

Basophil responses in susceptible AKR mice upon infection with the intestinal helminth parasite *Trichuris muris*

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Abstract

Intestinal helminth infection promotes a Type 2 inflammatory response in resistant C57BL/6 mice that is essential for worm clearance. The study of inbred mouse strains has revealed factors that are critical for parasite resistance and delineated the role of Type 1 versus Type 2 immune responses in worm clearance. In C57BL/6 mice, basophils are key innate immune cells that promote Type 2 inflammation and are programmed via the Notch signaling pathway during infection with the helminth *Trichuris muris*. However, how the host genetic background influences basophil responses and basophil expression of Notch receptors remains unclear. Here we use genetically susceptible inbred AKR/J mice that have a Type 1-skewed immune response during *T. muris* infection to investigate basophil responses in a susceptible host. Basophil population expansion occurred in AKR/J mice even in the absence of fulminant Type 2 inflammation during *T. muris* infection. However, basophils in AKR/J mice did not robustly upregulate expression of the Notch2 receptor in response to infection as in C57BL/6 mice. Blockade of the Type 1 cytokine IFN- γ in infected AKR/J mice was not sufficient to elicit infection-induced basophil expression of the Notch2 receptor. These data suggest that the host genetic background, outside of the Type 1 skew, is important in regulating basophil responses during *T. muris* infection in susceptible AKR/J mice.

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Abstract

Intestinal helminth infection promotes a Type 2 inflammatory response in resistant C57BL/6 mice that is essential for worm clearance. The study of inbred mouse strains has revealed factors that are critical for parasite resistance and delineated the role of Type 1 versus Type 2 immune responses in worm clearance. In C57BL/6 mice, basophils are key innate immune cells that promote Type 2 inflammation and are programmed

via the Notch signaling pathway during infection with the helminth *Trichuris muris*. However, how the host genetic background influences basophil responses and basophil expression of Notch receptors remains unclear. Here we use genetically susceptible inbred AKR/J mice that have a Type 1-skewed immune response during *T. muris* infection to investigate basophil responses in a susceptible host. Basophil population expansion occurred in AKR/J mice even in the absence of fulminant Type 2 inflammation during *T. muris* infection. However, basophils in AKR/J mice did not robustly upregulate expression of the Notch2 receptor in response to infection as in C57BL/6 mice. Blockade of the Type 1 cytokine IFN- γ in infected AKR/J mice was not sufficient to elicit infection-induced basophil expression of the Notch2 receptor. These data suggest that the host genetic background, outside of the Type 1 skew, is important in regulating basophil responses during *T. muris* infection in susceptible AKR/J mice.

Keywords: Basophils, *Trichuris muris*, Type 2 inflammation, Notch signaling, AKR-inbred strain

Introduction

Helminth infection elicits a Type 2 inflammatory response that is required for worm expulsion and repair of worm-induced tissue damage. The Type 2 immune response involves the activation of innate immune cells including basophils, eosinophils, mast cells, and group 2 innate lymphoid cells (ILC2s), the polarization of CD4⁺ T helper type 2 (Th2) cells that produce interleukin (IL)-4, IL-5, IL-9, and IL-13, and extensive tissue remodeling (1-8).

For decades, immunologists have successfully leveraged genetic differences in various inbred mouse strains to better understand the factors that regulate Type 2 inflammation (2, 9-13). The response to the murine model for whipworm infection, *Trichuris muris* (9, 13, 14) is dependent upon the genetic background in inbred mouse strains. C57BL/6 and Balb/C mice mount a robust Type 2 response and can expel parasites after infection with a high dose of eggs, while other strains such as AKR mice do not mount a fulminant Type 2 response, cannot clear worms, and harbor a chronic infection (9, 11, 13-17). These strain-dependent differences in outcome have revealed numerous cellular players and pathways that are required for a robust Type 2 immune response and resistance to *T. muris* infection observed in C57BL/6 but not AKR mice (9, 11, 13-17). Thus, genetically inbred mice offer an avenue to assess how particular cell types are activated in helminth infection and how these cells might contribute to resistance.

Our group and others have recently shown that basophils are important in mounting an effective Type 2 inflammatory response that expels *T. muris* in C57BL/6 mice (7, 18, 19). Basophils are rare innate granulocytes that comprise <1% of immune cells (20-22). In C57BL/6 mice, basophils are critical players in Type 2 inflammation, supporting worm clearance and the response to allergens in the context of allergic disease. Upon activation, basophils release inflammatory mediators, including histamine, proteoglycans, lipid mediators, proteases, growth factors, chemokines, and cytokines that promote Type 2 immunity. Basophils are activated by IgE crosslinking of the high affinity IgE receptor (Fc ϵ 1 α), stimulation with cytokines like thymic stromal lymphopoietin (TSLP), IL-33, and IL-3, and signaling through the Notch pathway (20-29), a receptor-ligand-based cell-cell communication pathway that can broadly regulate gene expression in a variety of contexts and cell types (30). Our previous data showed that basophils upregulated Notch2 during infection and that basophil effector gene expression and tissue positioning and fulminant Type 2 responses, but not basophil population expansion, were dependent on basophil-intrinsic Notch (7). These data reveal that Notch signaling is an important factor in basophil effector function during helminth infection in C57BL/6 mice. However, how Notch responsiveness in basophils is regulated *in vivo*, and how basophil responses differ in various genetically inbred mouse strains is unclear.

Here, we use AKR/J mice that are Th1-skewed and susceptible to *T. muris* (9-13, 15) to assess how basophil responses and Notch expression by basophils are regulated in a genetically susceptible inbred mouse strain. Upon *T. muris* infection in AKR/J mice, there was an infection-induced basophil population expansion in the cecum and spleen, similar to that observed in resistant C57BL/6 mice. Expression of genes associated with basophil as well as mast cell activation and serum IgE levels were also increased in AKR mice, despite a Type 1-skewed inflammatory response that was associated with persistent infection. However, basophils in AKR/J

mice did not upregulate surface expression of the Notch2 receptor after infection, which we previously have shown occurs in C57BL/6 mice (7). Neutralization of the Type 1 cytokine IFN- γ in infected AKR/J mice (31) did not result in Notch2 receptor upregulation on basophils at day 14 post-infection (p.i.), suggesting that the Type 1 skewed response in AKR/J mice is not a primary factor that controls Notch2 expression in AKR/J basophils. Together, these data reveal that basophilia is a hallmark feature of *T. muris* infection even in genetically susceptible AKR/J mice and that differences in genetic background, not IFN- γ levels, may contribute to basophil regulation of Notch receptor expression in AKR/J mice.

Materials and Methods

Experimental Mice and *in vivo* Treatments

Female AKR/J and C57BL/6 mice at 8-10 weeks old were purchased from The Jackson Laboratory. The animals were housed in specific pathogen free conditions at the Cornell University College of Veterinary Medicine Baker Institute for Animal Health or the University of Washington South Lake Union 3.1 animal facility. Animals were handled according to protocols approved by the Cornell University and University of Washington Institutional Animal Care and Use Committees. For IFN- γ neutralization experiments, AKR/J mice were treated with 1 mg of anti-IFN- γ (XMG1.2) or rat IgG1 (Isotype control) at Day -1, +3, +7, and +11. Day 0 served as the day of infection with 200 *T. muris* eggs.

Trichuris muris Infection and Tissue Preparation

In-house generated *T. muris* eggs were used for infection (13, 31). Experimental mice were infected with 200 *T. muris* eggs by oral gavage and euthanized at days 7, 14, 19, and 35 p.i. for analysis. At day 14, 19 and 35 p.i., the ceca from infected animals were collected to assess worm burden microscopically. Blood was collected for serum analysis. RNA tissue from the proximal colon was snap-frozen to analyze gene expression. Spleen, mesenteric lymph node (MLN) and cecum were collected to assess basophil infiltration using flow cytometry. Spleens and MLNs were digested with 1 U/mL Liberase TL (Roche) and 20 μ g/mL DNase (Sigma-Aldrich) for 15 min at 37°C. Single-cell suspensions were prepared by disrupting the tissue through a 70- μ m strainer. Spleens were lysed for red blood cells with ammonium-chloride-potassium lysis buffer (Lonza). For analysis of cecum basophil/immune cell populations, ceca were collected directly into ice-cold PBS, cut open longitudinally, cleaned of fecal contents, and washed twice in ice-cold HBSS (Thermo Fisher Scientific) with 10% FBS (Denville Scientific). Tissues were chopped roughly into 1-cm pieces in HBSS 2% FBS, stored on ice, and then shaken by hand vigorously for 10 s before being strained and incubated in HBSS supplemented with 2 mM EDTA (Thermo Fisher Scientific) for 15 min at 37°C in a shaking incubator. Samples were shaken by hand again, strained, and incubated for 25 min in fresh HBSS/EDTA at 37°C in a shaking incubator. After further shaking, samples were incubated in 1 U/mL Liberase TL and 20 μ g/mL DNase for 35 min at 37°C in a shaking incubator with further vigorous manual shaking every 7 min. Samples were washed through 40- μ m strainers to collect single-cell suspensions.

Flow Cytometry

Single cell suspensions were incubated with Aqua Live/Dead Fixable Dye (Life Technologies, Grand Island, NY) an fluorochrome-conjugated monoclonal antibodies (mAbs) against mouse CD3 (17A2), CD64 (X54-5/7.1), CD5 (53-7.3), CD11c (N418), CD19 (eBio1D3), NK1.1 (PK136), TCR $\gamma\delta$ (eBioGL3), CD45 (30-F11), IL-33R/ST2 (RMST2-2), CD49b (DX5), Fc ϵ RI α (MAR-1), IgE (R35-72), and Notch2 (16F11) from Thermo Fisher Scientific, BD Biosciences, or BioLegend. Staining was performed in the presence of 10% rat serum (Jackson ImmunoResearch) and 1 μ g/mL FcR block (anti-CD16/32, 2.4G2; Bio X Cell). Stained cells were acquired on an LSRII (BD biosciences) and analyzed using FlowJo software version 10.8.

ELISA and Real-Time PCR

Collected blood samples were allowed to clot and centrifuged at 10,000 rpm for 10 min at 4°C to separate out serum. Standard sandwich ELISA was performed to assess IgE levels (BD biosciences). For real-time PCR, RNA was isolated from snap-frozen proximal colon lysates using TRIzol extraction according to the manufacturers' instruction. Real-time PCR was performed on cDNA generated by using a Superscript II reverse

transcriptase kit (Life Technologies) with SYBR Green master mix (Applied Biosystems) and commercially available primer sets (QIAGEN QuantiTect). Samples were run on the ViiA7 real time PCR systems (Applied Biosystems -Life Technologies).

Statistics

Statistical outliers were identified in normal Gaussian data sets using the extreme studentized deviate (ESD) method, and outliers were uniformly omitted prior to analysis. Statistical analysis was carried out using JMP software (SAS), analyzed using linear mixed effects models with a fixed effect of experimental group and a random effect of experiment day. Model assumptions of normality and homogeneous variance were assessed by a visual analysis of the raw data and the model residuals. Right-skewed data were log or square root transformed. Experimental group was considered statistically significant if the fixed effect F test p -value was less than or equal to 0.05. Post-hoc pairwise comparisons between experimental groups were made using Tukey's HSD multiple-comparison test. Graphs of results were shown as mean $\pm$ SEM of untransformed data using Prism version 9 (GraphPad).

Results

Susceptible AKR/J mice demonstrate a Type 1 immune skew during *T. muris* infection.

To prepare to assess basophil responses in a susceptible host, we first validated that AKR/J mice are susceptible to *T. muris* and mount a Type 1-skewed cytokine response to infection in our hands. AKR/J mice were infected with 200 *T. muris* eggs orally. Worm burdens in the ceca and cytokine gene expression in the proximal colon were analyzed at day (d) 0 (naïve), 7, 14, 19 and 35 p.i. (Fig. 1A). These time points were selected based on the *T. muris* life cycle and the kinetics of the immune response that expels parasites in resistant mice (13). Consistent with previous studies (9-13, 15), AKR/J mice did not expel worms efficiently; the average worm burden decreased by d35 p.i., but many AKR/J mice retained worms out to this timepoint (Fig. 1B). Likewise, consistent with prior reports showing a Type 1-skewed cytokine response in *T. muris*-infected AKR/J mice (9-13, 15), infection-induced expression of the Th1-associated cytokine *Ifng* in the colon was observed at all time points p.i. and was ~70-fold higher in *T. muris*-infected AKR/J mice at d19 p.i. than in naïve mice (Fig. 1C). We did also observe a significant increase in *Il4* levels in the colon of mice at d19 and d35 p.i. compared to naïve mice (Fig. 1D), but no significant infection-induced increase in *Il13* expression (Fig. 1E). These data validate that, in our hands, AKR/J mice cannot uniformly expel *T. muris* by d19 p.i. as C57BL/6 mice can (9-13, 15), associated with a skew towards Type 1 inflammation.

We next assessed various parameters of the host immune response associated with granulocyte, specifically basophil, responses. We observed a robust increase in colonic expression of *Fcer1a*, which encodes for the high-affinity IgE receptor that is expressed on basophils and mast cells (20-23, 29), in the colon of mice at d19 and d35 p.i. with *T. muris* compared to naïve mice (Fig. 1F). We also observed increased serum IgE upon infection, though this increase was not statistically significant (Fig. S1A). As *Fcer1a* expression and IgE levels are not indicative of basophil-specific activation, we further quantified the expression of *Mcpt8*, a basophil-specific protease (27, 32, 33). *Mcpt8* levels in the colon were significantly increased in infected mice at d14, d19, and d35 p.i. compared to levels in naïve mice (Fig. 1G). Together, these data suggest that basophils and potentially also mast cells that express *Fcer1a* for IgE binding are either activated or increased in number in susceptible AKR/J mice during *T. muris* infection, despite the presence of a Type 1-skewed inflammatory environment.

The basophil population expands in susceptible AKR/J mice upon *T. muris* infection.

To confirm whether basophil population expansion was driving the increase in basophil-associated gene expression in the intestine during *T. muris* infection in AKR/J mice, we next quantified the accumulation of basophils (gated as CD45⁺Lineage⁻ IgE⁺FcεR1α⁺ CD49b⁺cKit⁻) in the cecum, spleen, and draining mesenteric lymph nodes (MLNs) by flow cytometry after *T. muris* infection. In the cecum, we observed a significant infection-induced increase in basophil frequency as a proportion of Lineage-negative cells and total CD45⁺ immune cells at d7 p.i., which returned to naïve levels on d14, increased again on d19, and

then decreased on d35 (Fig. 2A-C). Expansion of the splenic basophil population occurred later, at d19 p.i., and remained elevated at d35 p.i (Fig. 2D-F). These data largely mirror the kinetics of infection-induced changes in basophil abundance in the cecum and spleen of resistant C57BL/6 mice (7). We also observed an infection-induced increase in mast cell (CD45⁺ Lineage⁻IgE⁺ FcεR1α⁺CD49b⁺/cKit⁺) (Fig. S1B) frequencies out of Lineage-negative cells in the cecum but not the spleen starting on d14 p.i. (Fig. S1C-D), suggesting that mast cells are also mobilized during infection in AKR/J mice, but not as broadly across tissues as basophils. Consistent with our previous findings in C57BL/6 mice (7) we could not detect a substantial basophil population in the draining MLN (Fig. S1E), and there were no notable differences in basophil population frequencies throughout the time-course in the MLN (Fig. S1F). These data show that the basophil population expands in the spleen and cecum at various timepoints in *T. muris*-infected AKR/J mice.

Notch2 is not robustly expressed by cecum basophils in *T. muris*-infected AKR/J mice.

Previous findings from our group reported a key role for Notch signaling in programming helminth-induced basophil responses in C57BL/6 mice, associated Type 2 inflammation and helminth expulsion, but not basophil population expansion (7). Therefore, we sought to determine whether Notch2, the predominant Notch receptor expressed on C57BL/6 basophils during infection (7), was expressed on the surface of AKR/J basophils. Overall, there was no significant infection-induced increase in the percentage of basophils that expressed Notch2 (Fig. 3A-B) or in the Notch2 gMFI of Notch2 positive basophils (Fig. 3C) in the cecum following *T. muris* infection. In the spleen, the percentage of basophils that expressed Notch2 at d19 p.i. increased slightly over levels observed in naïve mice, but then declined (Fig. S2A-B), and there were no infection-induced changes in the Notch2 gMFI of Notch2-positive splenic basophils (Fig. S2C). Thus, these data suggest that while susceptible AKR/J mice do have expanded basophil populations after *T. muris* infection, these basophils do not display a substantial infection-induced increase in the level of surface Notch2 expression.

IFN-γ νευτραλίζατιον εν ΑΚΡ/Θ μίσε δόες νοτ ενήανσε βασοπηιλ Νοτση2 εξπρεσσιον.

As AKR/J mice have a predominant IFN-γ response following *T. muris* infection (Fig. 1C-E) (9-13, 15), we hypothesized that this Type 1 skew might suppress or prevent the upregulation of Notch2 on AKR/J basophils. To test this idea, we treated AKR/J mice with a neutralizing α-IFN-γ antibody throughout *T. muris* infection as described previously (15, 31) and then examined the levels of Notch2 on cecum basophils on day 14 p.i., the timepoint at which we see maximal basophil Notch2 expression in the infected tissue site in C57BL/6 mice (7) (Fig. 4A). We first validated our neutralization protocol, observing an infection-induced increase in IFN-γ protein levels in isotype control-treated AKR/J mice but not α-IFN-γ-treated mice in tissue and blood (Fig. 4B-C) and transcript levels in the colon at d14 p.i. (Fig. 4D). We did not observe an accompanying increase in transcription levels of *Il4* or *Il13* in the colon in AKR/J mice at day 14 p.i., though this might be due to the early time point examined. As expected, day 14 p.i. C57BL/6 mice had higher *Il4* and *Il13* levels in the colon than naïve C57BL/6 mice, though these data were not statistically significant (Fig. S3A-B).

At day 14 p.i., we did not observe an increase in cecum basophil Notch2 expression by frequency or gMFI in α-IFN-γ-treated AKR/J mice compared to what was observed in infected isotype-treated or naïve AKR/J mice. As previously reported, we observed that cecum basophils in C57BL/6 mice had elevated Notch2 expression on d14 p.i with *T. muris* compared to levels in naïve C57BL/6 mice (7) (Fig. 4E-G). These findings show that AKR/J cecum basophils do not upregulate Notch2 expression at day 14 p.i. with *T. muris* when IFN-γ is neutralized. Collectively, these studies suggest that IFN-γ levels do not control cecum basophil Notch2 expression in AKR/J mice in response to *T. muris* infection, at least at the day 14 p.i. timepoint.

Discussion

Basophils are rapidly mobilized after *T. muris* infection to the inflamed tissue to execute effector functions that support Type 2 inflammation (7, 26, 34). Our previous findings showed that Notch signaling in basophils

is required for optimal *T. muris* infection-induced basophil gene expression changes and effector function, allowing basophils to support Th2 cell responses in the tissue that promote worm expulsion in C57BL/6 mice (7). However, the regulation of basophil responses following infection and the factors that govern basophil Notch receptor expression remain unclear. Previous studies have leveraged differences in susceptibility to *T. muris* infection in inbred mouse strains to reveal cellular and molecular players that regulate the Type 2 immune response (9-11, 13-17). Here, we utilized AKR/J mice, which retain adult *T. muris* worms and mount a Type-1 skewed immune response to infection (9-13, 15), to investigate how basophil responses and basophil Notch expression are regulated in genetically susceptible mice.

Our data show that the size of the basophil population changed dynamically in the cecum and spleen upon *T. muris* infection in AKR/J mice, similar to the patterns observed in resistant C57BL/6 mice (Fig. 2) (7, 26, 34). However, our studies did not examine levels of factors that promote basophilia, including IL-3, IL-33, TSLP, and basophil-homing chemokines such as CXCL12 and CCL7, nor basophil expression levels of the cognate receptors for these factors (20-29), in *T. muris* -infected AKR/J vs. C57BL/6 mice. One study has shown that resistant Balb/C but not AKR/J mice infected with *T. muris* have infection-induced increases in *Tslp* gene expression in the cecum at 1 and 7 days p.i. (35), suggesting that there are strain-specific differences in the levels of factors that activate basophils. Future experiments that measure the levels and activities of cytokines and chemokines that activate and promote the accumulation of AKR/J vs. C57BL/6 or Balb/C basophils during *T. muris* infection will be required to determine whether there are strain-dependent differences in the levels and activities of basophil activating factors. Studies that delete IL-3 and TSLP and their receptors in AKR/J mice during *T. muris* infection will also be required to determine whether these, or other factors, are critical for mobilizing the basophilia that we observe in AKR/J mice upon infection.

In our study, basophil expansion was not associated with Type 2 inflammation and worm clearance in *T. muris* -infected AKR/J mice (Fig. 1). These data suggest that expansion of the basophil population per se is not sufficient to drive effective Type 2 inflammation and parasite expulsion nor indicative of an optimal Type 2 response. Indeed, there was negligible expression of Type 2 cytokines in the colon of infected AKR/J mice, but considerable expression of *Ifng* (Fig. 1C-E). Thus, basophils, while expanded in AKR/J mice, may not be receiving proper activation signals and may not be optimally functional. Further studies are required to investigate whether AKR/J basophils demonstrate similar infection-induced basophil degranulation, tissue positioning, cytokine and chemokine production, and interactions with other cell types, similar to what is seen in C57BL/6 mice.

We also found that basophils in AKR/J mice do not upregulate Notch2 on day 14 p.i. with *T. muris* as basophils in C57BL/6 mice do (Fig. 3) (7). One caveat of our study is that we did not measure expression of all four Notch receptors (30); we focused on Notch2, as this receptor was most strongly upregulated on C57BL/6 basophils following *T. muris* infection (7) and Notch receptor expression is highly tissue- and context-dependent (30). It is possible that AKR/J basophils upregulate other Notch receptors that control basophil effector function in infection. Regardless, our data suggest that AKR/J basophils may not be fully competent to receive critical Notch signals that program these cells to support Type 2 responses in C57BL/6 mice (7). It is notable that in the naïve state, C57BL/6 cecum basophils have lower Notch2 expression than AKR/J basophils (Fig. 4E-G), suggesting that C57BL/6 and AKR/J mice have different baseline levels of basophil Notch signaling activity. Further studies should focus on whether AKR/J basophils show evidence of proper Notch programming during *T. muris* infection to determine if the Notch pathway is active in mobilizing basophil responses in a genetically susceptible inbred mouse strain.

Interestingly, our IFN- γ neutralization experiment (Fig. 4) suggests that IFN- γ is not the primary player in suppressing Notch2 expression by AKR/J cecum basophils following *T. muris* infection on day 14 p.i. However, AKR/J basophils may upregulate Notch2 during infection and IFN- γ neutralization at other timepoints. If Type 2-associated factors are important in upregulating Notch2 on basophils, then it is not surprising that we did not observe increased Notch2 expression on basophils in infected AKR/J mice, in light of our findings that IFN- γ neutralization did not provoke increased colonic gene expression of *Il4* and *Il13* on day 14 p.i.

(SFig. 3). Thus, a kinetic analysis of AKR/J basophil Notch receptor expression should be performed. Future studies could also assess whether treatment with IL-4 complexes, which also provide protection to AKR/J mice (31), are sufficient to upregulate Notch2 on AKR/J basophils.

Indeed, previous studies have shown that IFN- γ neutralization does not control all facets of the host response to *T. muris* across susceptible mouse models. IFN- γ neutralization results in increased worm clearance and a decrease in IFN- γ -associated IgG2a in AKR/J mice (31), worm clearance and IL-13 upregulation in susceptible B cell-depleted C57BL/6 mice (36), and Type 2 cytokine responses on day 21 p.i. in susceptible TSLPR-deficient mice (37). However, it does not alter worm burden in C57BL/6 mice infected with a low dose of *T. muris* that retain worms (38) nor susceptible *Muc5ac*-deficient mice that have elevated IFN- γ levels (39). Thus, it is likely that Notch2 expression by basophils is governed by factors other than IFN- γ in AKR/J mice. C57BL/6 and AKR/J mice differ substantially at a wide array of loci that may regulate basophil responses during helminth infection (9-11, 13-15, 17). Further investigation and genetic analyses will be needed to determine why AKR/J basophils do not upregulate Notch2 receptor expression compared to C57BL/6 basophils during *T. muris* infection. Such studies could potentially illuminate new factors that promote basophil upregulation of Notch receptors, in addition to IL-3 and IL-33 (7).

In summary, our data reveal that the basophil population expands in the spleen and cecum during *T. muris* infection but does not upregulate Notch2 expression in susceptible AKR/J mice, even when IFN- γ is neutralized. Continued comparative studies in AKR/J and C57BL/6 mice could be leveraged to determine how and when Notch signaling shapes anti-helminth basophil responses. Our study emphasizes the significance of utilizing inbred mouse models to dissect the correlates of an effective Type 2 immune response and reveals new insight into how basophil responses are regulated during intestinal helminth infection.

Author Contributions

L.M.W. and E.D.T.W. designed the study. S.S., L.M.W., O.O.O., B.M. S.P.F. and E.D.T.W. planned and performed experiments and collected and analyzed data. M.K.M., B.M. and S.A.P. provided technical support and cared for the mouse colony. S.S., L.M.W. and E.D.T.W. wrote the manuscript, with input from all other authors. E.D.T.W. oversaw the research.

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Footnotes

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Figure Legends

Figure 1. Susceptible AKR/J mice retain *T. muris* and have a Type 1 immune skew. (A) Experimental schematic. (B) Worm burden in AKR/J mice. Real time PCR analysis of proximal colon homogenate from naïve and *T. muris* (Tm)-infected mice, quantifying (C) *Ifng* (D) *Il4*, (E) *Il13*, (F) *Fcer1a*, and (G) *Mcpt8* (normalized to *Actb*). Data from all combined experiments are presented as the mean $\pm$ SEM. Statistical analysis was performed using a linear fixed-effect model with pairwise comparison; *P [?] 0.05, **P [?] 0.01, ***P [?] 0.001, ****P [?] 0.0001. n=2-3 for each time point per experiment; 3 independent experiments.

Figure 2. The basophil population expands upon *T. muris* infection in AKR/J mice. Flow cytometric analysis for basophils in naïve and *T. muris* (Tm)-infected mice. (A) Cecum basophil gating (CD45⁺Lineage (Lin)⁻Fc ϵ RI α ⁺IgE⁺CD49b⁺ckit⁻). Basophil percentage of (B) Lin⁻ and (C) CD45⁺ cecum

cells. (D) Spleen basophil gating ($CD45^{+}Lineage (Lin)^{-}Fc\epsilon RI\alpha^{+}IgE^{+}CD49b^{+}ckit^{-}$). Basophil percentage of (E) Lin^{-} and (F) $CD45^{+}$ spleen cells. Data from all combined experiments are presented as the mean $\pm$ SEM. Statistical analysis was performed using a linear fixed-effect model with pairwise comparison; *P [?] 0.05, **P [?] 0.01, ***P [?] 0.001. n=2-3 for each time point per experiment; 3 independent experiments.

Figure 3. Basophil Notch2 expression does not substantially increase following *T. muris* infection in AKR/J mice. Flow cytometric analysis of basophils in the cecum in naïve and *T. muris* (Tm)-infected mice, displaying (A) representative plots for Notch2 staining, (B) percent Notch2⁺ basophils, and (C) Notch2 geometric mean fluorescence intensity (gMFI) on cecum basophils. Data from all combined experiments are presented as the mean $\pm$ SEM. n=2-3 for each time point per experiment; 3 independent experiments.

Φιγυρε 4. Βασοφιλ Νοτση2 εξπρεσσιον δοες νοτ ζηανγες ατ δαψ 14 ποστ-*T. muris* ινφερσιον υπον ΙΦΝ-γ νευτραλιζατιον ιν ΑΚΡ/Θ μιξε.(A) Experimental schematic. IFN-γ protein levels as measured by ELISA in (B) serum and (C) proximal colon tissue lysate, and (D) real time PCR analysis of proximal colon homogenate quantifying *Ifng*(normalized to *Actb*) from naïve and *T. muris* (Tm)-infected mice. Flow cytometric analysis of basophils in the cecum in naïve and Tm-infected mice, displaying (E) representative plots for Notch2 staining, (F) percent Notch2⁺ basophils, and (G) Notch2 geometric mean fluorescence intensity (gMFI) on basophils. Data from all combined experiments are presented as the mean $\pm$ SEM. Statistical analysis was performed using a linear fixed-effect model with pairwise comparison; *P [?] 0.05, **P [?] 0.01, ***P [?] 0.001, ****P [?] 0.0001. n=2-3 for each time point per experiment; 3 independent experiments.

Supplementary Figure Legends

Figure S1. (A) Serum IgE levels in naïve and *T. muris*(Tm)-infected mice measured by ELISA. Flow cytometric analysis for mast cells (B) ($CD45^{+}Lineage (Lin)^{-}Fc\epsilon RI\alpha^{+}IgE^{+}CD49b^{+/-}ckit^{+}$) in naïve and Tm-infected mice showing percentage of Lin^{-} in (C) cecum and (D) spleen. (E) MLN basophil gating ($CD45^{+}Lineage (Lin)^{-}Fc\epsilon RI\alpha^{+}IgE^{+}CD49b^{+}ckit^{-}$). Basophil percentage of (F) Lin^{-} and (G) $CD45^{+}$ MLN cells. Data from all combined experiments are presented as the mean $\pm$ SEM. Statistical analysis was performed using a linear fixed-effect model with pairwise comparison; P [?] 0.05, **P [?] 0.01, *** P [?] 0.001. n=2-3 for each time point per experiment; 3 independent experiments.

Figure S2. Flow cytometric analysis of basophils in the spleen in naïve and *T. muris* (Tm)-infected mice, displaying (A) representative plots for Notch2 staining, (B) percent Notch2⁺ basophils, and (C) Notch2 geometric mean fluorescence intensity (gMFI) on spleen basophils. Data from all combined experiments are presented as the mean $\pm$ SEM. Statistical analysis was done using a linear fixed-effect model with pairwise comparison; ***P [?] 0.001. n=2-3 for each time point per experiment; 3 independent experiments.

Figure S3. Real time PCR analysis of proximal colon homogenate from naïve and *T. muris* (Tm)-infected mice, quantifying (A) *Il4* and (B) *Il13* (normalized to *Actb*). Data from all combined experiments are presented as the mean $\pm$ SEM. Statistical analysis was done using a linear fixed-effect model with pairwise comparison; *P [?] 0.05. n=2-3 for each time point per experiment; 3 independent experiments.

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Figure 1

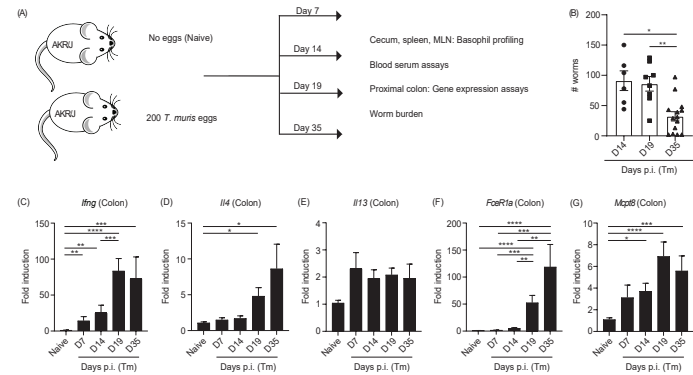


Figure 2

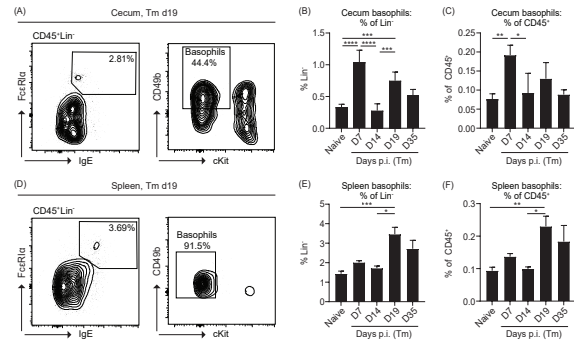


Figure 3

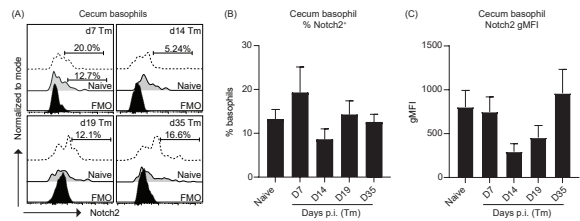


Figure 4

