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Basophils Are Dispensable for the Control of a Filarial Infection

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ABSTRACT

Basophils are innate effector cells that contribute to allergic reactions and provide protection against parasites. Using basophil-deficient *Mcpt8-cre* mice, we have previously shown that these granulocytes contributed to the immune mediated early control of the gastrointestinal helminth *Strongyloides ratti* in mice. In this study, we analyze the impact of basophils on the immune response and defense against the tissue-dwelling filarial helminth parasite *Litomosoides sigmodontis*. Although basophils and IgE increased at the site of infection, the absence of basophils did not change the outcome of *L. sigmodontis* infection. Worm burden in the thoracic cavity and microfilariae in the peripheral blood were alike in *L. sigmodontis*-infected *Mcpt8-cre* mice compared with *Mcpt8* wild type littermates during the entire course of infection. Analysis of the cytokine and Ab response to *L. sigmodontis* revealed no consistent alterations in the absence of basophils. Furthermore, basophil-deficient and -competent mice were protected to the same extent during a secondary infection with *L. sigmodontis*. In summary, our findings suggest that basophils are dispensable for the initiation of the appropriate immune response and host defense against *L. sigmodontis* infection in mice. *ImmunoHorizons*, 2018, 2: 296–304.

INTRODUCTION

Approximately one third of the world population is infected with helminths. Filariae such as *Brugia malayi*, *Wuchereria bancrofti*, and *Onchocerca volvulus* are transmitted by blood-sucking insects and affect ~150 million people worldwide (1). Infection of mice with the rodent nematode *Litomosoides sigmodontis* functions as a model for human filariasis (2). During a blood meal by mites (*Ornithonyssus bacoti*), third stage larvae (L3) are transmitted. The larvae migrate via the lymphatic vessels to the thoracic cavity and develop into adult worms within 30 days. In susceptible BALB/c mice, male and female adults mate and the females release their offspring, the microfilariae (MF) or first stage larvae, into the circulation by day 55 postinfection (p.i.). BALB/c mice remain infected for up to 200 days and thus represent an excellent model for chronic filarial infections (3).

Basophils are innate effector cells that are mainly located in the blood and migrate to the tissues in response to specific stimuli (4). The role of basophils during helminth infection has been studied using basophil-deficient mice or basophil-depleting Abs (5). Thus, most studies focused on the role of basophils in gastrointestinal nematode infection (6). In this context, we have shown that basophils are dispensable for the control of the tissue-migrating *Strongyloides ratti* L3 during primary infection and contribute to the control of parasitic adults in the intestine at the peak of infection (7). In other primary gastrointestinal infections, no obvious effector functions have been described for basophils (5). Upon secondary infection, basophils contribute to the defense against *Nippostrongylus brasiliensis* (8, 9) and *Heligmosomoides polygyrus* (10). During infection with the tissue-dwelling *L. sigmodontis*, basophils were proposed to amplify the type 2

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Abbreviations used in this article: FBZ, flubendazole; L3, third stage larvae; L4, fourth stage larvae; LsAg, *L. sigmodontis* Ag; *Mcpt8*, mast cell protease 8; MF, microfilaria; p.i., postinfection; wt, wild type.

The online version of this article contains supplemental material.

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immune response without having an impact on the course of primary infection (11). However, depletion of basophils prior to vaccination with irradiated larvae altered the worm burden in a challenge infection (12), suggesting a central role for basophils in immune memory formation to tissue dwelling helminths.

In this study, we investigate the role of basophils during infection with the rodent filariae *L. sigmodontis*. We use a recently developed mouse model of basophil deficiency on the susceptible BALB/c mouse background to re-evaluate the previously described function of basophils. In mast cell protease 8 (*Mcpt8*)-*cre* mice, the depletion of basophils is achieved by heterozygous bacterial artificial chromosome transgenic expression of the Cre recombinase under the control of the regulatory elements of the basophil-specific protease *Mcpt8* (9). Basophil-deficient *Mcpt8-cre* have an otherwise normal immune system; in particular, mast cells remain unaffected in this mouse model (5).

We show that despite an increase of IgE and a recruitment of basophils to the thoracic cavity during infection with *L. sigmodontis*, the absence of basophils did not change infection outcome. We recorded no differences in the worm burden regarding fourth stage larvae (L4), adults, and MF in basophil-deficient *Mcpt8-cre* in comparison with their cell-competent littermates. Deficiency of basophils did not compromise protection in a secondary *L. sigmodontis* infection after drug-mediated termination of the first infection. In summary, our results suggest that basophils play limited and/or redundant roles during the control of the tissue dwelling helminth parasite *L. sigmodontis*.

MATERIALS AND METHODS

Ethics and mice

Animal experimentation was conducted at the animal facility of the Bernhard Nocht Institute for Tropical Medicine in agreement with the German Animal Welfare Act under the supervision of a veterinarian. The experimental protocols have been reviewed and approved by the responsible federal health authorities of the State of Hamburg, Germany, the “Behörde für Gesundheit und Verbraucherschutz,” permission number 125/14.

BALB/c mice were obtained from Charles River (Sulzfeld, Germany). *Mcpt8-cre* (9) on the BALB/c background have been described previously and were bred heterozygously (*Mcpt8-cre*⁺, termed *Mcpt8-cre* in this paper) in the animal facility of the Bernhard Nocht Institute for Tropical Medicine. The genotype of the *Mcpt8-cre*/wt mice was confirmed by PCR (Supplemental Fig. 1).

Male and female wild type (wt) littermates (termed *Mcpt8-wt*) were matched for gender and age and cohoused with basophil-deficient mice. All mice were kept in individually ventilated cages under specific pathogen-free conditions and were used at 7–10 wk of age.

Infection with *L. sigmodontis* and drug treatment

The life cycle of *L. sigmodontis* was maintained in cotton rats (*Sigmodon hispidus*), the natural reservoir of the nematode. Mites

(*O. bacoti*), the intermediate hosts, were fed on infected cotton rats. Fourteen days after this blood meal, mice were infected naturally by exposure to infected mites. Mice belonging to different experimental groups were mixed and placed in the same tank to prevent a bias due to a different frequency or batches of mites. Flubendazole (FBZ) was dissolved in DMSO and further diluted in PBS. FBZ was administered s.c. from day 28 to day 32 p.i. in a concentration of 5 mg/kg body weight. Thirty days later, FBZ-treated mice were reinfected, and worm burden was determined 30 d after reinfection. Age- and sex-matched control mice were left untreated and infected in parallel to the reinfection from FBZ-treated mice.

Flow cytometry

For surface staining, cells were stained for 30 min on ice with FITC-labeled Abs against CD4 (clone: RM4-5), CD8 (clone: 53-6.7), and CD19 cells (clone: 1D3), PerCP Cy5.5-labeled anti-mouse CD11b (clone: M1/70), PE-labeled anti-mouse IgE (clone: RME-1), Brilliant Violet 421-labeled anti-mouse CD117 (c-Kit; clone: 2B8), and PE Cy7-labeled anti-mouse CD49b (clone: DX5). Abs were purchased from BioLegend or Affymetrix eBioscience. Cytokines in the thoracic cavity wash and serum were measured by cytokine bead assay (LEGENDplex Mouse Th Cytokine Mix and Match; BioLegend) according to the manufacturer's instructions. Samples were analyzed on an LSR II Flow Cytometer (Becton Dickinson) using FlowJo software (Tree Star).

Quantification of the vascular permeability

In vivo degranulation assay was performed with minor changes as recently described (13). Two hundred microliters of 1% Evans blue was injected i.v. into recipient mice (*Mcpt8-cre*/wt). Approximately 10–15 mites that were fed on *L. sigmodontis*-infected or naive cotton rats 14 d earlier were collected into an Eppendorf tube. Infected and mock-infected mites were allowed to have a blood meal on the ear of anesthetized mice. After 5 min, the tube and remaining mites were removed. As control, an Eppendorf tube without mites was administered to the ears of control mice in parallel. Twenty minutes later, mice were killed by cervical dislocation. Ears were collected and dried overnight at 50°C. Evans blue was extracted by 24 h incubation of the chopped ears in 200 μ l of formamide at 50°C. After centrifugation, the supernatant was transferred into a 96-well plate and the OD_{630nm} was measured.

Quantification of the humoral immune response

For analysis of serum Abs, blood was collected from mice by submandibular bleeding of the facial vein at days 40 and 75 p.i. and allowed to coagulate for 1 h at room temperature. Serum was collected after centrifugation at 10,000 $\times$ g for 10 min and stored at –20°C for further analysis.

ELISA plates were coated overnight with 4 μ g/ml *L. sigmodontis* Ag (LsAg) in PBS. Plates were washed, blocked by incubation with PBS 1% BSA for 2 h, and incubated for 2 h with serum. After washing, plates were incubated for 1 h with HRP-labeled anti-mouse IgG1, IgM, IgG2a, IgG2b (all Invitrogen),

and IgE (BD Biosciences). Plates were washed and developed by incubation with 100 μ l tetramethylbenzidine (0.1 mg/ml), 0.003% H_2O_2 in 100 mM NaH_2PO_4 (pH 5.5) for 2.5 min. Reaction was stopped by addition of 50 μ l 1 M H_2SO_4 , and $OD_{450\text{ nm}}$ was measured. The more abundant isotypes IgM and IgG1 were calculated by defining the highest serum dilution in a serial dilution (1:1000 to 1:128,000), resulting in an $OD_{450\text{ nm}}$ above the doubled background. For the less abundant LsAg-specific isotypes, IgG2a, IgG2b, and IgE arbitrary units were calculated by subtraction of OD_{450} of the diluent (0.1% BSA in PBS) from OD_{450} of one fixed serum concentration (1:100 for IgE and IgG2a, 1:1000 for IgG2b).

Quantification of the cytokine production

For analysis of cellular responses, mice were sacrificed at the indicated days p.i., and 5×10^5 splenocytes were cultured in quadruplicates in 96-well round-bottom plates in RPMI 1640 medium supplemented with 10% FCS, 20 mM HEPES, L-glutamine (2 mM), and gentamicin (50 μ g/ml) at 37°C and 5% CO_2 . The cells were stimulated for 72 h with medium, 12.5 μ g/ml LsAg, or 1 μ g/ml anti-CD3. Cytokines in the culture supernatants were measured as described (14).

Statistical analysis

Samples were tested for Gaussian distribution, and Student *t* test (unpaired) or Mann–Whitney *U* test was performed to compare two groups. A two-way ANOVA with Bonferroni posttest was conducted to compare two groups over time. One-way ANOVA with Bonferroni posttest or Kruskal–Wallis test with Dunn multiple comparison test was performed to compare more than two groups. Prism software was used for statistical analysis (GraphPad Software). The *p* values ≤ 0.05 were considered statistically significant.

RESULTS

Blood-sucking mites increase the vascular permeability independent of infective larvae

In the current study, we used a recently generated mouse model of basophil deficiency, *Mcpt8-cre* (9) mice, to illuminate the function of basophils during infection with *L. sigmodontis*.

During a natural infection with *L. sigmodontis*, blood-sucking mites transmit L3 into the skin of recipient mice. To analyze whether this blood meal triggers a local activation of basophils, we compared the vascular permeability in basophil-deficient mice and their cell-competent littermates. Blood-sucking mites increased the vascular permeability indicated by an increased extravasation of Evans blue into the tissue in BALB/c *Mcpt8-cre* mice and their respective wt littermates (Fig. 1). Thus, exposure to mock-infected mites and L3-infected mites increased the vascular permeability at the site of the blood meal to the same extent. Vascular permeability was not affected by basophil deficiency. The observed increase in vascular permeability was specifically induced by the blood meal of mites, as no change in the barrier function of the tissue was

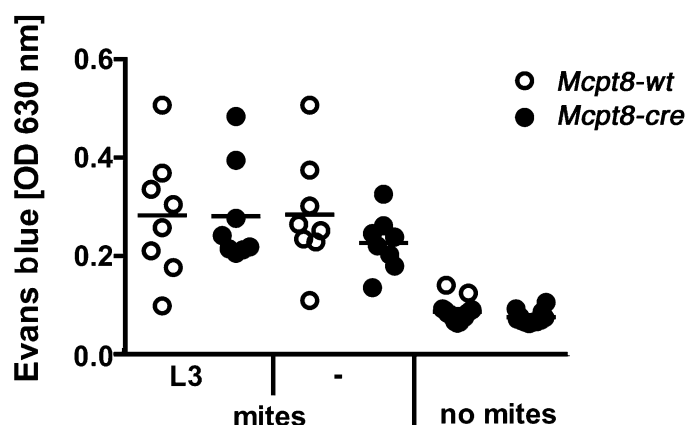


FIGURE 1. Vascular permeability induced during a blood meal of mites is not altered in the absence of basophils.

Evans blue was injected into the tail vein of basophil-deficient *Mcpt8-cre* and their competent littermates (*Mcpt8-wt*). After a blood meal by mites that had been either infected (L3) or mock infected (–), Evans blue was extracted from the ears. As control, an empty tube without mites (no mites) was administered to control mice in parallel. Each dot represents a single mouse (8 mice that were exposed to mites, 12–15 mice as control mice [no mites]). Data were analyzed using Kruskal–Wallis test with Dunn multiple comparison test and are combined from two independent experiments.

measured in the contralateral, nonexposed ear (Fig. 1, no mites). Thus, feeding mites enhance local vascular permeability during their blood meal independent of the presence of L3 in mite saliva or the presence of basophils in the host.

Basophil numbers increase at the site of infection

After being transmitted during the blood meal, L3 migrate within 3–5 d to the thoracic cavity. Flow cytometric analyses were performed to analyze whether basophils are recruited to the site of infection. Analyses were performed in the early phase at day 8 p.i. (L3/L4), at day 30 p.i. (immature adult worms), at day 60 p.i. (onset of patency), and at day 90 p.i. (chronic phase). The gating strategy used to identify basophils is shown in Supplemental Fig. 2.

At day 8 p.i., we observed a slight, nonsignificant accumulation of leukocytes at the site of infection (Fig. 2A). The number of leukocytes significantly increased until day 30 p.i. and remained constantly increased until day 90 p.i. compared with noninfected mice (Fig. 2A). The recruitment of basophils was maximal at the days 30 and 60 p.i., followed by a decrease until day 90 p.i. (Fig. 2B). As expected, basophils were absent in the thoracic cavity of basophil-deficient mice at day 30 p.i. (Supplemental Fig. 3). Because basophils are activated by cross-linking of membrane-bound IgE, we measured IgE in the serum and in the thoracic cavity of infected BALB/c mice. Polyclonal IgE in the serum increased compared with noninfected mice at day 30 p.i. and remained elevated during the course of infection (Fig. 2C). *L. sigmodontis*-specific IgE was below the detection limit in the serum but increased in the thoracic cavity between days 30 and 90 p.i. (Fig. 2D).

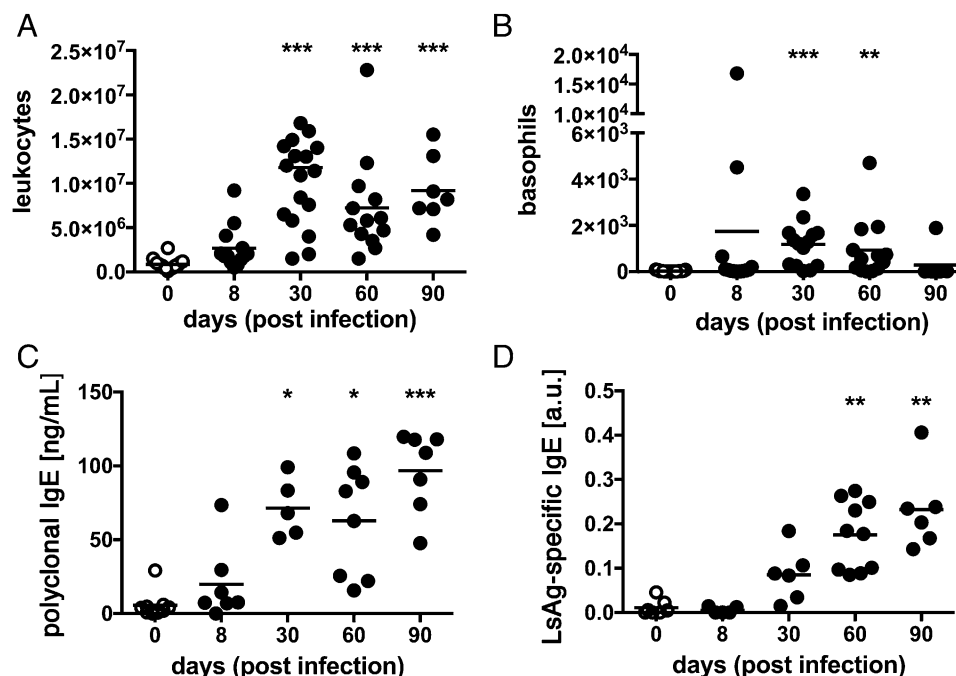


FIGURE 2. Basophils increase during infection with *L. sigmodontis*.

BALB/c mice were infected with *L. sigmodontis*, and mice were sacrificed at various time points p.i., indicated at the x-axis. Day 0 represents data from noninfected BALB/c mice. Numbers of leukocytes (A) and basophils (B) were analyzed in the thoracic cavity lavage at the indicated time points. Total amount of IgE (C) was detected in the serum at the indicated time points. LsAg-specific IgE (D) was analyzed in the thoracic cavity lavage. Each dot represents a value from a single mouse. Data are combined from two to three experiments. (A and B) $n = 13$ – 18 mice days 0–60 p.i.; $n = 6$ – 7 mice day 90 p.i. (C and D) $n = 5$ – 10 mice. Asterisks indicate significant differences in comparison with noninfected (day 0) BALB/c mice. Data were analyzed with a Kruskal–Wallis test with a Dunn multiple comparison test. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

Basophils are dispensable for the control of *L. sigmodontis* in a primary infection

To elucidate the role of basophils in the control of *L. sigmodontis*, we naturally infected *Mcpt8-cre* mice that lack basophils, as well as their cell-intact *Mcpt8-wt* littermates, with *L. sigmodontis*. After exposure to infected mites, we recorded the parasite burden at several time points. We analyzed day 10 p.i. to count *L. sigmodontis* L3 at the transition to L4, day 40 to count mature adults, and day 75 to count the mature reproducing adults in the thoracic cavity. The number of worms in the thoracic cavity was similar during all stages of infection in basophil-deficient mice compared with their wt littermates (Fig. 3A). Next, we monitored the release of MF into the circulation. Basophil deficiency (Fig. 3B) did not alter the microfilaremia, and thus fecundity, of the female worms. Irrespective of the basophil deficiency, mice were able to clear the infection with the same kinetic as wt mice.

Taken together, although basophils were recruited to the thoracic cavity (Fig. 2B) and IgE increased (Fig. 2C, 2D) during infection with *L. sigmodontis*, the genetic ablation of basophils did not alter the parasite burden at any time point.

***L. sigmodontis*-specific immune response is not concordantly altered by the absence of basophils during primary infection**

Because basophils have been proposed to orchestrate type 2 immune responses during helminth infection (11), we determined

the *L. sigmodontis*-specific cellular and humoral immune responses. *Mcpt8-cre* and *Mcpt8-wt* mice were infected for 40 or 75 d, and the *L. sigmodontis*-specific Ig responses directed against adult worm extract were analyzed. LsAg-specific IgE in the thoracic cavity lavage and serum was similar in *L. sigmodontis*-infected *Mcpt8-cre* and *Mcpt8-wt* mice at days 40 and 75 p.i. (Fig. 4A, 4B). Furthermore, *L. sigmodontis*-specific IgG2a, IgG2b, and IgM did not differ between basophil-deficient and -competent mice (Fig. 4C, 4D, 4F). By contrast, basophil-deficient mice displayed a significant reduction in *L. sigmodontis*-specific type 2-associated IgG1 titers at day 75 p.i. (Fig. 4E).

We next compared the T cell cytokine response in basophil-deficient and wt littermates by measuring cytokines in the serum and the supernatant of spleen cells. Spleen cells were isolated from *L. sigmodontis*-infected mice at day 40 p.i. and stimulated ex vivo. Spleen cells from basophil-deficient *Mcpt8-cre* mice showed comparable LsAg-specific release of the Th2 cytokines IL-4 and IL-13 and of the regulatory cytokine IL-10 as their *Mcpt8-wt* littermates (Fig. 5A, 5C, 5E). LsAg-specific production of the Th1-associated cytokine IFN- γ was reduced by trend ($p = 0.058$) in basophil-deficient mice compared with their basophil-competent littermates (Fig. 5G). Polyclonal stimulation of T cells with anti-CD3 resulted in a slightly increased splenic IL-4 synthesis in *Mcpt8-cre* mice, which was also reflected by higher IL-4 and IL-13 levels in the serum in comparison with *Mcpt8-wt* mice (Fig. 5B, 5D). The concentrations of

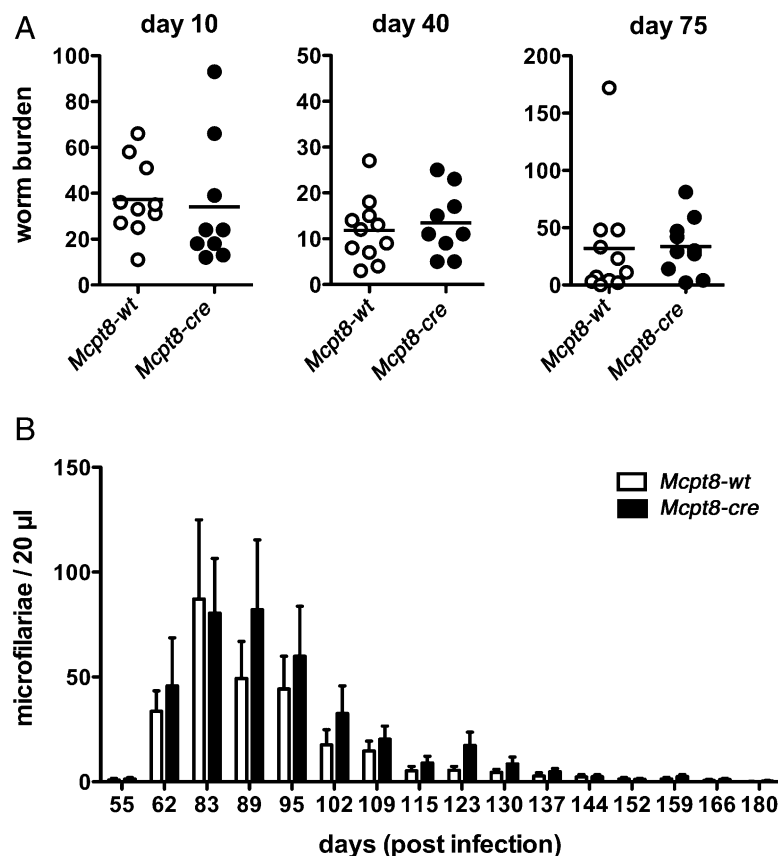


FIGURE 3. Unchanged worm burden and microfilaremia in basophil-deficient mice compared with their basophil-competent littermates.

Basophil-deficient *Mcpt8-cre* and their competent littermates (*Mcpt8-wt*) were infected with *L. sigmodontis*. (A) Numbers of worms were counted at the indicated time points in basophil-deficient and -competent mice. Each dot represents the worm burden from a single mouse ($n = 9$ –11 mice). Data were analyzed with an unpaired Student *t* test. MF were counted in 20 µl blood of basophil-deficient *Mcpt8-cre* and basophil-competent *Mcpt8-wt* mice (B) at the indicated days p.i. Data show the mean plus SEM and are combined from two independent experiments ($n = 11$ –12 mice). Microfilaremia was analyzed with a two-way ANOVA with Bonferroni posttest.

IL-13, IL-10, and IFN- γ were comparable in the supernatants from anti-CD3-stimulated spleen cells from *Mcpt8-cre* and *Mcpt8-wt* mice. We recorded no differences in the IL-10 and IFN- γ levels in the serum from infected mice (Fig. 5F, 5H). Both polyclonal and LsAg-specific cytokine responses were unaffected by the deficiency of basophils at a later time point, day 75 p.i. (Supplemental Fig. 4). Cytokines in serum samples from noninfected BALB/c *Mcpt8-cre* and *Mcpt8-wt* mice were below the detection limit of the assay.

In summary, we observed a mild decrease of the humoral type 2 immunity that was accompanied by an increase of the cellular type 2 immunity in basophil-deficient mice at some time points during infection. Despite these selective differences observed in *Mcpt8-cre* mice, no sustained and consistent alteration of the immune response to *L. sigmodontis* was observed in basophil-deficient mice.

Basophil-deficient mice are protected in a challenge infection

IgE-activated basophils have been shown to contribute to immunity against infection with the gastrointestinal parasites *H. polygyrus* and *N. brasiliensis*, specifically during a second infection of immune mice

(10). To analyze the impact of basophils in a secondary *L. sigmodontis* infection, mice were treated with FBZ, a benzimidazole anthelmintic, which was shown to be macrofilaricidal (15). Thirty days after treatment and effective clearance of *L. sigmodontis* worms, BALB/c *Mcpt8-cre* and *Mcpt8-wt* mice were reinfected with *L. sigmodontis*. Worm burden was counted 30 d after reinfection in comparison with age- and gender-matched mice that were infected for the first time (Fig. 6A). Dewormed and reinfected *Mcpt8-cre* and *Mcpt8-wt* mice displayed a significant reduction of the worm burden compared with control mice that were infected for the first time (Fig. 6B). Thus, 82% of the *Mcpt8-cre* and 92% of the *Mcpt8-wt* mice displayed sterile immunity to a second *L. sigmodontis* infection. In summary, our data show that basophils are not required for the establishment of a protective immune response that is induced during a primary infection with *L. sigmodontis*.

DISCUSSION

The aim of this study was to investigate the role of basophils during infection with the tissue-dwelling filarial nematode *L. sigmodontis*.

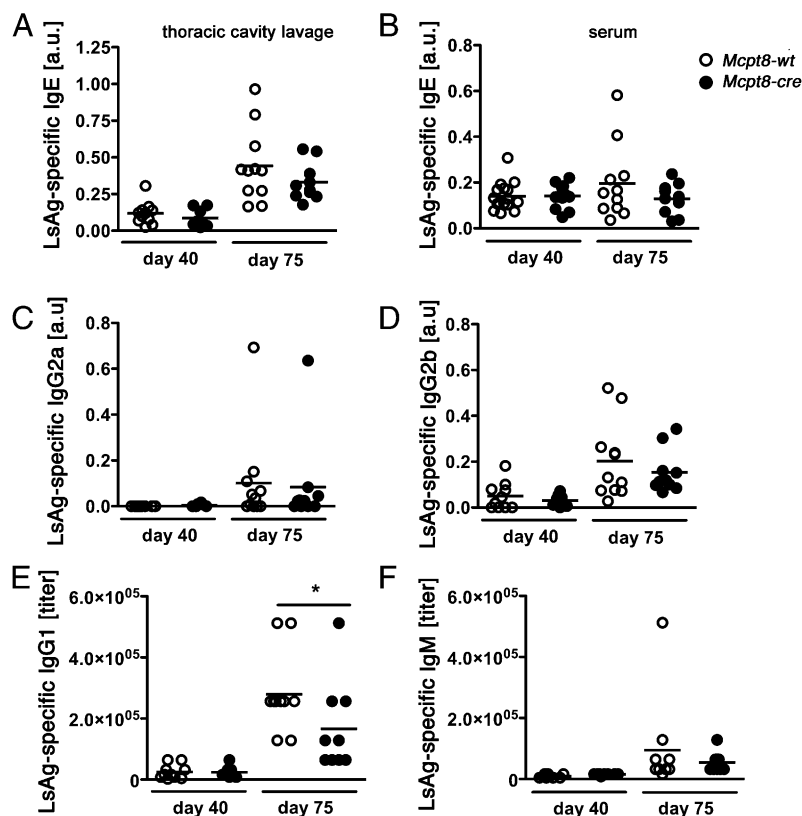


FIGURE 4. Absence of basophils resulted in a decreased *L. sigmodontis*-specific IgG1 response.

Basophil-deficient *Mcpt8-cre* mice and their basophil-competent littermates (*Mcpt8-wt*) were infected with *L. sigmodontis* for 40 and 75 d. LsAg-specific IgE was determined by ELISA in the thoracic cavity lavage (A) and serum (B). LsAg-specific IgG2a (C), IgG2b (D), IgG1 (E), and IgM (F) were measured in the sera of infected mice. Data show the mean and are combined from two independent experiments. Each dot represents the Ig response from a single mouse ($n = 8$ –16 mice). Data were analyzed by unpaired Student *t* test or Mann–Whitney *U* test. * $p \leq 0.05$.

We show that basophils were recruited to the site of infection and that *L. sigmodontis*-specific IgE, an isotype potentially required to activate basophils, increased as well in the thoracic cavity of infected BALB/c mice. Nonetheless, the absence of basophils changed neither the vascular permeability during a blood meal nor the parasite burden at any time point during *L. sigmodontis* infection. Consistent with the unchanged host defense, no consistent or sustained alterations in Ab or cytokine response were observed during infection in basophil-deficient mice compared with their cell-competent littermates.

A hallmark of infections with filarial nematodes is their transmission by an arthropod vector. During a blood meal, mites transmit *L. sigmodontis* L3 to the host. It has been shown that degranulating mast cells in the skin favor migration of *L. sigmodontis* larvae to the thoracic cavity in the absence of the chemokine CCL17 in C3H/HeN mice (16). Because mast cells and basophils share functional similarities, we analyzed on the fully susceptible BALB/c background whether the deficiency of basophils has an impact of vascular permeability induced by blood-sucking mites. Our data demonstrate that blood meal-induced vascular leakage was not affected by deficiency of basophils. The migratory capacity of L3 to the thoracic cavity was also not

changed by the absence of basophils because the numbers of *L. sigmodontis* larvae immediately after arrival in the thoracic cavity (day 10 p.i.) were alike. These results show that in susceptible BALB/c mice basophils do not contribute to migration of *L. sigmodontis* larvae by increasing vascular permeability.

Moreover, our data indicate that the enhanced vascular permeability induced by blood-sucking mites was independent of the transmission of *L. sigmodontis* L3. Mock-infected mites induced dye extravasation in the tissue to the same extent as *L. sigmodontis*-infected mites. During a blood meal by an arthropod vector, saliva compounds are cotransmitted to the host to facilitate feeding (17). It was recently shown that proteins excreted from the *L. sigmodontis* vector *O. bacoti* increased the production of the vasoactive cytokine TNF- α by APCs (18). Although it is likely that increased permeability would also promote infection efficacy, our data suggest that this is not an active L3-mediated process but an intrinsic function of the vector itself that is exploited by the helminth parasite. Therefore, basophils displayed dispensable functions in mediating the increased vascular permeability induced by feeding of *O. bacoti*.

Once the larvae arrive in the thoracic cavity, they are continuously attacked by the immune system and are cleared

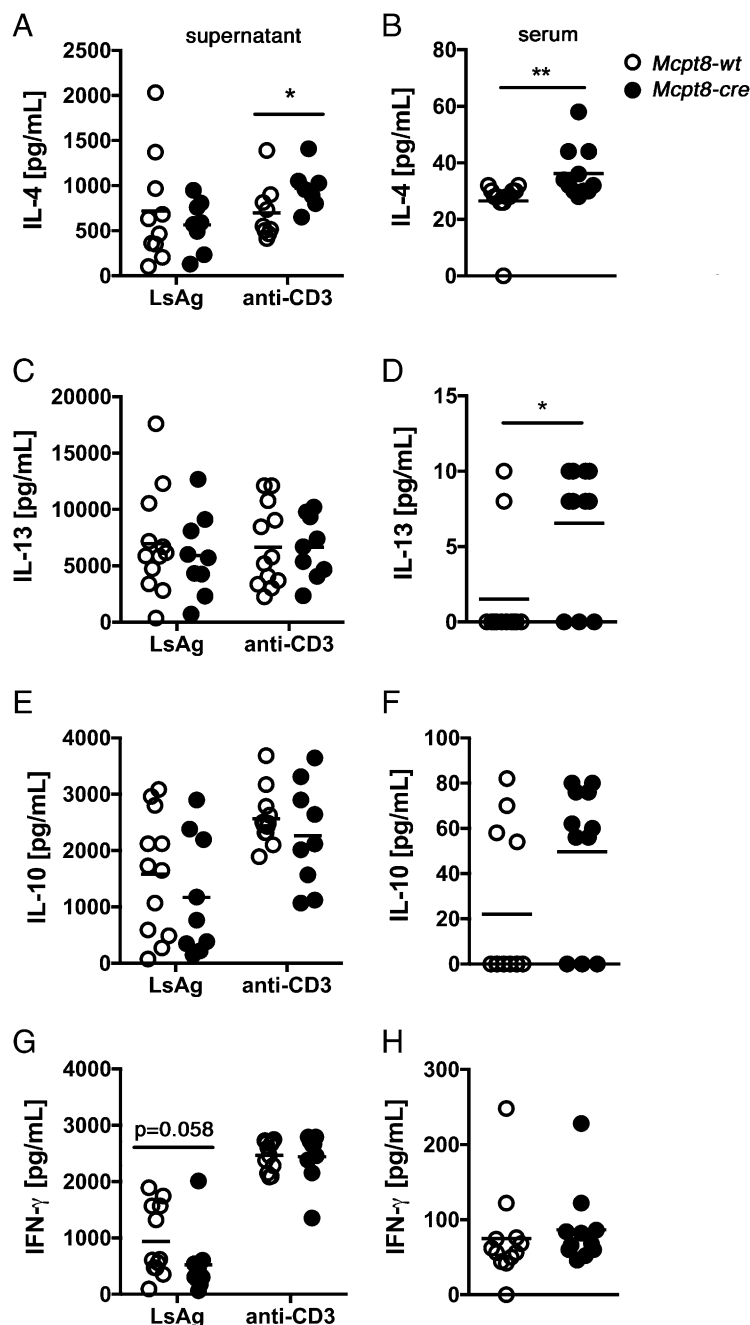


FIGURE 5. Th2 biased cellular immune response in basophil-deficient mice.

Basophil-deficient *Mcpt8-cre* mice and their basophil-competent littermates (*Mcpt8-wt*) were infected with *L. sigmodontis* for 40 d. Cytokines were measured in the supernatants from spleen cells restimulated either with LsAg or anti-CD3 by ELISA. Cytokine serum levels were determined by cytokine bead assay. The graphs show the amount of IL-4, IL-13, IL-10, and IFN- γ as indicated at the y-axis in the supernatant (A, C, E, and G) and serum (B, D, F, and H) of infected mice (spleen supernatant: $n = 8$ –12 mice; serum: $n = 11$ –12 mice). Data show the mean plus SEM and are combined from two independent experiments. Data were analyzed by unpaired Student *t* test or Mann–Whitney *U* test. * $p \leq 0.05$, ** $p \leq 0.01$.

within 200 days in the context of a mixed type 1 and 2 immune response (2). We did not observe differences in the number of larvae, adult worms, or MF in basophil-deficient mice compared with their cell-competent littermates during the entire course of infection. Therefore, our data clearly indicate that basophils

alone do not execute central functions during infection with *L. sigmodontis*.

In line with our findings, depletion or inhibition of basophils using an anti-CD200R Ab did not change the parasite burden at day 56 during *L. sigmodontis* infection (11). However, anti-CD200R

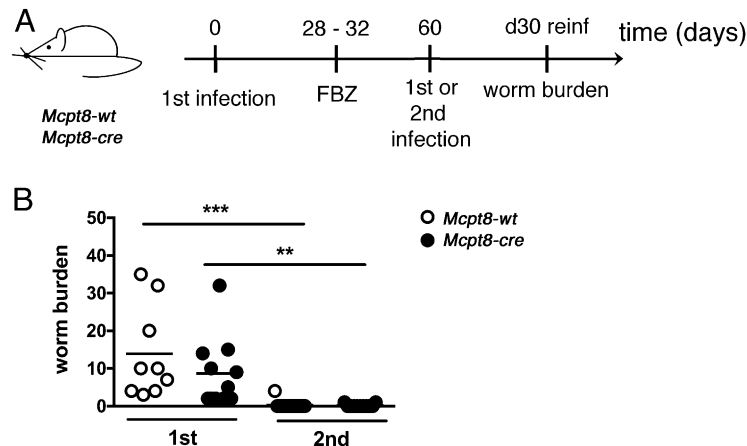


FIGURE 6. Basophil-deficient mice are protected in a reinfection.

Experimental setup (A). Basophil-deficient *Mcpt8-cre* mice and their basophil-competent littermates (*Mcpt8-wt*) were infected with *L. sigmodontis*. From day 28 p.i. on, mice were treated with FBZ for five consecutive days. Thirty days after anthelmintic treatment, mice were reinfected (second infection). Age- and gender-matched control mice were infected side by side for the first time (first infection). (B) Number of worms in the thoracic cavity 30 d after first or second infection. Each dot represents the worm burden from a single mouse ($n = 9\text{--}13$ mice). Data show the mean and are combined from two independent experiments. Data were analyzed using Kruskal–Wallis test with Dunn multiple comparison test. $**p \leq 0.01$, $***p \leq 0.001$.

treatment resulted in an impaired type 2 immune response 8 wk p.i. (i.e., diminished polyclonal and *L. sigmodontis*-specific IgE, less IL-4 production by T cells, and lower numbers of circulating eosinophils) (11). By contrast, in the current study, the evaluation of Ab and cellular cytokine responses revealed only minor changes, slightly decreased *L. sigmodontis*-specific IgG1, whereas the cellular type 2 immune response was increased, in mice lacking basophils. These differences may reflect the different impact of a transient Ab-mediated depletion/inhibition used in the study by Torrero et al. (11) versus constitutive ablation of basophils in the *Mcpt8-cre* mice used in our study. Furthermore, it is important to note that CD200R functions as an activating receptor that is expressed by basophils and mast cells (19). Cross-linking of anti-CD200R with the Ab clone Ba103 used by Torrero et al. efficiently activates mast cells and basophils and induces their degranulation (20, 21). Thus, in vivo treatment of mice with Ba103 may have several effects on basophils and mast cells besides “clean” depletion.

In general, the role of basophils during primary infection with helminths appears to be limited (6). Despite previous studies using depleting Abs that suggested a role of basophils during Th2 T cell priming, Th2 T cell expansion was not affected in *Mcpt8-cre* mice during infection with *N. brasiliensis*, *H. polygyrus*, *Schistosoma mansoni* (6), or *Strongyloides ratti* (M. Reitz, M.L. Brunn, D. Voehringer, and M. Breloer, unpublished observations). By contrast, the importance of basophils in the protection against a second infection with gastrointestinal helminths depends on the helminth species. Basophils promoted the trapping of larvae in the skin during a secondary infection with *N. brasiliensis* (8), whereas they were dispensable for the control of migrating *S. ratti* larvae (Reitz et al. unpublished observations). Within the intestine, basophils contributed to protection in a secondary infection with *H. polygyrus* and *N. brasiliensis* (10) but were redundant effector

cells during infection with *S. venezuelensis* (22). During infection with *L. sigmodontis*, basophils were reported to participate in the protective immunity induced by vaccination with irradiated larvae, which results in a 70% reduction of the worm burden (12). We evaluated the establishment of immunity induced by vector-transmitted L3 to mimic the natural situation. After anthelmintic treatment, mice were reinfected, which resulted in a more than 90% reduction of worm numbers, irrespective of the presence of basophils. Again, the different outcome of our study may be explained by the different depletion strategies (i.e., treatment with an Ab or constitutive depletion in basophil-deficient mice). It is also conceivable that vaccination with irradiated larvae and protection induced by natural infection followed by treatment with anthelmintic drugs rely on different effector mechanisms.

In summary, combined data from others and us provide accumulating evidence that basophils increase at the site of most helminth infections. However, despite their role in the initiation and execution of antihelminth immune responses during a first infection with some helminth species (7, 22), basophils are dispensable during infection with the tissue-dwelling nematode *L. sigmodontis*. Therefore, our study highlights that nematode-specific differences exist, and it is not valid to draw general conclusions about the function of basophils during helminth infections from single studies.

DISCLOSURES

The authors have no financial conflicts of interest.

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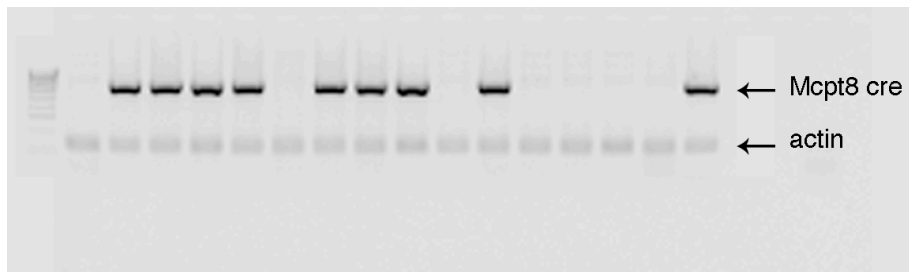
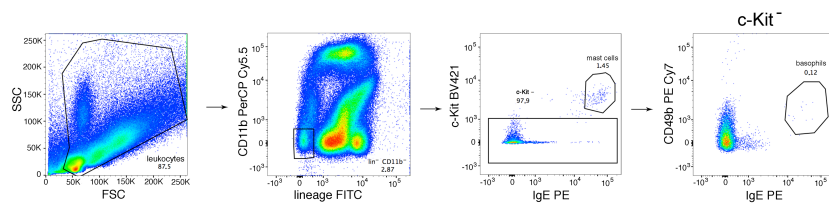


Figure S1: Screening of basophil-deficient mice

To verify the genotype of the mice, DNA was extracted from earhole biopsies. For the screening of the *Mcpt8-cre/wt* mice the following primer were used: *Mcpt8Cre-Fwd*: CTG CAA ACC CTA TGC TAC TC, *Rev*: AGG ACT CAC AGG GAT CAT AG. *Actin* was used as internal control for the *Mcpt8* screening: *AGA GGG AAA TCG TGC GTG AC*, *Rev*: CAA TAG TGA TGA CCT GGC GGT. Cycling conditions were as followed: 15 min polymerase activation at 95°C, 40 cycles at 95°C for 30 sec, 57°C for 30 sec and 72°C for 40 sec.

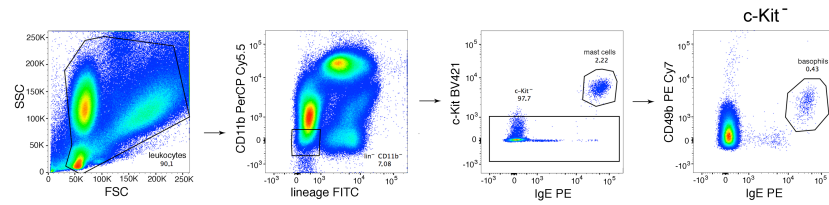
A

noninfected



B

day 30 p.i.

**Figure S2: Gating Strategy**

Representative dot blots showing the gating strategy. Thoracic cavity cells were isolated from noninfected mice (A) and day 30 *L. sigmodontis* infected mice (B). Basophils were defined as lineage⁺CD11b⁻ leukocytes that are c-Kit⁻ and IgE⁺CD49b⁺.

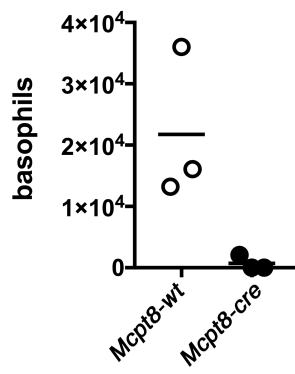


Figure S3: Recruitment of basophils during infection in *Mcpt8*-wt/cre mice

Basophil-deficient *Mcpt8-cre* as well as their cell-competent littermates (*Mcpt8-wt*) were infected with *L. sigmodontis* for 30 days.

Number of basophils was analyzed by flow cytometry. Each dot represents a value from a single mouse.

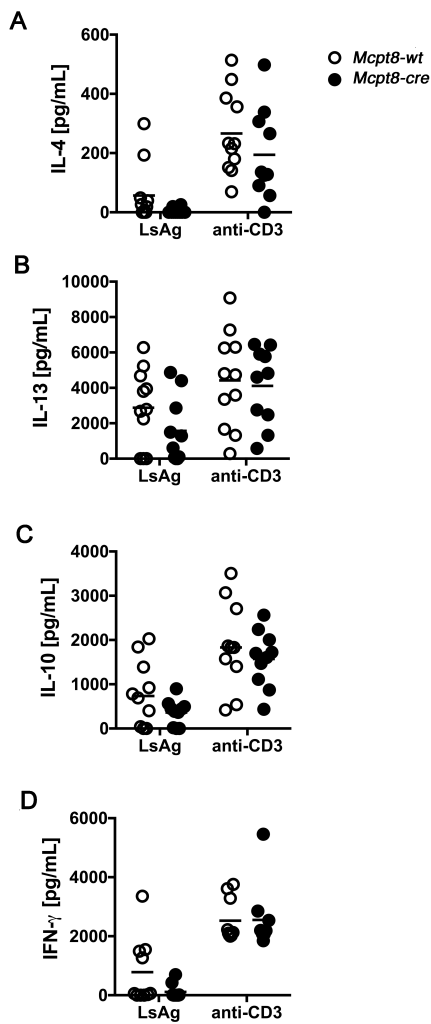


Figure S4: Cytokine response in the presence and absence of basophils

Basophil-deficient *Mcpt8-cre* and their -competent littermates (*Mcpt8-wt*) were infected with *L. sigmodontis* for 75 days. The amount of IL-4 (A), IL-13 (B), IL-10 (C), and IFN- γ (F) was measured in the supernatants from spleen cells either restimulated with LsAg or anti-CD3 by ELISA. Data show the mean and are combined from 2 independent experiments. Each dot represents a value from a single mouse