies have identified novel innate cell populations at barrier surfaces in mice and humans that have been variously termed nuocytes, natural helper cells, or innate type 2 helper cells, all of which are now collectively referred to as ILC2s (42). These cells lack classical lineage markers as well as a rearranged Ag receptor, and they regulate allergic inflammation, host defense, and wound repair when activated by IL-33. ILC2s expand early during infection and produce large quantities of IL-5 and IL-13 (20–22). Moreover, ILC2s have been shown to regulate various Th2 immunopathologies in the absence of adaptive immunity, including influenza A–induced airway hyperresponsiveness

and protease allergen-induced airway inflammation (43, 44). For example, after in vivo challenge with H3N1 influenza A virus, mouse innate lymphoid cells produced significant IL-5 lung protein that peaked at 4 dpi (43). In addition, ILC2s are thought to account for the majority of innate IL-5 and IL-13 in the lung and subsequent eosinophilia in a mouse respiratory model that uses fungal allergens derived from *A. alternata* (24, 25). During *C. neoformans* H99 infection of BALB/c mice, we observed IL-33–dependent expansion of a lineage-negative CD25$^+$CD127$^+$Sca-1$^+$c-Kit^{low} population at 7 dpi. Furthermore, a significantly greater number of WT lung ILC2s produced IL-5 and IL-13 in comparison with ILC2 from T1/ST2$^{-/-}$ mice, indicating that IL-33–dependent signaling regulates this cellular source of Th2 cytokines early after *C. neoformans* infection. These findings are noteworthy because they are the first demonstration, to our knowledge, of ILC2 activation during a live fungal infection.

Alveolar macrophages are among the first immune cells to confront microbes that enter the lower respiratory tract and use a variety of receptors to recognize and internalize inhaled *C. neoformans* (32). Recently, it has been shown that recruitment of CD11b$^+$CD11c$^+$MHCII^{int} exudate macrophages during the effector phase of the immune response mediates the clearance of *C. neoformans* (33). In accordance with this finding, we observed a significant increase in number and frequency of exudate macrophages in the lungs of relatively resistant T1/ST2$^{-/-}$ mice at 14 dpi. The activation phenotype of lung macrophages is also crucial for the control of fungal infection and is influenced by the local cytokine milieu. For example, induction of IL-33 during *P. murina* infection induced an AAMΦ phenotype that mediated host defense (40). Conversely, IL-13 transgenic animals that also have increased alternative macrophage activation were susceptible to progressive *C. neoformans* infection, whereas IL-13–deficient animals had increased classical macrophage activation that coincided with a decrease in lung fungal burden (29). On the basis of the current findings, it is plausible that IL-33–dependent activation of ILC2s with consequent IL-13 secretion is responsible for initiating the AAMΦ phenotype observed in the lungs of BALB/c, but not of T1/ST2$^{-/-}$, animals. The observation of differential macrophage polarization at 14 dpi also raises the possibility that IL-33 induction could directly polarize CD4$^+$ T cells toward a Th2 phenotype leading to the production of IL-5 and IL-13 that induces an AAMΦ phenotype (45).

BALB/c mice exhibit several hallmarks of a type 2 response to *C. neoformans* H99 respiratory infection, including pulmonary eosinophilia, airway mucus hypersecretion, Th2 lymphocyte polarization, and production of serum IgE (8, 9). Previous studies of *C. neoformans* infection of inbred mice have demonstrated that IL-5 is required for pulmonary eosinophilia and is associated with ineffective host defense (27, 46). Recent evidence using Rag1$^{-/-}$ mice suggests that early IL-33 expression induced after challenge with the fungal allergen *A. alternata* leads to expansion of Sca-1$^+$ c-Kit$^-$ ILC2, which triggers IL-5–mediated eosinophilia (24). During *C. neoformans* infection of BALB/c mice, a number of different IL-33–responsive cells are potential sources of IL-5 secretion that mediate eosinophilia. For example, shortly post infection, IL-5 production by activated ILC2 could trigger the initial maturation and subsequent homing of eosinophils to the lung, and Th2 cells could maintain this response during the adaptive immune phase. Basophils and mast cells have also been shown to be direct targets of IL-33 and could contribute to lung eosinophilia through release of inflammatory cytokines, and it is conceivable that IL-33 could act as a direct stimulator of eosinophil proliferation (17, 47). Similarly, early IL-13 secretion by ILC2 and/or late production of IL-13 by Th2-polarized CD4$^+$ lymphocytes may

also be responsible for the goblet cell hyperplasia and mucus secretion observed on histopathological analysis of the lungs.

T1/ST2$^{-/-}$ mice infected with *C. neoformans* H99 develop enhanced airway neutrophilia, increased lung exudative macrophage recruitment, and decreased Th2 polarization of CD4$^+$ T lymphocytes in the lung-associated lymph nodes that is associated with control of fungal burden. Despite a clear shift toward Th1 immunity, T1/ST2$^{-/-}$ mice, compared with uninfected mice, also retain elements of a Th2 response characterized by residual eosinophilia, mild airway mucus production, and elevation of serum IgE. Notably, the epithelial-derived cytokines TSLP and IL-25 have been shown to mediate allergic airway inflammation in mouse models (48, 49). Although we did not demonstrate mRNA induction of these mediators during *C. neoformans* infection, these data do not formally exclude the possibility that these factors contribute to various aspects of Th2 immunity observed in T1/ST2$^{-/-}$ mice.

An intact adaptive CD4$^+$ T lymphocyte immune response with Th1 polarization is necessary for *C. neoformans* clearance (35, 36). IL-33 has been shown to contribute to the polarization of naive T lymphocytes toward a Th2 phenotype (16, 50), and we demonstrate in this article that T1/ST2 deficiency results in diminished IL-13$^+$CD4$^+$ cells in lung-associated lymph nodes. Of interest, T1/ST2$^{-/-}$ mice did not exhibit a reciprocal switch to Th1/Th17 immunity at 14 dpi. This observation is consistent with studies of adaptive immune polarization following *C. neoformans* infection in IL-4, IL-10, or IL-13 knockout mice (28, 29) that found no change in the level of IFN-γ despite an expected decrease in Th2 immunity and reduced fungal burden. Conversely, IL-4$^{-/-}$IL-13$^{-/-}$ double knockout mice developed enhanced Th1/Th17 immunity following *C. neoformans* H99 infection (30). Taken together, these findings indicate that although deletion of a single Th2-promoting cytokine may be sufficient to enhance host defense, one or more related factors such as IL-4 or TSLP may concurrently suppress Th1/Th17 responses (51). Collectively, the observations in this report demonstrate that T1/ST2-mediated signaling makes an important contribution to the innate and adaptive type 2 responses during *C. neoformans* H99 infection, but does not uniquely regulate all elements of the local or systemic immune response to this fungal pathogen.

Another recent report highlighted the importance of T1/ST2 signaling during the adaptive immune phase of *C. neoformans* infection (52). Using intranasal infection with *C. neoformans* 1841D, Piehler et al. (52) showed that T1/ST2$^{-/-}$ mice have a lower lung fungal burden at 49 dpi, associated with reduced eosinophilic infiltration, airway mucus secretion, and serum IgE production. Furthermore, *C. neoformans* infection stimulated T1/ST2 induction on lung Th cells, and these cells also expressed higher levels of the activation markers CD25 and CD44. Our data are consistent with this report and extend the role of the IL-33–T1/ST2 signaling axis to the innate phase of the host response to pulmonary cryptococcal infection. Specifically, we have shown that IL-33–dependent signaling initiates a Th2-biased response by promoting the early accumulation and activation of lung ILC2s in association with enhanced alternative macrophage polarization.

From an evolutionary perspective, type 2 immune responses provide protection against helminth and parasite infections but are detrimental when associated with conditions characterized by sterile allergic inflammation, such as asthma. Although many theories have been proposed, the precise factors that initiate and propagate this effector arm of the immune response are still under debate (53). Our study demonstrates critical contributions of IL-33 to a complex host–pathogen interaction that enables fungal pathogens to persist in the presence of type 2 immunity. These observations give rise to a number of specific questions: 1) What *C. neoformans*–derived factors trigger skewing of type 2 polarization, and did they evolve in parallel with the host immune system? 2) Beyond IL-5 and IL-13 production, do ILC2s make additional contributions to the pathogenesis of fungal infection? 3) Are there other IL-33–responsive cell types, such as mast cells, basophils, or dendritic cells, that could contribute to this mechanism of fungal susceptibility? Further studies that yield a comprehensive understanding of the IL-33–ILC2 axis and how it leads to deleterious outcomes following *C. neoformans* infection in mice could facilitate the development of therapeutic approaches to combat human fungal pneumonia and allergic bronchopulmonary mycoses.

Disclosures

The authors have no financial conflicts of interest.

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