Basophils prime group 2 innate lymphoid cells for neuropeptide-mediated inhibition

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Type 2 cytokine responses promote parasitic immunity and initiate tissue repair; however, they can also result in immunopathologies when not properly restricted. Although basophilia is recognized as a common feature of type 2 inflammation, the roles basophils play in regulating these responses are unknown. Here, we demonstrate that helmint-induced group 2 innate lymphoid cell (ILC2) responses are exaggerated in the absence of basophils, resulting in increased inflammation and diminished lung function. Additionally, we show that ILC2s from basophil-depleted mice express reduced amounts of the receptor for the neuropeptide neuromedin B (NMB). Critically, NMB stimulation inhibited ILC2 responses from control but not basophil-depleted mice, and basophils were sufficient to directly enhance NMB receptor expression on ILC2s. These studies suggest that basophils prime ILC2s to respond to neuron-derived signals necessary to maintain tissue integrity. Further, these data provide mechanistic insight into the functions of basophils and identify NMB as a potent inhibitor of type 2 inflammation.

The World Health Organization estimates that helmint infections affect approximately 2 billion people worldwide, causing malnutrition, growth impairment, cognitive deficiencies and chronic immunopathology1–3. Although anthelminthic drugs are available, they have short-lived effects, resulting in high reinfection rates within months of treatment1. Further, the generation of long-lasting immunotherapeutics has been precluded by our incomplete understanding of the mechanisms that properly regulate type 2 cytokine responses and host protection1. Therefore, developing a better understanding of the immunological mechanisms that promote and/or inhibit type 2 inflammation would greatly inform the generation of new strategies to treat helmint infections and their associated morbidities.

Many helmints, such as hookworms, undertake complex migratory cycles that require their passage through multiple mucosal sites, including the lungs and gut. The passage of these large multicellular parasites results in substantial wounding and tissue damage1,4. In response to these signals, the mammalian host mounts potent type 2 cytokine responses, characterized by the secretion of interleukin-4 (IL-4), IL-5 and IL-13, which simultaneously promote potent type 2 inflammation; characterized by the secretion of cytokines (IL-6, tumor necrosis factor and IL-13), growth factors (amphiregulin and macrophage colony-stimulating factor 1)23–27. Given their capacity to secrete these potent effector molecules, it is not surprising that basophils are reported to operate as important mediators of allergic inflammation12–14. However, recent work has also begun to highlight the critical contributions of basophils during homeostatic conditions. For example, studies have demonstrated that basophils imprint on alveolar macrophages and play important roles during lung development, suggesting that the functions of basophils are context-dependent and extend beyond their ability to promote inflammation13–14.

Consistent with their functional diversity in response to distinct stimuli, the roles basophils play in the context of antihelmint immunity appear to be parasite-specific. While studies have demonstrated that basophils promote type 2 inflammation and worm expulsion following Trichurus muris and Trichinella spiralis infections, their functions following other helmint infections remain to be fully defined15–19. Although the population expansion of ILC2s in response to the hookworm Nippostrongylus brasiliensis has been appreciated for several decades, the functions of N. brasiliensis-induced basophils are poorly understood. Further, important studies have demonstrated that depleting basophils after a primary N. brasiliensis infection has no effect on worm clearance36,21. These data suggest that N. brasiliensis-induced basophils perform important functions that have yet to be appreciated.

A growing body of evidence has demonstrated that specialized innate immune cells engage in cellular cross talk and cooperate to initiate type 2 inflammation21–23. For example, basophils can promote the activation of ILC2s via their secretion of IL-4 (refs. 12,14). Additionally, recent reports showed that neuron-derived signals such as NMU and calcitonin gene-related peptide (CGRP) can also regulate the activation of ILC2s and thereby dictate the intensity of type 2 inflammation24–27. Collectively, these studies suggest that intracommunication (immune cell to immune cell) and extracommunication (immune cell to neuron) might cooperate to direct the scope of type 2 responses. Despite these advances, whether immune

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cell cross talk is required to facilitate subsequent neuroimmune interactions has yet to be defined.

In the present study, we report that *N. brasiliensis*-induced basophils accumulate in the lungs after the exit of parasitic larvae from the tissue, suggesting that they might contribute to host protection by promoting the healing of helminth-affected tissues rather than directly acting on the parasites. Consistent with this hypothesis, our data demonstrate that *N. brasiliensis*-induced ILC2 responses are exaggerated in the absence of basophils, resulting in increased inflammation and reduced pulmonary function. Critically, over-active ILC2s from basophil-depleted mice exhibited reduced expression of the receptor for NMB (NMBR), a member of the neuromedin family of neuropeptides that includes NMB, NMC, NMK, NML, NMN, NMU and NMS\(^{20,21}\). Importantly, treatment with exogenous NMB was sufficient to reduce ILC2 responses, eosinophilia and mucus production after *N. brasiliensis* infection. Finally, while recombinant NMB (rNMB) treatment inhibited sort-purified ILC2s from control mice, ILC2s from basophil-depleted mice were unresponsive to NMB stimulation, suggesting that basophils prime ILC2s for NMB-mediated inhibition. Altogether, our data highlight a previously unappreciated regulatory role for basophils in the context of anthelmintic immunity and identify NMB as a potent inhibitor of type 2 inflammation.

**Results**

**Depletion of basophils results in severe helminth-induced pathology and impaired lung function.** Despite basophilia being a hallmark of type 2 inflammation\(^ {23,24}\), the roles these enigmatic cells play in regulating type 2 cytokine responses are controversial\(^ {25-27}\). Therefore, we sought to better determine the functions of these rare immune cells after infection with the gastrointestinal nematode, *N. brasiliensis*. First, we evaluated several parameters of type 2 cytokine-mediated inflammation induced by *N. brasiliensis* in the presence or absence of basophils. Lineage-specific depletion of basophils was achieved by administration of diphtheria toxin to *Mcpt8\(^ {tm1(cre)Lky}\) ROSA26\(^ {mmt}\)* mice, as described previously\(^ {21}\). While depletion did not alter inflammation in the gastrointestinal tract (Extended Data Fig. 1a) or worm expulsion (Fig. 1a), basophil depletion resulted in significantly increased type 2 cytokine responses in the lung (Fig. 1b), where the parasites transiently reside as part of their developmental life cycle. In vivo staining protocols used to distinguish blood-circulating versus tissue-resident cells revealed that tissue-resident basophil populations increased in the lungs starting on day 3 and peaked on day 5 postinfection (Fig. 1c,d). While effector cells activated in the context of type 2 cytokine responses are required to promote parasite clearance, they are also important for maintaining the integrity of parasite-affected tissues\(^ {28,29}\). Since *N. brasiliensis* larvae exit the lung tissue on day 3 postinfection, before the majority of basophils are present, it is possible that basophils are playing a role in regulating inflammation in an attempt to restore lung function rather than serving to limit parasitic burdens. In support of this hypothesis, *N. brasiliensis*-infected mice depleted of basophils exhibited altered lung pathology, marked by increased mucus production (Extended Data Fig. 1b,c), inflammatory cell infiltrates (Fig. 1e and Extended Data Fig. 1d) and had significantly reduced oxygen levels compared to control mice (Fig. 1f). Collectively, these data provoke the hypothesis that basophils limit infection-induced type 2 cytokine responses and assist in maintaining lung function post-*N. brasiliensis* infection.

**Basophil depletion results in elevated ILC2 responses.** Microscopic analysis of lung pathology suggested that basophil-depleted mice had elevated infection-induced eosinophil responses. Flow cytometry analysis of bronchoalveolar lavage (BAL) fluid and lung infiltrates confirmed that while infection-induced neutrophilia was not significantly altered (Fig. 2a and Extended Data Fig. 2a), basophil-depleted mice exhibited significantly increased BAL and lung eosinophil responses (Fig. 2b and Extended Data Fig. 2b), as defined by flow cytometry gating strategies (Extended Data Fig. 2c). *N. brasiliensis*-induced eosinophil responses and mucus production are dependent on IL-5 and IL-13, which are produced by ILC2s or CD4\(^ +\) T cells\(^ {30}\). Lung basophil responses occur in the first few days postinfection (days 3–5), during the innate window when *N. brasiliensis*-induced inflammation is reported to be ILC2-dependent\(^ {24,25}\). Further, the ability of basophils to communicate with and alter the activation state of ILC2s has been demonstrated previously\(^ {21,24}\). Therefore, we sought to determine if basophil-depleted animals exhibited elevated infection-induced ILC2 responses that correlated with increased mucus production and eosinophilia. Interestingly, ILC2 populations were increased in both the BAL and lung tissue of basophil-depleted mice compared to controls (Fig. 2c and Extended Data Fig. 2d). Further, increases in IL-5 and IL-13 producing ILC2s were also detected (Fig. 2d,e and Extended Data Fig. 2e,f), as defined by specific gating strategies (Extended Data Fig. 2g). Similar results were observed in the BAL and lungs of infected *Mcpt8\(^ {cre}\)* mice that constitutively lack basophils\(^ {22}\) (Extended Data Fig. 3a–f). However, despite increased IL-5 and IL-13 production by lung ILC2s, no increases in infection-induced eosinophil responses were observed in the lungs of *Mcpt8\(^ {cre}\)* mice (Extended Data Fig. 3f). This is probably due to compensation mechanisms that are thought to exist in this constitute mouse model\(^ {22}\). Importantly, basophil depletion in naive mice did not alter ILC2 responses, suggesting that the observed differences are not due to diphtheria toxin receptor (DTR)-mediated cell death (Fig. 2c–e and Extended Data Fig. 2d–f). Nonetheless, to further rule out the possibility of off-target depletion effects, we next took a gain-of-function approach and transferred DTR\(^ +\) basophils into basophil-depleted mice. Critically, the intratracheal transfer of DTR\(^ +\) basophils was sufficient to suppress ILC2 responses, mucus production and eosinophilia back to normal levels in both the BAL and lungs of basophil-depleted mice (Fig. 2f–i and Extended Data Fig. 4a–g). Collectively, these loss- and gain-of-function approaches suggest that basophils negatively regulate lung ILC2 responses after *N. brasiliensis* infection.

**Helminth-activated ILC2s exhibit reduced NMBR expression in the absence of basophils.** To further examine whether the effects of basophil depletion were occurring independently of adaptive lymphocytes, recombination-activating gene 2 (Rag2)-deficient mice were treated with the basophil-depleting antibody Ba103 (ref. 39). Rag2\(^ {−/−}\) mice treated with Ba103 exhibited significantly elevated *N. brasiliensis*-induced ILC2 responses and eosinophilia in both the BAL (Fig. 3a,b) and lung (Fig. 3c,d). Further, Rag2\(^ {−/−}\) mice treated with Ba103 also exhibited significantly elevated mucus production and altered lung pathology (Fig. 3e–f). Similar results were observed when basophils were depleted by treatment with the FcR\(^ {α}\)R-specific antibody MAR-1 (ref. 39) (Extended Data Fig. 5a–f). Collectively, these data suggest that basophils negatively regulate lung ILC2 responses independently of adaptive lymphocytes. To elucidate the mechanism through which basophils regulate ILC2s, we first examined whether basophil depletion resulted in increased production of cytokine alarmins such as IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), which promote ILC2 activation\(^ {40}\). Importantly, no changes in the expression of *Il25* or *Tslp* were observed in the lungs of basophil-depleted mice. However, basophil-depleted mice exhibited significantly reduced expression of *Iil3* in whole lung tissue (Extended Data Fig. 6a–c). Consistent with these data, analysis of IL-33-green fluorescent protein (GFP) reporter mice revealed that type 1 and type 2 pneumocytes expressed significantly less *N. brasiliensis*-induced *Iil3* following treatment with the anti-FcR\(^ {α}\)R-targeting antibody MAR-1 (Extended Data Fig. 6d,e). In aggregate, these studies suggest that elevated ILC2...
responses in basophil-depleted mice are not a result of increased cytokine/alarmin production. We next evaluated whether basophils suppress ILC2 activation via regulating mediators including anti-inflammatory cytokines, such as IL-10, or growth factors, like amphiregulin. Although no significant changes in Il10 expression were detected in basophil-depleted mice, expression of amphiregulin was significantly elevated in the absence of basophils (Extended Data Fig. 6f,g). Further, while basophils secreted robust amounts of activation-induced IL-6, we could not find detectable levels of basophil-derived IL-10 or amphiregulin (Extended Data Fig. 6h–j). Collectively, these data suggest that basophils are not regulating lung inflammation by producing these regulatory factors or amplifying their expression levels. Next, we performed single-cell RNA sequencing (scRNA-seq) analysis to evaluate if the depletion of basophils substantially altered the composition of ILC2 populations. scRNA-seq of lung-resident ILC2s revealed the presence of six distinct cell clusters defined by specific marker genes using the Seurat workflow (Extended Data Fig. 7a,b). Importantly, while basophil...
depletion did not result in substantial changes to the composition of ILC2s, helminth-induced expression of *Il5*, *Il13* and *Areg* were significantly elevated across clusters of ILC2s sort-purified from basophil-depleted mice compared to control samples (Extended Data Fig. 7c). However, we did not detect significant changes in the expression of *Il1rl1* or *Arg1*.
While scRNA-seq is helpful to identify general differences across samples, the depth of gene lists can make it difficult to perform pathways analysis. To address this, we also performed conventional RNA-seq of lung ILC2s post-\textit{N. brasiliensis}, to better identify how basophils may regulate their state of activation. Control ILC2s expressed a substantial number of genes at significantly higher levels than those from basophil-depleted mice (Fig. 4a, Extended Data Fig. 8a and Supplementary Table 1). Interestingly, pathway analysis revealed that ILC2s from control animals were enriched for pathways including neuroactive ligand-receptor interactions, seven-transmembrane domain (7TM), G protein-coupled receptor signaling and rhodopsin-like signaling compared to ILC2s sort-purified from basophil-depleted mice (Fig. 4b). Notably, all three of these pathways are associated with neuropeptide signaling, which has recently been identified as an important component of ILC2 activation post-\textit{N. brasiliensis} infection. Specifically, the neuropeptide NMU, produced by cholinergic neurons, promotes ILC2 activation post-\textit{N. brasiliensis} infection. Importantly, NMU is part of the neuromedin family of neuropeptides including NMB, NMC, NMK, NML, NMN, NMS and NMU. Similar to NMU, NMB is expressed in the central nervous system, lungs, gastrointestinal tract and adipose tissues of mammals. Further, both NMU and NMB signal through receptors belonging to the rhodopsin-like subfamily A7 of G protein-coupled protein receptors, which was identified by pathway analysis. While no significant changes in the receptors for NMU (NMUR1) or NMB (NMBR) were identified by RNA-seq analysis on day 5 postinfection, when ILC2s were sort-purified from the lungs of mice on day 7 post-\textit{N. brasiliensis} (following the peak of lung basophilia), significant reductions in Nmbr expression were observed in basophil-deficient animals (Fig. 4c). Further, while surface expression of NMBR by ILC2s was increased postinfection, ILC2s from basophil-depleted mice exhibited significantly reduced expression.

**Fig. 3** Basophils regulate ILC2 responses independently of T cells. a–d, \textit{N. brasiliensis}-infected \textit{Rag2}−/− mice were treated with isotype control or the basophil-depleting antibody Ba103 and IL-5+ and IL-13+ ILC2s (a), and eosinophilia (b) was determined in the BAL and lungs (c,d) on day 7 postinfection. e, Mucus production in the lung was evaluated by Muc5ac expression. f, H&E staining of lung sections on day 7 post-\textit{N. brasiliensis} infection with individual images digitally tiled together to provide a larger overview. a–e, \(P\) values were determined by two-tailed Student’s t-tests. *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\). Representative of at least 3 separate experiments with at least \(n=2\) mice per group. d, Illustrated data were pooled from two separate experiments.
Fig. 4 | Basophils promote the expression of NMBR on ILC2s. **a**, RNA-seq analysis of sort-purified ILC2s from the lungs of control or basophil-depleted mice was performed on day 5 post-*N. brasiliensis*. The heatmap illustrates genes expressed differently at twofold or higher between control or basophil-depleted ILC2s. DAVID pathways analysis of the genes enriched in control ILC2s was performed. **b**, Genes enriched in control ILC2s that define the rhodopsin-like pathway. For the RT-qPCR studies, lung-resident ILC2s from control or basophil-depleted mice were sort-purified on day 7 post-*N. brasiliensis*. **c**, *Nmbr* and *Nmur1* expression levels were evaluated. **d**, Representative histograms of surface NMBR expression by ILC2s (**d**) or alveolar macrophages (**f**) from the lungs of control or basophil-depleted mice on day 7 post-*N. brasiliensis*. **e**, Geometric means of fluorescence intensity (gMFI) quantification of NMBR expression by ILC2s. **g**, *Nmb, Nmu* and *Mcpt8* expression in the lungs of control or basophil-depleted mice was determined on day 7 post-*N. brasiliensis* by real-time PCR. *P* values were determined by two-tailed Student’s t-tests. **P** < 0.01, ***P** < 0.001. **c–g**, Representative of at least 3 separate experiments with at least *n* = 3 mice per experimental group. **a,b**, Data generated from 3 individual samples of ILC2s sort-purified from *n* = 5 mice per experimental group.
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NMB is a potent inhibitor of type 2 cytokine responses. To investigate if NMB operates as a negative regulator of type 2 cytokine responses, wild-type (WT) mice were infected with *N. brasiliensis* and treated with rNMB. On day 7 postinfection, the parameters of type 2 cytokine-dependent inflammation were evaluated in both the BAL and lung. Strikingly, rNMB-treated WT mice exhibited reduced ILC2 responses and eosinophilia in both compartments (Fig. 5a–f). Further, rNMB-treated mice also showed increased disruption of the alveoli, significantly reduced mucus production and failed to clear worms as efficiently as control mice (Fig. 5g–j). Collectively, these data suggest that basophils regulate type 2 cytokine responses post-*N. brasiliensis* infection.

While NMB is reported to regulate cell growth, body temperature, blood pressure and glucose levels via its effects on non-hematopoietic cells, its ability to alter the activation state of immune cells is unknown. To address this, we first evaluated expression of NMBR by CD45* immune cells and found that NMBR is expressed by ILC2s, CD4+ T cells, alveolar macrophages, nonalveolar macrophages and eosinophils (Extended Data Fig. 8b–f). Collectively, these data suggest that NMB may influence *N. brasiliensis*-induced inflammation via its effects on several cells of hematopoietic origin. To further evaluate whether NMB-NMBR signaling is important in the hematopoietic compartment, we generated NMBR-floxed mice (Extended Data Fig. 9a) and crossed them with Vav-iCre mice to remove NMBR expression from CD45-expressing cells. As expected, NMBR was significantly reduced on CD45* cells, including ILC2s, but remained unchanged in the nonhematopoietic compartment (Fig. 6a–c). Similar to basophil depletion, NMBR by CD45* × Vav-iCre mice exhibited significantly increased expression of IL4, IL5 and IL13 in the lungs on day 7 post-*N. brasiliensis* infection (Extended Data Fig. 9b–d). Critically, NMBR-loxp/loxP × Vav-iCre mice also presented with significantly elevated cytokine responses by ILC2s and elevated eosinophilia in both the BAL (Fig. 6d–h) and lungs (Extended Data Fig. 9e–g) post-*N. brasiliensis*. Additionally, selectively targeting NMBR expression in the hematopoietic compartment also resulted in significantly elevated *Muc5ac* expression and increased cellular infiltrates in the lung (Fig. 6i,j). Collectively, these data suggest that NMB-NMBR signaling on immune cells is required to properly regulate *N. brasiliensis*-induced inflammation.

NMB is a potent inhibitor of innate immunity to *N. brasiliensis*. The data presented above suggest that basophils regulate NMB-NMBR signaling that is required to limit type 2 cytokine responses post-*N. brasiliensis*. Further, these studies suggest that NMB may act on cells in both the innate and adaptive immune compartments. *N. brasiliensis*-induced basophils enter the lungs between days 3 and 7 postinfection (Fig. 1d) when type 2 cytokine responses are reported to be primarily ILC2-driven. Therefore, we sought to determine if NMB can negatively regulate *N. brasiliensis*-induced inflammation independently of adaptive lymphocytes. To test this, *Rag2*−/− mice were infected with *N. brasiliensis* and treated with rNMB. Consistent with experiments in WT mice, *Rag2*−/− mice treated with rNMB exhibited significantly reduced ILC2 and eosinophil responses in both the BAL and lung (Fig. 7a–c and Extended Data Fig. 9a–c). Further, rNMB-treated *Rag2*−/− mice also exhibited significantly reduced mucus production and impaired worm expulsion, suggesting that rNMB treatment is sufficient to inhibit type 2 inflammation in the absence of adaptive lymphocytes (Fig. 7d,e). rNMB-treated *Rag2*−/− mice also presented with significantly elevated expression of *Il17* and increased neutrophilia suggesting that, in the absence of type 2 cytokine production, early *N. brasiliensis*-induced IL-17 responses remain elevated (Fig. 7f,g and Extended Data Fig. 9i). Finally, rNMB treatment resulted in severe lung pathology and significantly elevated numbers of red blood cells in the BAL, an indication of a reduced capacity to initiate type 2-dependent wound healing (Fig. 7h–j). Collectively, these data suggest that NMB operates as a potent negative regulator of innate type 2 responses.

NMB directly inhibits ILC2s. These studies demonstrate that NMBR is expressed by several innate cell populations known to promote anthelmintic immunity. Further, gain-of-function studies demonstrate that rNMB is sufficient to inhibit *N. brasiliensis*-induced ILC2 responses in vivo. However, whether NMB acts directly on ILC2s is unknown. To test this, we sort-purified lung ILC2s from *N. brasiliensis*-infected control and basophil-depleted mice and cultured them in vitro for 24 h. This allowed us to remove the cells from the inhibitory presence of the NMB ligand. In the absence of the NMB ligand, ILC2s from control and basophil-depleted mice produced similar amounts of IL-5 and IL-13 (Fig. 8a). However, the addition of rNMB significantly reduced production of IL-5 and IL-13 by ILC2s from control mice but had no impact on cytokine production by ILC2s obtained from basophil-depleted animals (Fig. 8a). Importantly, WT ILC2s treated with rNMB showed increased survival, suggesting that reduced cytokine levels were not a result of increased cell death (Fig. 8b). Similar results were also seen when WT ILC2s were sort-purified and stimulated with IL-33 and NMB simultaneously (Extended Data Fig. 10a–d). These data illustrate that NMB directly alters the activation state of ILC2s and suggest that basophils regulate this process.

These data suggest that basophils prime ILC2s for NMB-mediated inhibition via their ability to regulate NMBR expression. However, whether basophils regulate NMBR expression in a direct or indirect manner is unknown. To address this, we cocultured ILC2s either alone or in the presence of activated basophils and monitored surface expression of NMBR as well as cytokine secretion and cell proliferation. As reported previously, coculture of ILC2s with activated basophils resulted in elevated secretion of cytokines but failed to promote changes in ILC2 proliferation (Extended Data Fig. 10e–g). Critically, ILC2s cocultured with basophils also exhibited significantly increased surface expression of NMBR (Fig. 8c,d). Importantly, the ability of basophils to promote receptor expression was not a conserved feature of ILC2 activation since IL-33 treatment had no effect on NMBR levels (Fig. 8e). Basophils are known to produce several important effector molecules including cytokines and lipid mediators that are capable of influencing the activation state of distinct immune cells including ILC2s. Therefore, we also tested if the basophil-associated effector molecules IL-4 and prostaglandin E2 (PGE2) are sufficient to increase NMBR expression. While IL-4 had no effect, PGE2 stimulation resulted in significantly increased surface expression of NMBR by sort-purified ILC2s (Fig. 8e). Collectively, these data suggest that basophils might prime ILC2s for NMB-mediated inhibition, in part through their expression of PGE2.

PGE2 and NMB cooperate to inhibit ILC2 activation. While no changes in ILC2 viability were observed after NMB stimulation (Fig. 8b), it is possible that NMB inhibits ILC2s via its effect on cell proliferation and/or cytokine production. To evaluate whether rNMB affects proliferation, we stained sort-purified ILC2s with CellTrace Violet and monitored cell division by employing a culture system described previously. As expected, ILC2s showed strong proliferation in response to IL-23, but proliferation was significantly reduced in ILC2s treated with rNMB (Extended Data Fig. 10h). Collectively, these data suggest that basophils might prime ILC2s for NMB-mediated inhibition, in part through their expression of PGE2.
proliferative capacity with approximately 40% of the cells undergoing 2–3 divisions and 15% of cells undergoing 4–5 divisions over the 4-d culture period. As a positive control for inhibition, cultures were treated with PGE2 (ref. 41). Consistent with previous reports, PGE2 stimulation resulted in significantly decreased percentages of cells achieving between 2 and 5 divisions (Fig. 8f,g). Importantly, rNMB treatment alone showed no effect on the proliferation of ILC2s (Fig. 8f,g). Given the ability of PGE2 to restrict ILC2 proliferation and our data demonstrating that PGE2 appears to prime ILC2s for NMB-mediated inhibition (Fig. 8e), we sought to test if...
**Fig. 6 | NMBR expression is required to limit helminth-induced inflammation.** a–c, NMBR^loxP/loxP^ mice were generated and crossed with Vav-iCre mice to selectively ablate NMBR expression in hematopoietic cells. Surface expression of NMBR was evaluated in CD45^−^ cells (a), CD45^+^ cells (b) and ILC2s (c) of NMBR^loxP/loxP^ and NMBR^loxP/loxP^ × Vav-iCre^+^ mice by flow cytometry analysis 7 days post- *N. brasiliensis*. d–h, IL-5^+^ (d,f) and IL-13^+^ (e,g) ILC2s and eosinophils (h) were quantified in the BAL of NMBR^loxP/loxP^ × Vav-iCre^+^ mice 7 days post- *N. brasiliensis*. i,j, RT–qPCR analysis of Muc5ac expression (i) and lung pathology (H&E staining) (j) were determined 7 days post- *N. brasiliensis*. P values were determined by two-tailed Student’s t-tests. *P < 0.05, **P < 0.01, ***P < 0.001. a–j, Representative of at least 3 separate experiments with at least n = 5 mice per group.
the two signals cooperate to simultaneously restrict both ILC2 proliferation and cytokine production. Similar to our previous data, ILC2s cultured with PGE₂ showed significantly increased expression of NMBR in our proliferation assays (Fig. 8h). Further, when PGE₂ and NMB were added together significantly less IL-5 and IL-13 were detected in culture supernatants, suggesting that the two signals cooperate to inhibit ILC2s via combined effects on proliferation and cytokine production (Fig. 8i).
Fig. 8 | Basophils prime ILC2s for negative regulation by NMB. ILC2s were sort-purified from the lungs of control or basophil-depleted mice on day 7 post-*N. brasiliensis* and cultured (O/N) with IL-2 and IL-7 in the presence or absence of rNMB. a, b, IL-5 and IL-13 (a) levels in culture supernatants were determined by ELISA and cell viability (b) was evaluated by negative staining for 7-AAD and annexin V. c, Representative histograms of surface NMBR expression in sort-purified ILC2s cultured (O/N) with IL-2 and IL-7 alone or with activated basophils. d, gMFI quantification of NMBR expression by ILC2s. e, NMBR surface expression was evaluated in sort-purified ILC2s cultured (O/N) with IL-33, basophils, IL-4 and PGE2 in the presence of IL-2 and IL-7. f–i, Sort-purified ILC2s were cultured for 4 d with vehicle, rNMB, PGE2 or both in the presence of IL-2 and IL-7 and cell proliferation was monitored by CellTrace Violet dilution (f, g), surface NMBR expression (h), and cytokine levels (i) in the supernatant were monitored. j, Volcano plot of differentially expressed genes of ILC2s treated with vehicle or rNMB (O/N). k, Levels of cytokines in the supernatant of ILC2s cultured (O/N) with vehicle or in the presence of the P2rx7 inhibitor, brilliant blue G. *P < 0.05, **P < 0.01, ***P < 0.001. a, b, Representative of 3 separate experiments with at least *n* = 5 mice per group. c–i, Representative of 3 separate experiments with data generated from at least *n* = 5 individual samples of ILC2s sort-purified from *n* = 5 mice per experimental group.
While our data demonstrate that NMB-NMBR signaling inhibits ILC2 cytokine production, the molecular pathways that promote these changes remain to be defined. Therefore, we stimulated sort-purified ILC2s with either PBS or rNMB and performed RNA-seq analysis. After rNMB stimulation, ILC2s exhibited significantly downregulated expression of the purinergic receptor P2X 7 (P2rx7) gene, which was previously linked to ILC2 activation42 (Fig. 8j, Extended Data Fig. 10h,i and Supplementary Table 2). To evaluate if P2rx7 inhibition phenocopies the effects of NMB treatment, we treated sort-purified ILC2s with the P2rx7 inhibitor Brilliant Blue G42 and monitored IL-5 and IL-13 levels. Consistent with the effects of rNMB treatment, inhibition of P2rx7 resulted in significantly reduced levels of IL-5 and IL-13 (Fig. 8k). Collectively, these data suggest that NMB may restrict ILC2 cytokine production via its inhibition of P2rx7.

Discussion

The findings presented in this study identify a previously unappreciated aspect of cross talk between basophils and ILC2s. Moreover, these data suggest that communication between innate immune cells is required to promote neuroimmune interactions necessary to maintain tissue integrity. While it is well appreciated that robust type 2 cytokine production is vital to promote host-protective responses to helminths43, our understanding of how this inflammation is restricted to prevent persistent tissue remodeling is understudied. The data presented here identify a complex cellular and molecular network that is required to properly inhibit helminth-induced inflammation and identify a role for basophils in regulating these events.

While basophils have long been associated with type 2 cytokine-mediated inflammation, their diverse cellular functions have remained largely underappreciated. Although the dramatic basophilic that occurs after an N. brasilensis infection has been recognized for over 40 years44, the functions of N. brasilensis-elicited basophils are controversial. Although several studies have reported that basophils are not required to promote N. brasilensis expulsion25,26, our data indicate that basophils enter the lungs after the parasitic larvae exit the tissue, suggesting that basophils probably participate in promoting tissue homeostasis. Interestingly, recent studies have also determined that basophils infiltrate the lung during tissue development, where they serve important functions by programming alveolar macrophages15,16. Our data suggest that basophils might perform regenerative functions following an inflammatory response to promote tissue homeostasis. A similar role for eosinophils in the lung has recently been established47, suggesting that type 2-associated granulocytes may perform tissue-protective functions in some contexts.

Communication between several innate immune populations is required to initiate potent type 2 cytokine responses. For example, basophils have been reported to activate ILC2s via their secretion of IL-4 (refs. 12,14). In response to these signals, ILC2s secrete vast amounts of IL-5 and IL-13 that elicit several events, such as eosinophil recruitment and mucus production by goblet cells. Nonetheless, whether innate immune cells also communicate to efficiently limit type 2 inflammation and prevent chronic tissue remodeling is not well understood. The studies presented here highlight a previously unappreciated interaction between basophils and ILC2s that allows them to receive inhibitory signals induced by the neuropeptide NMB. It is possible that in the process of activating ILC2s via IL-4 secretion, basophils also prime ILC2s to receive the regulatory signals required to limit the duration of their activation. This concept may be similar to the effects observed during T cell activation, where T cell receptor stimulation results in the induction of the inhibitory molecules programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte protein 4 (CTLA-4) that become important negative regulators after the initial activation phase is completed46,47. Importantly, basophils, but not IL-4, were sufficient to induce NMBR on ILC2s, further supporting the notion that additional signaling pathways are initiated when basophils interact with ILC2s.

PGE2 is a well-defined lipid mediator known to be released by activated basophils48. Importantly, our data demonstrate that PGE2 is sufficient to induce the expression of NMBR on ILC2s, although to a lesser extent than basophils. Interestingly, PGE2, can inhibit ILC2 proliferation49 and our data show that NMB selectively targets ILC2 cytokine production. Therefore, our studies suggest a model where NMB and PGE2 might cooperate to suppress ILC2 activation via their combined effects of proliferation and cytokine production. A similar concept was proposed by Nagashima et al.27 where the neuropeptide CGRP shaped ILC2 functions by limiting NMU-induced proliferation and IL-13 secretion while supporting IL-5 production, suggesting that ILC2s may be regulated in specific manners that tailor to optimal outcomes. Although further studies are required to fully define the molecular actions of NMB, our data suggest that it might operate via its ability to regulate P2rx7 expression.

A growing body of evidence has demonstrated that highly coordinated interactions between the nervous and immune systems are required to initiate host-protective responses against helminths45. However, whether similar communication events exist to limit helminth-induced inflammation has yet to be fully defined. This is perhaps most evident in the context of ILC2 biology, where numerous mediators of activation, including NMU40,41, vasoactive intestinal peptide (VIP)42 and serotonin43, have been identified compared to a relatively limited number of inhibitors. Importantly, our studies identify an unappreciated regulatory pathway where NMB suppresses cytokine secretion by ILC2s, with basophils operating as the transition switch required for ILC2s to respond to NMB-mediated inhibition. Critically, our studies have also identified the expression of NMBR in several immune cells, including CD4+ T cells and alveolar macrophages, highlighting the need to further evaluate this critical pathway in other model systems. In summary, these data provide insight into the highly coordinated cellular and molecular events needed to allow for the necessary initiation, but carefully regulated persistence, of type 2 inflammation in the lung.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41590-020-0753-y.

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Articles

N. brasilensis infection and pharmacological treatments. Methods for the maintenance, recovery, infection and isolation of N. brasilensis larvae were performed as described previously55. Briefly, mice were infected intratracheally at days 3, 4, 5 and 6 after N. brasilensis (BD Biosciences). Purity for all cell populations was 98% or greater. Then, 15,000 Basophils were analyzed as CD45

R3+CD49b+CD200R1+FceRI+R. Eosinophils were analyzed as CD45+CD11b−Siglec-F+CD11c−. Neutrophils were evaluated as CD45+CD11b+Ly6G−. ILCs were identified as CD45+CD3+CD19+CD11b+CD11c−NK1.1−B220−CD3−TER-198+YTCR+FcεR+CD90+CD127−IL-5−IL-13−. Alveolar macrophages were analyzed as CD45+F4/80+CD64+Siglec-F+CD11c−. Type 1 pneumocytes were evaluated as CD45−TER-191+CD13+EpCAM+PD-PNP−. Type 2 pneumocytes were identified as CD45−TER-191+CD13+EpCAM+PD-PNP+. Samples were acquired on a BD Fortessa flow cytometer (BD Biosciences) and analyzed with FlowJo v10.5.0 (FlowJo, LLC). Cell sorting was performed using a FACSaria II.

ILC2 in vitro cultures. Lung cells were isolated from WT or basophil-depleted mice with infected with N. brasilensis 7 days previously. Then, ILC2 populations (CD3−, CD19− and CD11b−) were isolated from the presence of 10 ng ml−1 of mouse recombinant IL-2, IL-7 (cat. no. 402-ML; R&D Systems) and vehicle (PBS) or 1 μg ml−1 of NMBR-floxed mice were generated by Cyagen Biosciences. Briefly, the guide gene, the donor vector containing the loxp sites flanking excon 2 of the Nmbr gene and Cas9 messenger RNA were co-constructed into fertilized mouse eggs to generate targeted conditional knockout mice. F0 founder animals were identified by PCR followed by sequence analysis. Positive offspring were then bred to WT mice to test germline transmission and F1 animal generation. F1 mice were bred with WT mice to generate more positive 2F2 animals, which were identified by PCR. NMBR-floxed hemizygous mice were bred to generate homozygous offspring, which were then crossed with Vav-iCre hemizygous mice to target NMBR expression specifically on all hematopoietic cells. NMBR-floxed homozygous Vav-iCre negative littermates were used as control.

All mice were maintained in specific pathogen-free facilities at the Rutgers New Jersey Medical School. All protocols were approved by the Rutgers Institutional Animal Care and Use Committee, protocol no. 00968.

N. brasilensis infection and pharmacological treatments. Methods for the maintenance, recovery, infection and isolation of N. brasilensis larvae were performed as described previously55. Mice were infected with 500 N. brasilensis larvae via intranasal injection. For NMBR depletion, basophil- and basophil-depleted mice were treated with 0.375 μg of diphertheria toxin (catalog no. 150; LIST Biological Laboratories) intraperitoneally every other day; mice were killed 3–7 days post-N. brasilensis infection. Intestinal worm burdens were evaluated 7 days postinfection as described previously54. For NMBR treatment, mice were anesthetized with 5% isoflurane and then treated with 10 μg of NMB (cat. 87096-B2-2; SBM Pharmaceuticals) dissolved in 50 μl of PBS administered via intratracheal instillation. For antibody-mediated basophil depletion, RAG-2− mice were treated with 20 μg of anti-Flt3R1 antibody (clone MAR-1; Thermo Fisher Scientific) or anti-CD200R1 antibody (clone Ga103; BioLegend) three days previously. ILC2s and basophils were cocultured for 24 h in the presence of mouse recombinant IL-2, IL-7 and IL-3 (10 ng ml−1).

ILC2 proliferation was evaluated as described previously40. Briefly, 10,000 sort-purified, lung-resident ILC2s were labeled with CellTrace Violet (catalog no. C34571; Thermo Fisher Scientific) according to the manufacturer’s protocol and cultured with 5,000 irradiated (1,500 rad) OP9-DL1 cells (provided by D. Sant’Angelo), in the presence of mouse recombinant IL-2 and IL-7 (10 ng ml−1). For some conditions, NMB (1 μg ml−1), PGE2 (100 nm) or 10,000 sort-purified basophils were added. After 4 d of culture, CellTrace Violet dilution was determined by flow cytometry.

RNA isolation and quantitative PCR with reverse transcription (RT–qPCR) analysis. RNA from sections of lung tissue was isolated by homogenization in TRizol (Invitrogen) followed by phenol/chloroform extraction and isopropanol precipitation. Complementary DNA was generated per standard protocol with TRIzol (Invitrogen) followed by phenol/chloroform extraction and isopropanol precipitation. Complementary DNA was generated per standard protocol with TRIzol (Invitrogen) followed by phenol/chloroform extraction and isopropanol precipitation. Complementary DNA was generated per standard protocol with TRIzol (Invitrogen) followed by phenol/chloroform extraction and isopropanol precipitation. Complementary DNA was generated per standard protocol with TRIzol (Invitrogen) followed by phenol/chloroform extraction and isopropanol precipitation.

Lung-resident ILC2s from control and basophil-depleted mice were sorted on a BD FACSaria II (BD Biosciences) and sequenced using an Illumina NextSeq 500 platform with 150-bp read length. RNA-seq libraries were prepared using the nf-core RNA-Seq pipeline, with aligned reads analyzed in the nf-core RNA-seq guidelines v1.4.2 (ref. 22). The output reads were aligned to the GRCm38 genome using STAR v2.1.0d, followed by gene count generation using featureCounts v1.6.4 and StringTie v2.0 (ref. 24). Read counts were normalized and compared for differential gene expression using bioconductor-DESeq2 v1.22.1 with significance at a false discovery rate-adjusted P < 0.05 (ref. 61). For the analysis of functional pathways, the Database for Annotation, Visualization, and Integrated Discovery (DAVID) platform25 was used. For RNA-seq, 10,000 sort-purified ILC2s were processed using the 10X Genomics Chromium Controller. Cell suspensions were loaded onto the Chromium Single Cell A Chip for cell lysis and barcoding. RNA from individual
cells was reverse-transcribed and sequencing libraries were prepared using the Chromium Single Cell 3’ Library Kit v2 according to the manufacturer’s protocol. Samples were sequenced using an Illumina NextSeq 550 with standard 10X Genomics configuration (2698bp). After sequencing, raw BCL files were processed using the Cell Ranger mk fasta v3.1.0 command for sample demultiplexing and conversion to FASTQ files, followed by Cell Ranger count for cell barcode and unique molecular identifier deconvolution and mapping to the respective reference genome. Processed digital gene expression matrices were imported into RStudio v1.2.5019 for analysis using the Seurat package v3.1.4. Samples were aligned along common sources of variation and compared using canonical correlation analysis to identify unique clusters of cells within the samples. Marker genes for each sample and cluster were identified and used to generate downstream plots within the Seurat package. All packages are maintained to be best in class and are regularly updated to their most recent releases.

Pulse oximetry. Oxygen saturation was evaluated with the MouseOx Plus (Starr Life Sciences Corp) according to the manufacturer’s instructions. Briefly, the hair around the thigh was removed 1 day before N. brasiliensis infection. Then, mice were anesthetized with 5% isoflurane and oxygen saturation was monitored using the thigh sensor for an interval of approximately 5 min. This time point was used to collect representative and error-free data.

Histology. Lung sections were fixed in 10% formalin buffered for at least 3 days, followed by processing in a Leica ASP900 tissue processor (Leica Biosystems) according to the manufacturer’s instructions. Tissue blocks were paraffin-embedded using a Leica EG1150H modular tissue embedding system and sliced into 5-μm sections using a Leica RM2235 rotary microtome (Leica Biosystems). Tissue sections were then deparaffinized, hydrated and stained with hematoxylin and eosin (H&E) or periodic acid–Schiff (PAS). Images were acquired using a Keyence BZ-X710 All-in-One Fluorescence Microscope and analyzed using the Keyence BZ-X Viewer v01.03 software.

Statistics. Results are shown as the mean±s.e.m. P < 0.05 was considered as significantly different. Statistical analysis was performed using Student’s t-tests in Prism 8 (GraphPad Software).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Bulk RNA-seq and single-cell RNA-seq data are deposited in the Gene Expression Omnibus under accession code GSE150793. Source data are provided with this paper.

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Author contributions

J.M.I.R., J.J.P., N.V.P., C.M.H., C.B.S., A.D.L. and M.C.S. designed and performed the research. A.M.B. contributed to experimental design and data analysis, conceptualization and manuscript editing. J.M.I.R. and M.C.S. analyzed the experimental data and wrote the paper.

Competing interests

Mark C. Siracusa is the founder and president of Nemagen Discoveries.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41590-020-0753-y.
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Peer review information Jamie D. K. Wilson was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.
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Extended Data Fig. 1 | Basophils limit helminth-induced pulmonary inflammation. **a**, Supernatant levels of IL-4, IL-5 and IL-13 from re-stimulated mesenteric lymph nodes (mLN) isolated from control or basophil-depleted mice. Mucus production was evaluated in control and basophil-depleted mice on day 7 post-Nb infection by **b**, periodic acid shiff (PAS) staining and **c**, Muc5ac expression in the lungs by real-time PCR. **d**, Lung pathology was evaluated by H&E-stained sections with individual images digitally tiled together to provide a larger overview. *P* values were determined by two-tailed Student's t-tests. **a-d**, Representative of at least 3 separate experiments with at least 5 mice per group. *P < 0.05, **P < 0.01, ***P < 0.001. **b**, Illustrate data pooled from 2 separate experiments.
**Extended Data Fig. 2 | Basophil depletion results in elevated ILC2 responses.**

- **a**, Lung neutrophils and (b), eosinophils were quantified by flow cytometry on day 7 post-Nb infection in control or baso-dep mice.
- **c**, Representative flow cytometric gating strategy to evaluate neutrophils and eosinophils.
- **d**, ILC2s in the lung were quantified on day 7 post-Nb infection in control or baso-dep mice. Intracellular cytokine staining for (e, f), IL-5 and IL-13 was performed on lineage negative, CD90+ CD127+ ILC2s in lung on day 7 post-Nb infection and cytokine positive cells were quantified. **g**, Representative flow cytometric gating strategy to evaluate ILC2 populations. *P* values were determined by two-tailed Student's t-tests. *P* < 0.05, **P** < 0.01, ***P** < 0.001.

- **a-g**, Representative of at least 3 separate experiments with at least 5 mice per group.
**Extended Data Fig. 3** | Constitutive ablation of basophils is associated with increased ILC2 activation. a, b, IL-5+ and IL-13+ ILC2s, as well as c, eosinophils in the BAL and d-f, lungs were quantified in control and Mcpt8Cre-4get mice that constitutively lack basophils, 7 days post-Nb infection. P values were determined by two-tailed Student’s t-tests. *P < 0.05, **P < 0.01, ***P < 0.001. a-f, Representative of at least 3 separate experiments with at least 5 mice per group.
Extended Data Fig. 4 | Basophils are sufficient to limit helminth-induced ILC2 responses. a, ILC2 numbers, (b), ILC2 production of IL-5, and (c), IL-13, as well as (d) eosinophil numbers were quantified in the lung on day 7 post-Nb infection in control mice, baso-dep mice, or baso-dep mice that received adoptive transfers of basophils. e, H&E staining of lung sections on day 7 post-Nb infection with individual images digitally tiled together to provide a larger overview. Mucus production in the lung was evaluated by (f), PAS staining of lung sections and (g), Muc5ac expression. P values were determined by two-tailed Student’s t-tests. *P < 0.05, **P < 0.01, ***P < 0.001. a-g, Representative of at least 3 separate experiments with at least 4 mice per group.
Extended Data Fig. 5 | Basophils regulate ILC2s independently of adaptive lymphocytes. Nb-infected Rag2−/− mice were treated with isotype control or the basophil-depleting antibody MAR-1 and (a), ILC2 responses and (b), eosinophilia were determined in the BAL and (c,d), lung on day 7 post-infection. (e), H&E staining of lung sections on day 7 post-Nb infection with individual images digitally tiled together to provide a larger overview. (f), Mucus production in the lung was evaluated by Muc5ac expression. P values were determined by two-tailed Student’s t-tests. *P < 0.05, **P < 0.01, ***P < 0.001. a-f, Representative of at least 3 separate experiments with at least 2 mice per naive groups and at least 4 mice per infected groups.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Elevated ILC2 responses are not associated with increased cytokine alarmin expression. a-c, Expression of cytokine alarmins in the lungs of control and baso-dep mice was determined on day 7 post-Nb infection by real-time PCR. d, e, Numbers of IL-33-GFP+ type 1 and type 2 pneumocytes were evaluated in IL-33-GFP-reporter mice infected with Nb and treated with the basophil-depleting antibody MAR-1. Expression of (f), Il10 and (g), Areg in the lungs of control and baso-dep mice was determined on day 7 post-Nb infection by real-time PCR. Splenic basophils were sort-purified and cultured (O/N) with IL-3 and anti-IgE antibody and supernatant levels of (h), IL-6, (i), amphiregulin (Areg), and (j), IL-10 were evaluated by ELISA. P values were determined by two-tailed Student’s t-tests. *P < 0.05, **P < 0.01, ***P < 0.001. a-g, Representative of at least 2 separate experiments with at least 2 mice per naive groups and at least 5 mice per infected groups. h-j, Representative of at least 3 separate experiments with at least 5 individual samples of sort-purified basophils from 5 mice per experimental group.
Extended Data Fig. 7 | Single cell RNAseq analysis of lung-resident ILC2s. a, Uniform Manifold Approximation and Projection (UMAP) plot illustrating defined clusters of cells generated by single cell RNA-sequencing of lung-resident live ILC2 populations (CD45+Lin-CD90+CD127+) sort-purified from control (and basophil-depleted (baso-dep) mice 5 days post-Nb infection. b, Top 10 marker genes expressed by each cluster of ILC2s. c, Single-cell expression of Il5, Il13, Areg, Arg1, Il1rl1, and Il17rb in ILC cell clusters as defined in A. Horizontal bars represent mean normalized expression. P values were determined by Wilcoxon signed rank sum test. *P < 0.05, **P < 0.01, ***P < 0.001.
Extended Data Fig. 8 | Analysis of NMBR expression in the hematopoietic compartment. **a**, Heat map illustrating representative genes of interest expressed in control or baso-dep ILC2s. Surface NMBR expression by **b**, CD4+ T cells, **c**, alveolar macrophages, **d**, non-alveolar macrophages, **e**, neutrophils, and **f**, eosinophils was determined in lung suspensions of naïve and mice infected with Nb 7 days prior. P values were determined by two-tailed Student’s t-tests. *P < 0.05, **P < 0.01, ***P < 0.001. (b-f), Representative of at least 3 separate experiments with at least 2 mice per naïve groups and at least 4 mice per infected groups.
Extended Data Fig. 9 | NMB-NMBR signaling suppresses helminth-induced ILC2 responses. a, Schematic illustrating targeting strategy and placement of loxP cassettes upstream and downstream of exon 2 of the Nmbr gene. b–d, Type 2 cytokine expression in the lungs of NMBRfl/fl controls and NMBRfl/fl x Vav-iCre+ mice was determined on day 7 post-Nb infection by real-time PCR. e, IL-5+ and (f), IL-13+ ILC2s, as well as (g), eosinophils were quantified in the lungs of NMBRfl/fl x Vav-iCre+ mice 7 days post-Nb. Nb-infected Rag2−/− mice were treated with PBS or rNMB (i.t.) and (h, i), the percentage of IL-5+ and IL-13+ ILC2s were determined in the BAL and (j), the total number of IL-5+ and IL-13+ ILC2s were determined in the lung on day 7 post-infection. k, eosinophils and (l), neutrophils were determined in the lungs of Rag2−/− mice treated with PBS or NMB on day 7-post infection. P values were determined by two-tailed Student’s t-tests. *P < 0.05, **P < 0.01, ***P < 0.001. b–l, Representative of 3 separate experiments with at least 3 mice per naive groups and at least 5 mice per infected groups.
Extended Data Fig. 10 | See next page for caption.
**Extended Data Fig. 10 | Basophils are required for NMBR-mediated inhibition of ILC2s.** Sort-purified ILC2s were cultured (O/N) with vehicle or rNMB in the presence of IL-2 and IL-7 or IL-2, IL-7, and IL-33. a, b, The percentage of IL-5+ and IL-13+ ILC2s were quantified by intracellular staining. c, d, IL-5 and IL-13 levels in the supernatant were quantified by ELISA. Sort-purified ILC2s were cultured (O/N) alone or with activated basophils. e, Cytokine levels in the supernatant were monitored by ELISA and (f, g), cell proliferation was evaluated by CTV dilution 4 days post-culture. h, Heat map illustrating genes differentially expressed at 2.0-fold or higher between control or NMB-treated ILC2s. i, Heat map illustrating genes not differentially expressed in control or NMB-treated ILC2s. P values were determined by two-tailed Student’s t-tests. *P < 0.05, **P < 0.01, ***P < 0.001. a-g, Representative of at least 3 separate experiments with at least 5 individual samples of sort-purified ILC2s in each experimental group.
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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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  - *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
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  - *Give P values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | No open-source or custom code was used to collect data for this paper. |
|-----------------|---------------------------------------------------------------------|
| Data analysis   | Bulk RNA-sequencing analysis was performed in accordance with the NF-Core RNA-sequencing guidelines (version 1.4.2). The output reads were aligned to the GRCh38 genome using STAR, followed by gene count generation using featureCounts and StringTie. Read counts were normalized and compared for differential gene expression using DESeq2 with significance at False Discovery Rate (FDR) adjusted p-value < 0.05. For analysis of functional pathways, the Database for Annotation, Visualization, and Integrated Discovery (DAVID) platform was used. For single cell RNA-sequencing, after sequencing, raw bc files were processed using the cellranger multi-processor command for sample demultiplexing and conversion to .fastq files, followed by cellranger count for cell barcode and UMI deconvolution as well as mapping to the respective reference genome. Processed digital gene expression matrices were imported into R studio for analysis using the Seurat package. Samples were aligned along common sources of variation and compared using canonical correlation analysis to identify unique clusters of cells within the samples. Marker genes for each sample and cluster were identified and used for generation of downstream plots within the Seurat package. All packages are maintained to be best in class and are regularly updated to their most recent release. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data

Bulk RNAseq and single cell RNAseq data is deposited in the GEO accession code GSE150793. Source data for all figures is provided with the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/mr-reporting-summary-list.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample sizes were determined according to previously published studies.

Data exclusions
No data were excluded from the present study.

Replication
Experiments in this study were repeated at least three independent times.

Randomization
Samples and organisms used in the present study were assigned to their respective groups randomly before the experiments were performed.

Blinding
All samples are coded during analysis and decoded during final data analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | Antibodies            |
| ☒   | Eukaryotic cell lines |
| ☐   | Palaeontology and archaeology |
| ☐   | Animals and other organisms |
| ☒   | Human research participants |
| ☒   | Clinical data |
| ☒   | Dual use research of concern |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | ChiP-seq |
| ☒   | Flow cytometry |
| ☒   | MRI-based neuroimaging |

Antibodies

Cells were stained with monoclonal anti-mouse fluorescently conjugated antibodies: B220 (RA3-6B2), CD3 (145-2C11), CD4 (GK1.5), CD5 (53-73), CD19 (1D3), NK1.1 (PK136), CD11b (M1/70), CD11c (N418), IgE (23G3), FceRI (MAR-1), CD31 (MEC 13.3), CD49b (DXS), CD45 (30-F11), CD64 (X54-5/7.1), CD90 (5E10), CD127 (A7R34), CD200R1 (OX110), CD200R3 (Ba13), EpCAM (G8.8), F4/80 (BM8), V6TCR (eBioGL3), Siglec-F (E50-2440), Ly6G (1A8), Ly6C (AL-21), IL-5 (TRFK5), IL-13 (eBio13A), PDPN (8.1.1), Ter-119 (TER-119) from eBioscience or BD Biosciences. For intracellular staining, cells were incubated for 5 hours at 37°C with Leukocyte Activation Cocktail, with BD GolgiPlugTM (BD Biosciences) following manufacturer’s instructions. For NMBr staining rabbit anti-mouse NMBr antibody (NLS825) was used from Novus Biologicals at 10μg/ml.

Validation

Antibodies were used according to vendor instructions based on their provided methods of validation.
Animals and other organisms

Policy information about studies involving animals, ARRIVE guidelines recommended for reporting animal research

Laboratory animals
- 8-10 week old C57Bl/6j wild type (WT) (stock #003664), Mcp8tm1(cre)l ksy (stock #017578), ROSA26ΔdT (stock #007900), Rag2−/−/− mice (stock #008449), Il-33If/i-eGFP mice (stock #030619), and Vav-iCre mice (stock #008610) were purchased from The Jackson Laboratory. Mcp8Cre4get mice were kindly provided by Dr. David Voehringer. Lineage-specific depletion of basophils was obtained by crossing Mcp8tm1(cre)l ksy mice with ROSA26ΔdT mice as previously described.
- NMBR-floxed mice were generated by Cyagen Biosciences Inc. Briefly, the gRNA to mouse Nmbr gene, the donor vector containing loxP sites flanking exon 2 of the Nmbr gene, and Cas9 mRNA were co-injected into fertilized mouse eggs to generate targeted conditional knockout mice. F0 founder animals were identified by PCR followed by sequence analysis. Positive offspring were then bred to wild type mice to test germine transmission and F1 animal generation. F1 mice were bred with wild type mice to generate more positive F2 animals, which were identified by PCR. NMBR floxed hemizygous mice were bred to generate homozygous offspring, which were then crossed with Vav-iCre hemizygous mice to target NMBR expression specifically on all hematopoietic cells.
- NMBR floxed hemizygous Vav-iCre negative littermates were used as control.
- All mice were maintained in specific pathogen-free facilities at the Rutgers New Jersey Medical School.

Wild animals
- n/a

Field-collected samples
- n/a

Ethics oversight
- All protocols were approved by the Rutgers Institutional Animal Care and Use Committee (IACUC). Protocol number 00968.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots
- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
- For BAL collection after necropsy, 3-5mL of PBS was injected and aspirated from the trachea of each mouse, and the volume of BAL obtained was recorded. Following BAL collection, lungs were collected at necropsy and single-cell suspensions for flow cytometric analysis were prepared as previously described. Briefly, pulmonary tissue was minced and incubated in HBSS containing 2.5% of FBS, collagenase D (2 mg/mL, Roche) and DNase I (80 U/mL, Roche) for 30 min at 37°C. Cell suspensions were then filtered through a 100μM filter and analyzed by flow cytometry.

Instrument
- Flow cytometry was performed using a BD Fortessa flow cytometer (BD Biosciences). Cell sorting was performed using a FACSAnia (BD Biosciences).

Software
- Flow cytometry data were analyzed using Flowjo software [v10.0.5, Tree Star].

Cell population abundance
- Purity for all cell populations were determined to be 98% or greater.

Gating strategy
- Basophils were analyzed as CD45+CD3−CD19−NK1.1−Ly6G−Siglec-F−CD200R3+CD49b+CD200R1+Fcer1+. Eosinophils were analyzed as CD45+CD11b+Siglec-F+CD11c+. Neutrophils were evaluated as CD45+CD11b+Ly6G+. ILC2s were identified as CD45+CD19−CD11b−CD11c−NK1.1−B220−CD5−Ter-119−pDCR1−CD90−CD127−IL-5+IL-13+. Alveolar macrophages were analyzed as CD45+F4/80+CD64+Siglec-F+CD11c+. Non-alveolar macrophages were analyzed as CD45+F4/80+CD64+Siglec-F−CD11c+. CD4 T cells were defined as CD45+CD3+CD90+CD4+. Type 1 pneumocytes were evaluated as CD45−Ter-119−CD31−EpCAM+PDNP+. Type 2 pneumocytes were identified as CD45−Ter-119−CD31−EpCAM+PDNP−.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.