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Chronic Helminth Infection Reduces Basophil Responsiveness in an IL-10–Dependent Manner

David Larson,* Marc P. Hübner,*[†] Marina N. Torrero,* Christopher P. Morris,* Amy Brankin,* Brett E. Swierczewski,* Stephen J. Davies,* Becky M. Vonakis,[‡] and Edward Mitre*

Basophils play a key role in the development and effector phases of type 2 immune responses in both allergic diseases and helminth infections. This study shows that basophils become less responsive to IgE-mediated stimulation when mice are chronically infected with *Litomosoides sigmodontis*, a filarial nematode, and *Schistosoma mansoni*, a blood fluke. Although excretory/secretory products from microfilariae of *L. sigmodontis* suppressed basophils in vitro, transfer of microfilariae into mice did not result in basophil suppression. Rather, reduced basophil responsiveness, which required the presence of live helminths, was found to be dependent on host IL-10 and was accompanied by decreases in key IgE signaling molecules known to be downregulated by IL-10. Given the importance of basophils in the development of type 2 immune responses, these findings help explain the mechanism by which helminths protect against allergy and may have broad implications for understanding how helminth infections alter other disease states in people. *The Journal of Immunology*, 2012, 188: 000–000.

Like allergic diseases, helminths induce type 2 immune responses characterized by eosinophilia, elevated IgE levels, and CD4⁺ T cell production of IL-4, IL-5, and IL-13. In people with infrequent exposures to helminths, this immune response is often associated with allergic symptoms such as rash and pruritus during acute infection (1). Over time, though, allergic symptoms in patients chronically infected with helminths appear to decrease (1). The filarial nematode *Loa loa*, for example, causes less angioedema, hives, and itching in chronically infected individuals living in endemic regions than in travelers with infections of shorter duration (2, 3). In addition to decreased allergic responses to the infection itself, chronic helminth infections are associated with decreased allergic responses to environmental allergens (4–9).

Although several studies have investigated the mechanisms by which chronic helminth infection may suppress allergic reactivity, to date no studies have evaluated the effect chronic helminth infection has on basophil responsiveness. Like mast cells, basophils function as acute effector cells in allergic disease, releasing preformed histamine and other inflammatory mediators within minutes of allergen exposure, primarily through cross-linking of allergen-specific IgE molecules bound to high-affinity IgE receptors on the cell surface.

In addition to being effector cells of allergic disease, basophils also help development of type 2 immune responses (10–15). IL-4 is a principal driver of type 2 immunity, and studies have shown basophils to be major contributors of IL-4 in helminth and allergic diseases (16–20). Studies conducted in mice depleted of or deficient in basophils have found decreased type 2 immune responses in both allergy and helminth models (19, 21–26).

As chronic helminth infections are associated with decreases in allergic manifestations, and as basophils play important roles in the development and effector stages of allergy, in this study we tested the hypothesis that chronic helminth infection suppresses basophil responsiveness to IgE-mediated stimulation.

Materials and Methods

Animals and parasites

Female BALB/c mice (National Cancer Institute Mouse Repository, Frederick, MD), BALB/c IL-10-deficient mice (The Jackson Laboratory, Bar Harbor, ME), and BALB/c J_H^{−/−} mice (Taconic, Hudson, NY) were housed at the Uniformed Services University Center for Laboratory Animal Medicine. Blood used for basophil activation studies was obtained by retroorbital bleed. At study endpoints, mice were euthanized with carbon dioxide. All experiments were performed under protocols approved by the Uniformed Services University Institutional Animal Care and Use Committee.

For *Litomosoides sigmodontis* infections, L3-stage larvae were obtained by pleural lavage of jirds (*Meriones unguiculatus*, obtained from TRS Laboratory, Athens, GA) that had been infected 4 d earlier by the bite of infectious mites, as previously described (27). Forty L3-stage larvae were collected in RPMI 1640 and injected s.c. in the dorsal neck region into female BALB/c mice that were 5–12 wk of age.

For *Schistosoma mansoni* infections, 6- to 10-wk-old female BALB/c mice were infected by exposing the tails to 30–40 *S. mansoni* cercariae (Puerto Rican strain) obtained from infected *Biomphalaria glabrata* snails provided by F. Lewis (Biomedical Research Institute, Rockville, MD).

Parasite Ags

L. sigmodontis worm Ag (LsAg) and *S. mansoni* worm Ag (SWAP) were prepared from the PBS-soluble fraction of homogenized male and female adult worms harvested from infected animals, as previously described (26, 28).

For worm excretory/secretory (E/S) products, adult male worms, adult female worms, and microfilariae were cultured separately in RPMI 1640

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Abbreviations used in this article: E/S, excretory/secretory; LsAg, *L. sigmodontis* worm Ag; MFI, mean fluorescence intensity; PKC, protein kinase C; SWAP, *S. mansoni* worm Ag; WT, wild-type.

supplemented with HEPES, penicillin/streptomycin, and glucose. Supernatants from cultures were collected every 24 h while worms were still living. E/S products from each day were pooled and concentrated using 3000-kDa MWCO Amicon Ultra centrifugal filter units (Millipore), and protein concentration was measured using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL). Concentrated E/S products were stored at -20°C until use.

LsAg sensitization, L. sigmodontis implantation, and microfilariae injection

Mice 10 wk of age were sensitized to *LsAg* by three i.p. injections of 100 μg *LsAg* adsorbed to Inject Alum Adjuvant (Thermo Scientific). Injections were spaced 2 wk apart. Three weeks after the last injection, adult worms for implantation and microfilariae were obtained from jirds that had been infected with *L. sigmodontis* for >8 wk. After euthanizing infected jirds, adult worms were removed from the pleural cavity using dissecting probes and placed in RPMI 1640 to determine sex. Five to seven male worms or five to seven microfilaria-producing female worms were surgically implanted into the peritoneal cavity of anesthetized mice, as previously described (29). Microfilariae were purified from the blood by diluting peripheral blood 1:2 with RPMI 1640 containing 25 mM HEPES and 2.05 mM glutamine (Mediatech). Diluted blood was layered on top of a 25% and 30% Percoll/sucrose isosmotic solution and centrifuged for 35 min at $400 \times g$. Microfilariae were washed with RPMI 1640 to remove any remaining Percoll. Fifty thousand microfilariae were then injected into the jugular vein of *LsAg*-sensitized mice. After 2 wk, blood was obtained from mice, plasma was removed, and basophil activation was measured using flow cytometry.

Abs

The following Abs were used to assess basophil activation by flow cytometry: anti-CD45b/B220 PerCP (RA3-6B2), anti-CD4 PerCP (RM4-5), anti-IgE FITC (R35-72), anti-IL-4 allophycocyanin (11B11), anti-CD45 FITC (30-F11), anti-CD49b PE (HM ALPHA2), and anti-CD200R PE (OX-110). Anti-IgE (R35-72) and anti-Fc ϵ R1 α (MAR-1) were used for stimulation. Anti-DNP IgE (SPE-7) used to boost basophil surface IgE levels was purchased from Sigma-Aldrich. Anti-Fc ϵ R1 α (MAR-1) was purchased from eBioscience, anti-CD200R PE (OX-110) was purchased from AbD SeroTec, and all other Abs were purchased from BD Biosciences.

The following Abs were used to measure expression levels of basophil signaling molecules by flow cytometry: anti-Akt AF647 (5G3), anti-protein kinase C (PKC)- α , anti-phospho-Akt PE (D9E), anti-phospho-STAT5 AF647 (C71E5), and anti-rabbit F(ab') AF647, all purchased from Cell Signaling Technology. Anti-FYN PE, anti-Syk PE, anti-STAT5, and Fc γ RIIb/CD16-2 (2.4G2) blocking Ab were purchased from Santa Cruz Biotechnology.

The following Abs were used for mouse IgE ELISAs: anti-mouse IgE (R35-72) for coating, mouse IgE (27-74) used as the standard, and biotinylated anti-mouse IgE (R35-118) for detection (all purchased from BD Biosciences).

Measuring basophil activation by flow cytometry

Basophil activation was determined, as previously described (26, 30), with minor modifications. Briefly, after collecting whole blood in heparinized tubes, blood was centrifuged and plasma was removed. Cells were washed twice with RPMI 1640 to ensure complete removal of plasma. After resuspending blood cells back to original volume using RPMI 1640, cells were diluted 1:2 with RPMI 1640 and aliquoted 200 μl per condition. Diluted blood cells were stimulated with media alone, 1 $\mu\text{g}/\text{ml}$ ionomycin (Calbiochem), or several concentrations of either anti-IgE (0.0005, 0.002, 0.0078, 0.031, 0.125, and 0.5 $\mu\text{g}/\text{ml}$) or anti-Fc ϵ R1 α (0.4, 1.6, 6.25, 25, and 100 $\mu\text{g}/\text{ml}$) for 45 min before adding GolgiStop (containing monensin; BD Biosciences). Stimulation proceeded for additional 2 h. At the end of the stimulation period, cells were washed with PBS, RBCs were lysed, and remaining cells were fixed using a whole blood lysing reagent kit (Beckman Coulter). Cells were resuspended in 1% BSA (Cohn V fraction; Sigma-Aldrich)/PBS at 4°C overnight, and then surface stained with anti-IgE FITC, anti-CD4 PerCP, anti-B220 PerCP, and, when CD200R was also evaluated, anti-CD200R PE. Cells were subsequently washed, permeabilized with Perm/Wash buffer (BD Biosciences), and then stained with anti-IL-4 allophycocyanin. Cells were analyzed using a BD LSRII flow cytometer and FACSDiva software (BD Biosciences). In Fig. 1B, CD200R was measured without simultaneously measuring intracellular IL-4. For these assays, no GolgiStop was added and total stimulation time was reduced to 2 h. Cutoff gates for CD200R-PE and IL-4 allophycocyanin positivity were established using the fluorescence-minus-one technique.

Measuring signaling molecules by flow cytometry

Blood was obtained from mice chronically infected with *L. sigmodontis* and age-matched uninfected controls, RBCs were lysed, and leukocytes were immediately fixed using a whole blood lysing kit (Beckman Coulter). After washing twice with PBS, cells were resuspended with 1% BSA/PBS with Fc γ RIIb/CD16-2 (2.4G2) blocking Ab and incubated overnight. Surface staining was performed by adding anti-IgE FITC (R35-72), anti-CD45b/B220 PerCP (RA3-6B2), and anti-CD4 PerCP (RM4-5), and incubating for 30 min at 4°C . After washing cells twice with PBS, they were permeabilized with Perm/Wash buffer (BD Biosciences). Abs to signaling molecules were added to permeabilized cells and incubated for 30 min at 4°C . Cells were washed twice with PBS before using an anti-rabbit secondary Ab for the detection of anti-stat5 and anti-PKC- α Abs. After staining, cells were washed twice with PBS, and fluorescence was analyzed using a LSRII flow cytometer and FACSDiva 6.1.3 software (BD Biosciences). Titrations were performed to determine optimum concentrations of each Ab, and BD CompBead compensation beads (BD Biosciences) were used for all colors to account for spectral overlap. Nonspecific binding of anti-rabbit secondary Ab was subtracted from mean fluorescence intensities (MFIs) obtained for stat5 and PKC- α expression. Infected and control samples were assayed at the same time to eliminate interday variability.

Worm-specific and total IgE ELISAs

ELISAs were performed using Costar half-area, high-binding plates. IgG was removed from plasma using GammaBind Plus Sepharose (GE Healthcare). For worm-specific IgE ELISAs, plates were coated with 20 $\mu\text{g}/\text{ml}$ *LsAg* or SWAP diluted in PBS and incubated overnight at 4°C . Plates were blocked with 5% BSA/PBS plus 0.05% Tween 20. Plasma was added to the plate in duplicate at a starting dilution of 1:4, followed by further 3-fold dilutions made in 1% BSA/PBS plus 0.05% Tween 20. Bound IgE Ab was detected by adding biotinylated anti-mouse IgE diluted in 1% BSA/PBS plus 0.05% Tween, followed by adding alkaline phosphatase-conjugated streptavidin that was diluted 1:1000 in 1% BSA/PBS plus 0.05% Tween. Plates were developed by the addition of 4-nitrophenyl phosphate disodium (Sigma-Aldrich) in 0.1 M carbonate buffer to the plate. Absorbances were read at 405 nm using a Victor³ V microplate reader from PerkinElmer. Titters were calculated as the dilution that would yield an OD of 0.5 calculated by SoftMax Pro 4.6 (Molecular Devices). For total IgE measurements, plates were coated with 10 $\mu\text{g}/\text{ml}$ anti-IgE in PBS instead of worm Ag. All subsequent steps were identical to the worm-specific IgE ELISA. Total IgE concentration was determined from the IgE standard curve using WorkOut 2.0 ELISA software (PerkinElmer).

Passive sensitization with DNP-specific IgE

Mice were injected simultaneously with 30 μg anti-DNP IgE i.v. and with 50 μg i.p. These injections were repeated 2 wk later. Blood was obtained 5 d after last injection, plasma was removed, and basophil activation assays by flow cytometry were performed.

Splenocyte and whole blood cultures

For splenocyte cultures, after obtaining single-cell suspensions of splenocytes from mice chronically infected with *L. sigmodontis* or age-matched uninfected controls, RBCs were lysed with ACK lysis buffer (Quality Biological), and cells were plated in IMDM (Mediatech) containing 10% FCS (Valley Biomedical), 1% L-glutamine (Mediatech), 1% insulin-transferrin-selenium medium (Invitrogen), and 80 $\mu\text{g}/\text{ml}$ gentamicin (Quality Biological) at a concentration of $2 \times 10^6/\text{ml}$, and were incubated with either media, 0.05 $\mu\text{g}/\text{ml}$ PMA (Sigma-Aldrich), 1 $\mu\text{g}/\text{ml}$ ionomycin (Calbiochem), 5 $\mu\text{g}/\text{ml}$ anti-CD3, 2 $\mu\text{g}/\text{ml}$ anti-CD28 (both from eBioscience), or 20 $\mu\text{g}/\text{ml}$ *LsAg* and cultured at 37°C , 5% CO_2 . After 3 d, culture supernatants were collected and stored at -80°C until IL-10 ELISAs were performed.

For whole blood cultures, after removing plasma, blood cells were thoroughly washed twice with RPMI 1640, and aliquoted 500 μl per condition into 48-well cell culture plates (Corning). Blood was stimulated with media and several concentrations of anti-IgE (0.0001, 0.0005, 0.002, 0.0078, 0.031, and 0.125 $\mu\text{g}/\text{ml}$) for 2 h at 37°C , 5% CO_2 . Samples were centrifuged at $400 \times g$ for 5 min, and supernatants were collected and stored at -80°C until IL-4 ELISAs were performed.

For whole blood cultures with worm E/S products, plasma from mouse whole blood was removed and whole blood cells were incubated with 30 $\mu\text{g}/\text{ml}$ E/S products or BSA diluted in RPMI 1640 for 7 h. Blood containing E/S products was then diluted 1:2 from the original blood volume and aliquoted 200 μl per condition. Basophil activation was performed using flow cytometry, as described earlier.

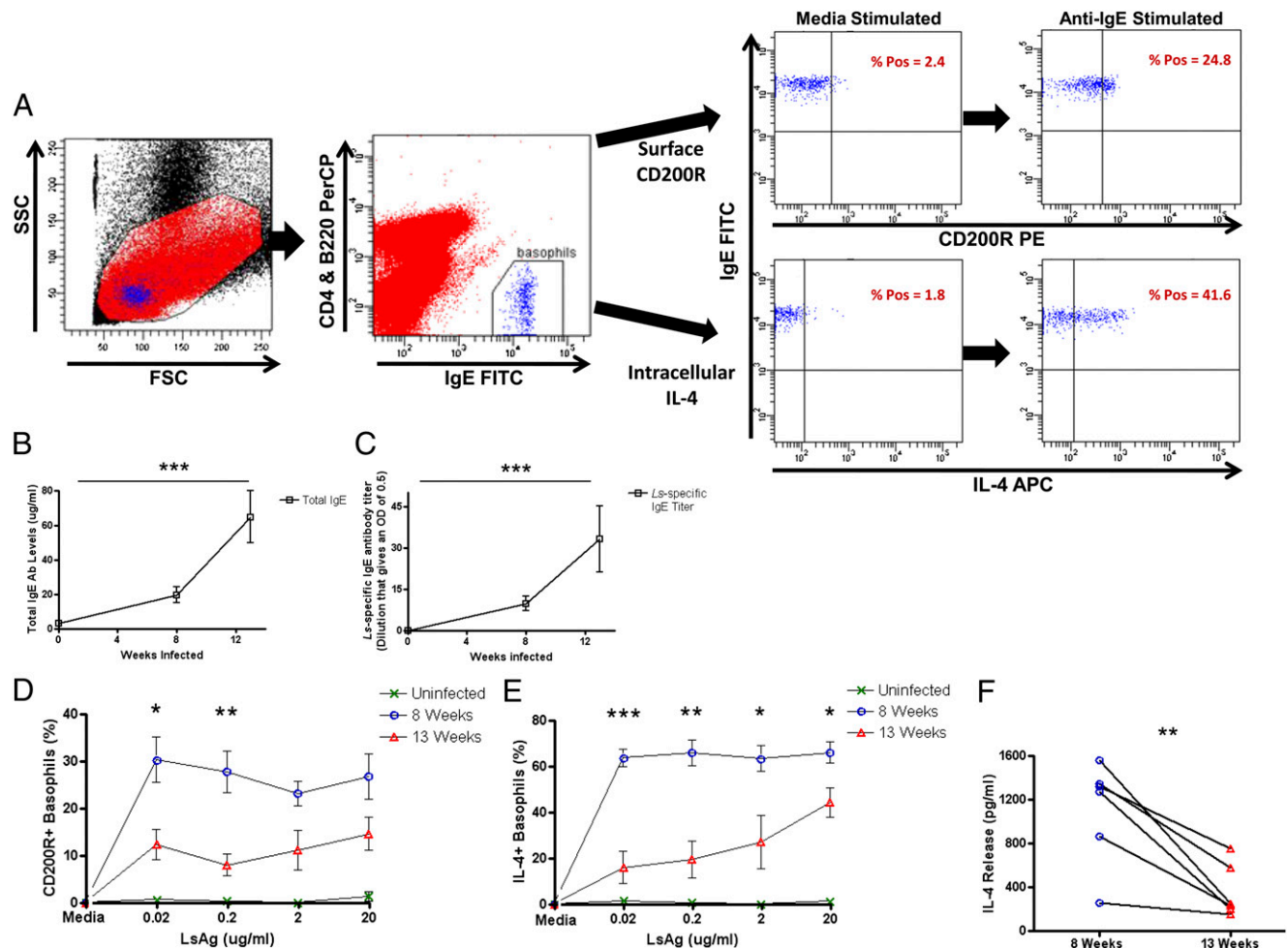


FIGURE 1. Responsiveness of basophils from *L. sigmodontis*-infected mice to *LsAg* stimulation. **(A)** Gating strategy for flow cytometric assessment of basophil activation. Initial gating by forward and side scatter (*left panel*). Basophils identified as CD4⁺B220⁺IgE⁺ peripheral blood cells (*second panel*). *Right panels*, Demonstrate CD200R and IL-4 staining after incubation of whole blood with media and 0.031 μ g/ml anti-IgE. Levels of total IgE **(B)** and *LsAg*-specific IgE **(C)** were measured in the plasma of uninfected ($n = 6$), 8-wk ($n = 6$), and 13-wk ($n = 6$) *L. sigmodontis*-infected BALB/c mice. Percentages of basophils that stain positively for CD200R **(D)** and IL-4 **(E)** after stimulation with several concentrations of *LsAg* as measured by flow cytometry and after subtracting background levels. Each data point represents the mean of at least three independent experiments (statistically significant differences are shown between 8-wk- and 13-wk-infected mice). **(F)** Supernatant IL-4 concentrations from whole blood after stimulation with 2 μ g/ml *LsAg* for 2 h (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Mouse IL-10 and IL-4 ELISAs

IL-10 and IL-4 ELISAs were performed using Costar half-area, high-binding plates. Mouse IL-10 BD OptEIA ELISA kits and OptEIA ELISA reagents were purchased from BD Biosciences. Mouse IL-4 ELISAs were purchased from eBioscience. ELISAs were performed using manufacturer's reagents following the protocols provided with each kit.

In vivo treatment with rIL-10

Wild-type (WT) mice were i.p. injected twice daily with 2 μ g rIL-10 (GenScript) in 100 μ l PBS or PBS alone for 5 d, as previously described (31). Whole blood was obtained from injected mice and stimulated with anti-IgE, and basophil activation was assessed using flow cytometry, as described earlier.

In vivo IL-10 cytokine capture

Using the mouse IL-10 in vivo capture assay set (BD Biosciences), WT mice were injected with 10 μ g/ml biotin anti-IL-10 0, 2, 13, or 22 wk postinfection with *L. sigmodontis*. Twenty hours after injecting biotin anti-IL-10 Ab, plasma was collected and stored at -80°C until all timepoints were collected. IL-10 ELISA was performed according to manufacturer's instructions.

Statistical analyses

All statistics were performed using GraphPad Prism 4.03. Statistical significance between multiple groups was determined using the Kruskal–Wallis

test, followed by Dunn's multiple comparison posttest. Either paired or unpaired two-tailed Student *t* test was used for comparisons between two groups. For basophil activation curves when more than two groups were compared, one-way ANOVA was performed for each stimulation concentration, followed by Bonferroni correction. For basophil activation curves when only two groups were compared, two-tailed Student *t* test was performed for each stimulation concentration. For all tests, p values < 0.05 were considered significant.

Results

Basophils from mice chronically infected with *L. sigmodontis* are less responsive to *LsAg* than basophils from acutely infected mice

To determine whether basophils become less responsive to worm Ag over the course of a filarial infection, BALB/c mice were infected with 40 L3-stage *L. sigmodontis* larvae. After obtaining whole blood from uninfected, acutely infected, and chronically infected mice, plasma was removed from blood cells by multiple washes. Blood cells were then stimulated with several concentrations of *LsAg* to develop dose-response basophil activation curves to *LsAg*. Basophil activation was determined by measuring surface CD200R expression and intracellular IL-4 production by

flow cytometry (Fig. 1A). Consistent with prior work we have done (26), total and *LsAg*-specific IgE levels are low and undetectable, respectively, in uninfected animals (Fig. 1B, 1C). *LsAg*-specific IgE levels are measurable by 8 wk of infection, and levels of both total and *LsAg*-specific IgE increase throughout the course of infection.

One of the principal mechanisms of basophil activation is by aggregation of surface high-affinity IgE receptors (FcεRI) in response to cross-linking of receptor-bound IgE Abs by specific Ag. As expected, basophils from uninfected animals, which lack specific Abs against *LsAg*, do not become activated when stimulated with *LsAg* (Fig. 1D, 1E). The highest percentage of basophils activated in response to *LsAg* was observed in acutely infected mice, in which a maximal percentage of basophils became activated even at the lowest *LsAg* concentration tested (Fig. 1D, 1E). In contrast, basophils from chronically infected mice never achieved the same frequencies of activation, even at the highest *LsAg* concentration tested. Results were consistent when measuring basophil activation by either surface CD200R or intracellular IL-4 expression (Fig. 1D, 1E). Data shown for both CD200R and IL-4 demonstrate activation above basal expression levels because CD200R staining exhibited some variability when conducted on different days. For all experiments done with WT mice in this study, basal IL-4 expression in basophils demonstrated very little interday variability, was consistently low, and was not different between infected and uninfected groups.

Similar results were obtained when the data were analyzed by evaluating changes in CD200R and IL-4 expression on a per cell basis by MFI (Supplemental Fig. 1A, 1B). These results suggest that not only do greater percentages of basophils become activated in response to *LsAg* during the acute stage of infection, but that the amplitude of activation of individual basophils is greater at 8 wk than at 13 wk. Interestingly, the decrease in basophil responsiveness to *LsAg* at 13 wk occurred despite the fact that more *LsAg*-specific IgE is present at that timepoint (Fig. 1C).

To confirm that the changes observed by flow cytometry reflected actual changes in basophil functionality, we assessed IL-4 release from basophils by measuring IL-4 in supernatants of whole blood cells after 2-h in vitro stimulation with *LsAg*. As seen in Fig. 1F, there is a significant decrease in IL-4 release from whole blood of chronically infected mice after *LsAg* stimulation as compared with acutely infected mice ($p = 0.008$; Fig. 1F). Because basophils are the only circulating cells capable of releasing IL-4 within 2 h of activation (26, 32), and as the percentages of blood cells that were basophils were equivalent at 8 and 13 wk postinfection (1.1 versus 0.9%, $p = 0.133$, data not shown), these results confirm that basophil responsiveness to worm Ag is suppressed in chronic *L. sigmodontis* infection.

Basophils from chronically infected mice are less responsive to anti-IgE stimulation than basophils from acutely infected mice and uninfected mice

To determine whether reduced basophil responsiveness was an Ag-specific phenomenon or if overall basophil responsiveness becomes downmodulated by chronic filaria infection, basophil activation curves in response to increasing IgE cross-linking were assessed in uninfected, acutely infected, and chronically infected mice.

Basophils from chronically infected mice were less responsive to anti-IgE stimulation as measured by both surface CD200R (Fig. 2A) and intracellular IL-4 (Fig. 2B) expression. Significantly lower percentages of basophils from chronically infected mice expressed CD200R than uninfected mice in response to stimulation with 0.002, 0.0078, and 0.031 $\mu\text{g/ml}$ anti-IgE (mean percentage of CD200R⁺ basophils from uninfected mice = 5.28, 12.78, 18.28 versus 0.93, 2.33, 4.08 from chronically infected mice, $p < 0.05$ for all concentrations; Fig. 2A). Percentages of basophils expressing intracellular IL-4 were also significantly lower in chronically infected mice than uninfected mice when stimulating with 0.002 and 0.0078 $\mu\text{g/ml}$ anti-IgE (mean percentage of IL-4⁺ basophils from uninfected mice = 23.12, 39.02 versus 5.16, 18.63 from chronically infected mice, $p < 0.05$, $p < 0.01$; Fig. 2B). Basophils from 2-wk-infected mice demonstrated an intermediate basophil response phenotype, although the differences between these mice and uninfected mice were not statistically significant.

This right shift in basophil activation curves was also observed when measuring IL-4 release from whole blood stimulated with anti-IgE (Fig. 2C), confirming the flow cytometric data. Given that blood of 13-wk-infected mice had on average 1.5–2 times as many basophils as uninfected animals, the decreased IL-4 release from blood of chronically infected animals reflects a substantial decrease in basophil responsiveness.

Basophils from mice chronically infected with S. mansoni are less responsive to SWAP and anti-IgE stimulation than basophils from acutely infected and uninfected mice

To determine whether basophil suppression is filaria specific or a phenomenon that occurs with other helminthiases, we evaluated basophil responses in mice infected with *S. mansoni*, a blood fluke that establishes chronic infection in BALB/c mice. Because a prior study with *S. mansoni* infection in mice demonstrated that it takes 16 wk for Th2 CD4⁺ T cells to develop substantial hyporesponsiveness (33), mice were infected for 16 wk before performing basophil activation assays.

The effect of *S. mansoni* infection on basophil functionality mirrored that of *L. sigmodontis*. As with *L. sigmodontis*, levels of total and parasite-specific IgE were greater in chronic infection

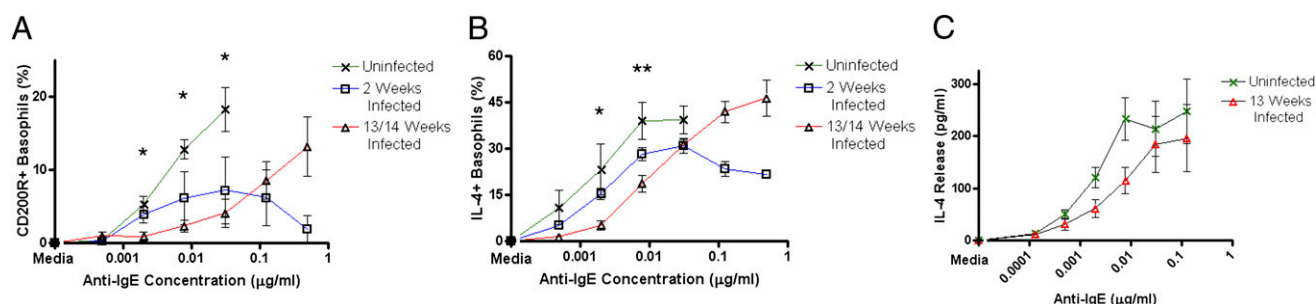


FIGURE 2. Responsiveness of basophils from *L. sigmodontis*-infected mice to anti-IgE stimulation. Percentages of basophils that stain positively for CD200R (A) and IL-4 (B) in response to increasing concentrations of anti-IgE, as assessed by flow cytometry after subtracting media stimulation levels (statistical significance between uninfected and 13/14-wk-infected groups). (C) Supernatant IL-4 concentrations from whole blood after 2-h stimulation with several concentrations of anti-IgE. Each data point represents the mean of at least four independent experiments (* $p < 0.05$, ** $p < 0.01$).

than at the 8-wk timepoint (Fig. 3A, 3B). Despite the higher specific IgE concentration at 16 wk, basophils of chronically infected mice became less activated in response to SWAP than basophils of 8-wk-infected mice ($p = 0.017$; Fig. 3C). Additionally, basophil activation curves of 16-wk-infected animals were significantly right shifted in comparison with those of uninfected controls after stimulating with anti-IgE (mean percentage of IL-4⁺ basophils after stimulation with 0.0005 and 0.002 $\mu\text{g/ml}$ anti-IgE from uninfected mice = 14.1, 44.5 versus 1.9, 13.6 from chronically infected mice, $p = 0.008$, $p = 0.0007$; Fig. 3D).

These data suggest that reduced basophil responsiveness may be a phenomenon that occurs in many chronic helminth infections.

Reduced basophil responsiveness is not due to increased levels of surface IgE

Because basophils from chronically helminth-infected mice have more IgE on their surface than basophils from uninfected ($p < 0.001$) and even acutely infected mice (Fig. 4A), we tested whether increased surface IgE levels can account for changes in basophil responsiveness to IgE cross-linking by anti-IgE Ab. To increase IgE levels on the surface of basophils, uninfected BALB/c mice were injected with an IgE mAb to DNP, and basophil responsiveness to anti-IgE stimulation was compared between IgE-injected and control mice.

Surface IgE levels were significantly increased in mice that were injected with DNP IgE ($p = 0.024$; Fig. 4B), and this IgE was functional and of sufficient quantity for basophils to become activated when stimulated with DNP-human serum albumin ($p = 0.0054$; Fig. 4C). As seen in Fig. 4D, basophils with increased surface IgE levels were as sensitive to activation with anti-IgE, if not more so, than basophils from control mice (Fig. 4D).

Additionally, basophils from chronically infected mice became significantly less activated ($p = 0.033$) than basophils from unin-

fected mice when stimulated with ionomycin (Fig. 4E), a calcium ionophore that bypasses surface IgE to cause activation of the cell.

Together, these data indicate that decreases in basophil responsiveness during chronic *Litomosoides* infection are not due to increased surface IgE levels.

Repeated basophil activation through IgE receptors is not the cause of reduced basophil responsiveness

As previous studies have demonstrated that IgE-mediated activation of basophils reduces their responsiveness to subsequent IgE cross-linking events (34–36), we hypothesized that repeated Ab-mediated activation could be responsible for the induction of basophil hyporesponsiveness during chronic helminth infection. To test this, we measured basophil responsiveness to anti-Fc ϵ RI α and ionomycin stimulation in uninfected and chronically infected $J_H^{-/-}$ mice. As $J_H^{-/-}$ mice cannot generate worm-specific IgE or IgG during helminth infection (37), their basophils cannot undergo Ab-mediated activation. Additionally, prior work we have conducted has shown that *LsAg* does not activate basophils in the absence of specific Ab (26). Because we could not use surface IgE staining as a marker for basophils in $J_H^{-/-}$ mice, basophils were identified by flow cytometry as being CD49b^{high} and CD45^{low} (38) and then assessed for IL-4 expression by intracellular flow cytometry (Fig. 5A).

In contrast to studies done with WT mice, infection of $J_H^{-/-}$ mice increased baseline basophil IL-4 expression (mean percentage of IL-4⁺ basophils after media stimulation = 19% in 13-wk-infected $J_H^{-/-}$ mice versus 13% in uninfected mice, $p = 0.01$, data not shown). Stimulation above basal levels, however, remained suppressed in chronically infected mice.

Comparing the basophil response curves with anti-Fc ϵ RI α stimulation from $J_H^{-/-}$ chronically infected and age-matched uninfected controls revealed that basophils from chronically infected $J_H^{-/-}$ mice still become less responsive to anti-Fc ϵ RI α when

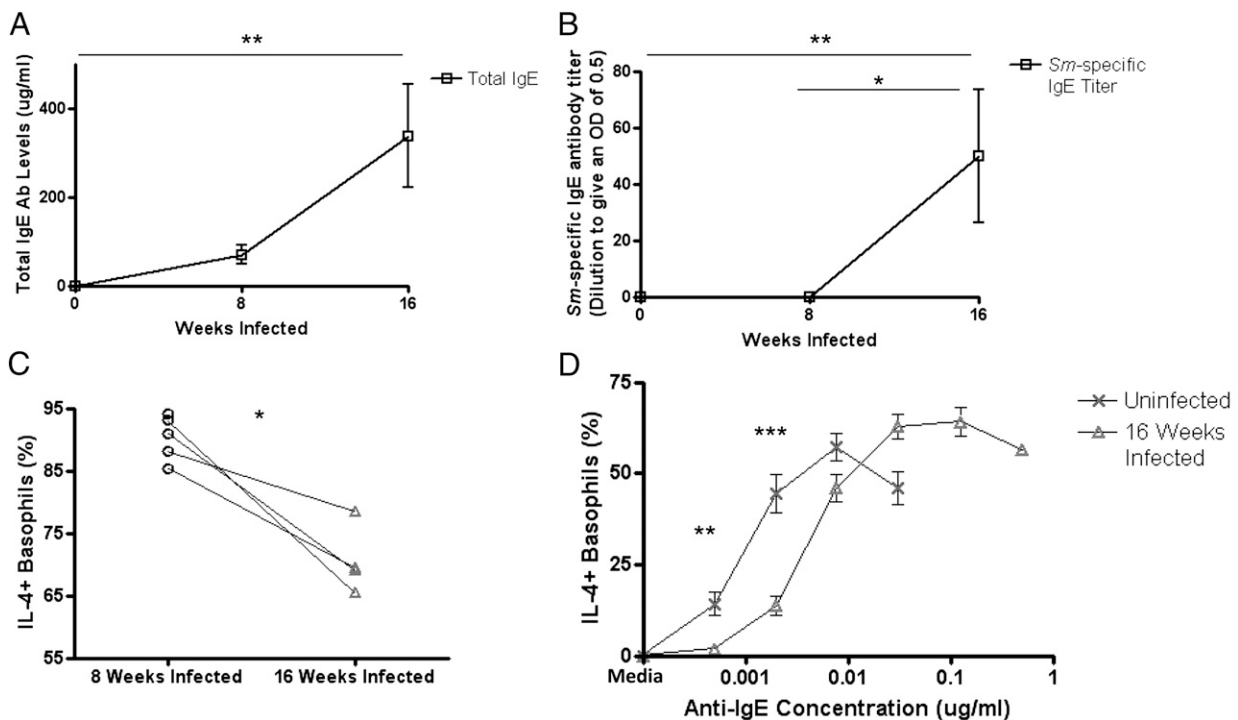


FIGURE 3. Responsiveness of basophils from *S. mansoni*-infected mice. Levels of total IgE (A) and SWAP-specific IgE (B) measured in the plasma of uninfected ($n = 5$), 8-wk-infected ($n = 5$), and 16-wk-infected ($n = 5$) BALB/c mice. Percentages of basophils that stain positively for IL-4 in response to 2 $\mu\text{g/ml}$ SWAP (C) and to several concentrations of anti-IgE (D) from uninfected ($n = 5$) and 16-wk-infected ($n = 5$) mice after subtracting media stimulation levels (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

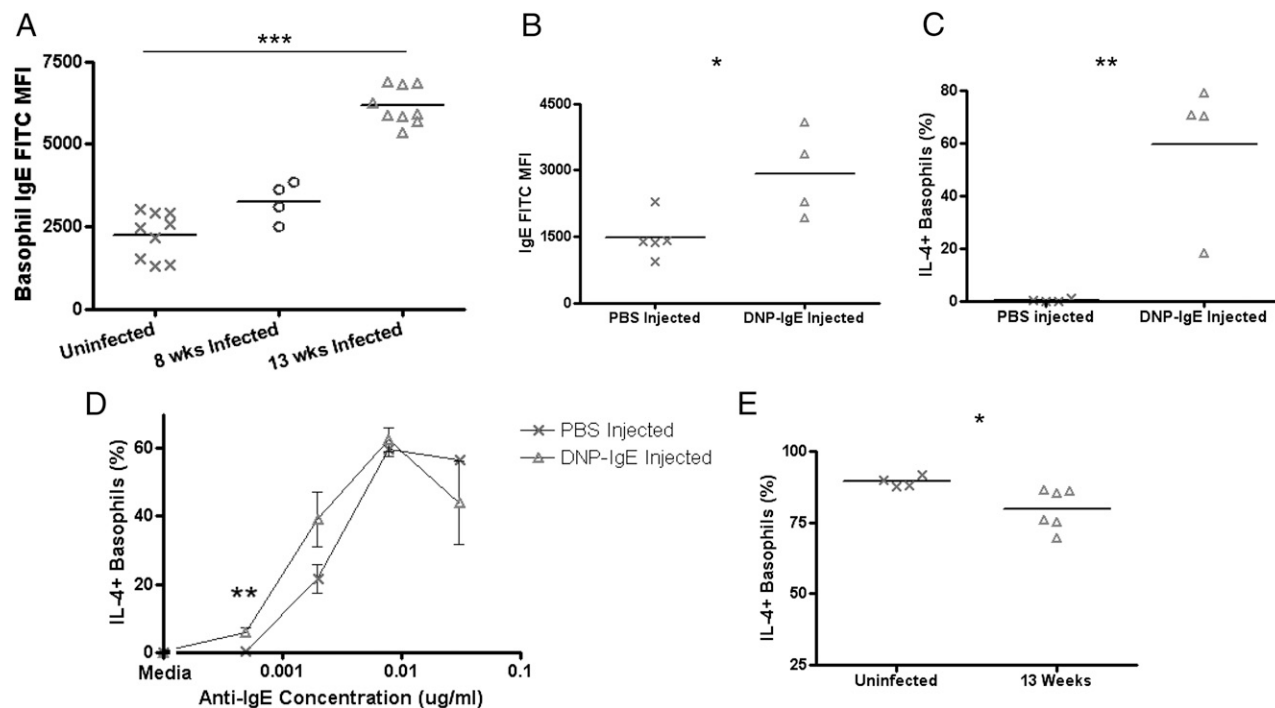
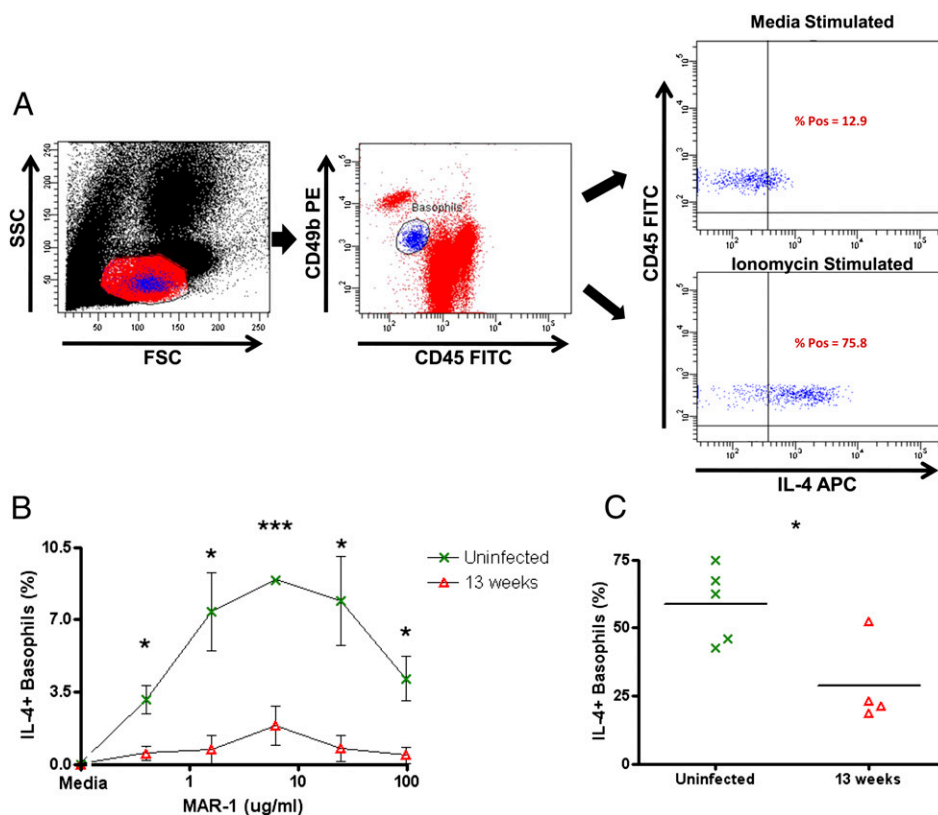


FIGURE 4. Activation curves of basophils with different surface IgE levels. **(A)** Relative surface IgE levels of uninfected, 8-wk-infected, and 13-wk *L. sigmodontis*-infected BALB/c mice, as assessed by mean fluorescence intensity staining of IgE on basophils. **(B)** Relative basophil surface IgE levels after injection of BALB/c mice with DNP IgE mAb, assessed by measuring IgE FITC MFI by flow cytometry. **(C)** Percentages of basophils from PBS-injected and DNP-IgE-injected mice that stain positively for IL-4 after stimulation with 2 μ g/ml DNP-human serum albumin. **(D)** Percentages of basophils from PBS-injected ($n = 5$) and DNP IgE-injected ($n = 4$) mice that stain positively for IL-4 after stimulation with several concentrations of anti-IgE after subtracting media stimulation levels. **(E)** Activation of basophils from uninfected and 13-wk *L. sigmodontis*-infected mice measured by flow cytometry after stimulation with 1 μ g/ml ionomycin (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

infected with *L. sigmodontis* despite the absence of repeated Ab-mediated activation (mean percentage of IL-4⁺ basophils after stimulation with 0.4, 1.6, 6.25, 25, and 100 μ g/ml anti-Fc ϵ RI α

from uninfected mice = 3.1, 7.4, 8.9, 7.9, 4.1 versus 0.53, 0.7, 1.9, 1.9, 0.44 from chronically infected mice, $p = 0.028$, $p = 0.035$, $p = 0.0003$, $p = 0.013$, $p = 0.013$; Fig. 5B). Also, fewer basophils from

FIGURE 5. Basophil responsiveness in *L. sigmodontis*-infected $J_H^{-/-}$ BALB/c mice. **(A)** Gating strategy for flow cytometric assessment of basophil activation in $J_H^{-/-}$ BALB/c mice. Basophils identified as CD49b^{high} and CD45^{low}. Right panels, Demonstrate IL-4 staining after incubation of whole blood with media and 1 μ g/ml ionomycin. **(B)** Percentages of basophils from uninfected and *L. sigmodontis*-infected $J_H^{-/-}$ BALB/c mice that stain positive for IL-4 after stimulating with several concentrations of anti-Fc ϵ RI α after subtraction of media stimulation levels ($n = 4$ per data point). **(C)** Percentages of basophils from uninfected and 13-wk-infected $J_H^{-/-}$ mice in response to 1 μ g/ml ionomycin (* $p < 0.05$, *** $p < 0.001$).



chronically infected $J_H^{-/-}$ mice became activated in response to ionomycin stimulation compared with basophils from uninfected $J_H^{-/-}$ controls (mean percentage of IL-4⁺ basophils after ionomycin stimulation = 28.9% versus 58.6, $p = 0.02$; Fig. 5C).

In a second experiment, BALB/c mice were sensitized to parasite Ag by three injections of *LsAg* adsorbed to alum. Functional evidence for specific Ab came from the observation that basophils of sensitized mice became activated when exposed to either *LsAg* ($p < 0.05$) or microfilarial Ag (Fig. 6A). To test whether basophil hyporesponsiveness is associated with chronic exposure of helminth-sensitized basophils to helminths, 3 wk after sensitization mice had either adult male worms or adult female worms surgically implanted into the peritoneal cavity or had microfilariae injected into the jugular vein. Control mice had sham surgery with no worms implanted. After 14 d, blood was obtained to compare basophil responsiveness to anti-IgE stimulation. This timepoint was chosen because implantation of adult female worms, as well as direct injection of microfilariae, resulted in circulating microfilaremia for at least 2 wk. Because basophil lifespan is ~2–3 d (39), and as even a single activation event can reduce basophil responsiveness (36), this duration should have been sufficient to observe suppressed activation curves if repeated activation was the primary mechanism decreasing basophil function.

As seen in Fig. 6B, after 14 d *LsAg*-sensitized basophils exposed to adult male and female worms had little or no reduced responsiveness to anti-IgE stimulation. *LsAg*-sensitized basophils exposed to microfilariae actually seemed to become slightly more responsive to anti-IgE stimulation (Fig. 6B).

These data indicate the reduced basophil responsiveness that develops in chronic helminth infection is not due to chronic Ab-mediated activation of basophils.

Incubation of basophils with high concentrations of microfilaria E/S products results in reduced basophil responsiveness, but in vivo microfilariae do not suppress basophil responsiveness

Because a previous study has demonstrated that a filarial E/S product from *Acanthocheilonema vitae* can suppress bone marrow-derived mast cells (40), we tested whether E/S products from *L. sigmodontis* could suppress basophils.

After incubating whole blood with E/S products from different life cycle stages of *L. sigmodontis*, basophils were stimulated with several concentrations of anti-IgE and basophil activation assessed by measuring IL-4 expression with intracellular flow cytometry.

No changes in basophil responsiveness to anti-IgE were observed after incubating whole blood with E/S products from adult male or adult female worms (Fig. 7A). However, basophil responsiveness to anti-IgE stimulation was reduced after incubating whole blood with E/S products from microfilariae (mean percentage of IL-4⁺ basophils after stimulation with 0.002 and 0.0078 μ g/ml anti-IgE from whole blood incubated with BSA = 21.1, 33.5 versus 3.4, 10.1 from whole blood incubated with microfilaria E/S products, $p < 0.001$, $p < 0.01$; Fig. 7A).

To determine whether microfilariae were responsible for inducing basophil hyporesponsiveness in vivo, 50,000 microfilariae in RPMI 1640 were injected i.v. into BALB/c mice and, after 11 d, basophil responsiveness was assessed by stimulating basophils with several concentrations of anti-IgE.

Injection of microfilariae did not reduce basophil responsiveness to IgE cross-linking (Fig. 7B). In fact, basophils seemed more responsive to anti-IgE stimulation after microfilariae injection. Importantly, microfilaria counts when blood was obtained for basophil activation studies were equal to or higher than microfilaria counts from blood obtained from mice with chronic infections (Fig. 7C). Thus, the lack of basophil responsiveness was not due to insufficient numbers of microfilariae in the injection study. Further evidence that products secreted from microfilariae are not the main drivers of basophil hyporesponsiveness comes from the observation that, in natural *L. sigmodontis* infections, basophils are more suppressed at 13 wk than 8 wk (Fig. 1D, 1E), even though microfilaria counts are lower at 13 wk than 8 wk (Fig. 7C). In total, these results suggest that E/S products from microfilaria have the capability to suppress basophil responsiveness, but likely are not the main cause of basophil hyporesponsiveness in chronic helminth infection.

IL-10 generated during helminth infection is important for development of reduced basophil responsiveness

IL-10 is an anti-inflammatory cytokine that is produced during helminth infections and plays a role in immune and cellular hyporesponsiveness (31, 41–44). To test whether IL-10 generated during helminth infection was responsible for reduced basophil responsiveness, IL-10-deficient BALB/c mice were infected with *L. sigmodontis* and basophil responsiveness to anti-IgE, *LsAg*, and ionomycin compared with uninfected IL-10-deficient BALB/c age-matched controls.

Whereas no splenocyte populations spontaneously released measurable quantities of IL-10, splenocytes from chronically infected

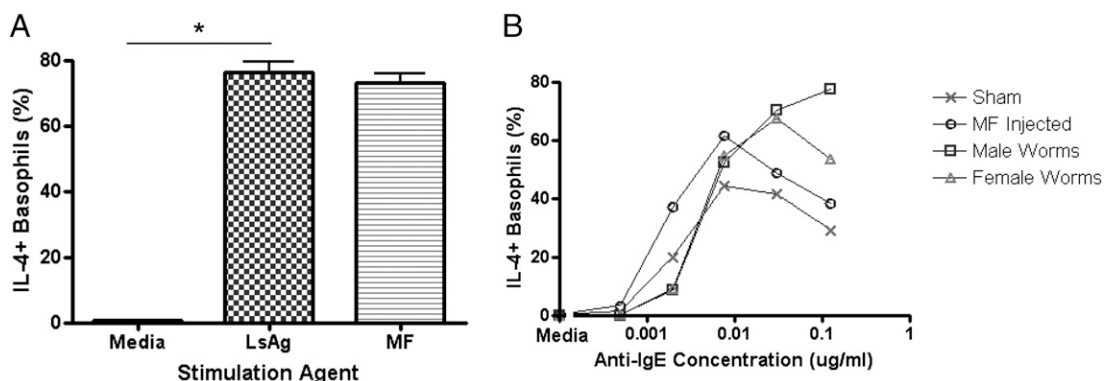


FIGURE 6. Basophil responsiveness following adult worm implantation or microfilaria transfection in *LsAg*-sensitized BALB/c mice. BALB/c mice were sensitized to worm Ags by three injections of 100 μ g *LsAg* + alum. (A) Percentages of basophils from *LsAg*-sensitized mice that are IL-4⁺ after stimulation with media, 2 μ g/ml *LsAg*, or 2 μ g/ml microfilaria Ag 3 wk after sensitization, as assessed by flow cytometry. (B) Percentages of basophils that stain IL-4 positive after stimulation with several concentrations of anti-IgE, as assessed by flow cytometry after subtracting media stimulation levels 14 d after i.p. implantation of five to seven adult male worms, i.p. implantation of five to seven adult female worms, or i.v. injection of 50,000 microfilariae. Each data point represents the mean of four independent experiments (* $p < 0.05$).

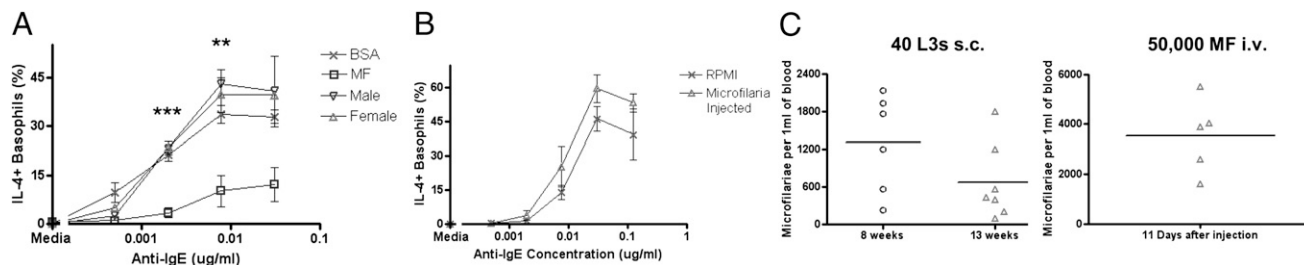


FIGURE 7. Effects of *L. sigmodontis* E/S products on basophil responsiveness. **(A)** E/S products from different life cycle stages of *L. sigmodontis* were incubated with whole blood containing basophils for 7 h and then stimulated with several concentrations of anti-IgE (each data point represents mean of at least two independent experiments). Degree of basophil activation was assessed by measuring basophil IL-4 positivity by flow cytometry after subtracting media stimulation levels (only showing statistical significant difference between the BSA control and microfilaria E/S-incubated conditions). **(B)** Percentages of basophils that stain positively for IL-4 by flow cytometry after subtracting media stimulation levels in response to several concentrations of anti-IgE 11 d after i.v. injection of 50,000 microfilariae in RPMI 1640 or RPMI 1640 only ($n = 5$ for each group). **(C)** Microfilaria levels in mice 8 wk and 13 wk after s.c. injection of 40 L3-stage larvae and 11 d after injection of 50,000 microfilariae (** $p < 0.01$, *** $p < 0.001$).

WT BALB/c mice produced significant amounts of IL-10 ($p < 0.001$) in response to *LsAg* compared with WT uninfected controls (Fig. 8A). These splenocyte cultures also produced more IL-10 on average than cultures from uninfected mice in response to polyclonal activation (data not shown). No IL-10 was detected from splenocytes obtained from IL-10-deficient BALB/c mice under any stimulation conditions (data not shown). Using an in vivo cytokine capture kit confirmed that more circulating IL-10 was present in mice with chronic worm infections of 13-wk duration than in uninfected mice, acutely infected mice (2 wk), and mice that had cleared the infection (22 wk) (Fig. 8B).

As seen in Fig. 8C and 8D, the reduced basophil responsiveness to IgE cross-linking and ionomycin stimulation previously observed in chronic helminth infections was abrogated in IL-10-deficient BALB/c mice infected with *L. sigmodontis* (Fig. 8C, 8D). Interestingly, basophils from chronically infected IL-10-deficient

BALB/c mice still had slightly reduced responsiveness to *LsAg* (mean IL-4⁺ basophils in response to 2 μ g/ml *LsAg* 64.3% at 8 wk versus 40.5% at 13 wk, $p = 0.003$; Fig. 8E), although not to the same degree as basophils from chronically infected WT mice (mean IL-4⁺ basophils in response to 2 μ g/ml *LsAg* 63.3% at 8 wk versus 26.9% at 13 wk, $p = 0.036$; Fig. 1E). This finding was not due to a drop in parasite-specific IgE levels at the chronic timepoint, because *LsAg*-specific IgE levels were greater at 13 wk postinfection than at the 8 wk timepoint in IL-10-deficient mice (Supplemental Fig. 2).

Because IL-10 has not been previously shown to have a suppressive effect on basophils, we assessed the effects of IL-10 on basophil function in vivo by administering IL-10 twice daily to WT mice for 5 d. As seen in Supplemental Fig. 3, basophils of mice administered IL-10 exhibit decreased responsiveness to IgE-mediated stimulation.

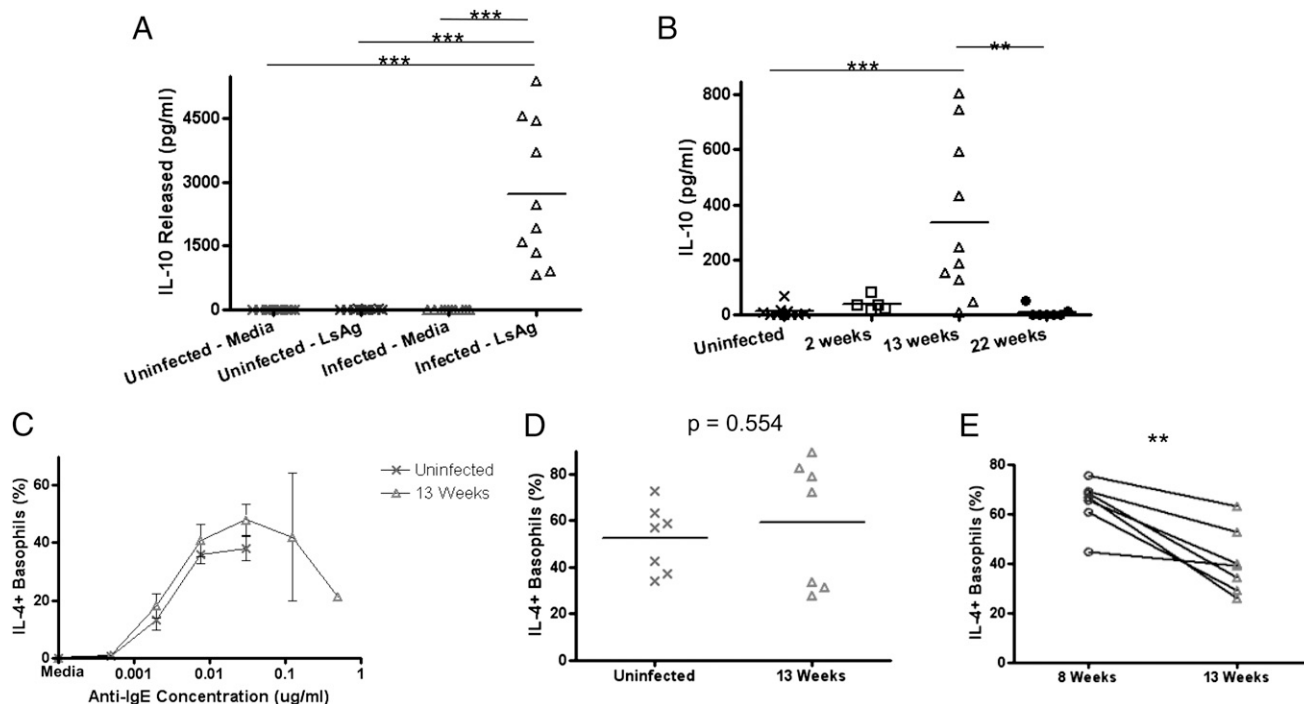


FIGURE 8. Decreased basophil responsiveness to IgE-mediated stimulation is dependent on IL-10. **(A)** IL-10 concentrations from supernatants of splenocytes from uninfected or 13-wk-infected WT BALB/c mice after stimulation with *LsAg* as measured by ELISA. **(B)** Circulating levels of IL-10 at different timepoints of infection as measured by in vivo cytokine capture assay. Percentages of basophils from uninfected ($n = 7$) and 13-wk-infected ($n = 7$) IL-10-deficient BALB/c mice that stain positively for IL-4 after stimulation with several concentrations of anti-IgE **(C)**, ionomycin **(D)**, or *LsAg* **(E)** and after subtracting media stimulation levels (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

These results demonstrate that IL-10 is responsible for much of the reduced basophil responsiveness observed in chronic *L. sigmodontis* infection.

Helminth infection decreases Syk, Akt, and STAT5 expression in basophils

We assessed the expression of signal transduction molecules in basophils from WT BALB/c mice chronically infected with *L. sigmodontis* and age-matched uninfected controls by flow cytometry. Expression levels of Akt ($p = 0.001$) and Syk ($p = 0.033$) from mice chronically infected with *L. sigmodontis* were significantly lower compared with uninfected controls (Fig. 9A, 9B). Fyn expression in basophils from chronically infected mice was also slightly lower on average than uninfected controls (Fig. 9C), but this difference did not reach statistical significance. No decrease in baseline STAT5 expression ($p = 0.453$) could be detected in basophils of chronically infected mice (Fig. 9D). However, expression levels of STAT5 ($p < 0.0001$) and phosphorylated STAT5 ($p = 0.049$) in basophils of chronically infected mice were significantly lower than in basophils of uninfected controls after stimulation for 10 min with 0.0078 $\mu\text{g/ml}$ anti-IgE (Fig. 9F, 9G). Basophil phosphorylated Akt expression levels ($p = 0.0008$) were also lower after anti-IgE stimulation (Fig. 9H).

Because ES-62, a filarial E/S product, has been shown to suppress bone marrow-derived mast cells in vitro by decreasing PKC- α expression (40), we also evaluated expression of PKC- α in basophils of mice chronically infected with *L. sigmodontis*. As seen in Fig. 9E, no reduction in PKC- α expression was observed in basophils of chronically infected mice (Fig. 9E).

Basophil suppression requires active helminth infection

Whereas *L. sigmodontis* can produce patent infections in BALB/c mice, the adult worms and microfilariae eventually die by 16–20 wk. To determine whether helminth infection permanently reduces basophil responsiveness, basophil activation assays were performed 22 wk postinfection, a timepoint when both adult worms and microfilariae are no longer present.

No adult worms were found in the pleural cavity, and no microfilariae were found in the blood of mice inoculated with *L. sigmodontis* 22 wk prior. The basophil response curve to IgE

cross-linking of mice that no longer had worms present was identical to that of uninfected controls (Fig. 10A). Basophils from 22-wk-infected mice also no longer had reduced basophil activation when stimulated with ionomycin (Fig. 10B).

These data suggest that active worm infections are needed to reduce basophil responsiveness, and that basophil responsiveness, which is reduced in chronic helminth infections, returns to normal after death of the worms.

Discussion

This study demonstrates that chronic helminth infection suppresses basophil responsiveness to both IgE- and non-IgE-mediated stimuli. This phenomenon was observed with *L. sigmodontis* and *S. mansoni* infections. Given that these worms belong to different phyla (Nematoda and Platyhelminthes, respectively), these results suggest that basophil suppression may be a common immunologic phenotype in many chronic helminth infections.

Basophil suppression appeared to be due primarily to IL-10 as basophils of mice deficient in this cytokine did not exhibit alterations in their activation curves to anti-IgE stimulation. Additionally, intracellular flow cytometry revealed decreases in basophil expression of Syk and Akt as well as decreased total STAT5, phosphorylated STAT5, and phosphorylated Akt in response to IgE-mediated activation. These findings are consistent with IL-10-mediated suppression, as IL-10 suppresses IgE signaling in mast cells by decreasing expression of these same molecules (31). The reduced expression of Syk is particularly noteworthy because there is substantial evidence that Syk may be the principal signaling molecule responsible for fine-tuning basophil responsiveness (45). IL-10 production during chronic helminthiasis is well documented and has been shown to contribute to the highly immune regulated state observed in these infections (46–48). Important sources of IL-10 during filaria infection include CD4⁺CD25⁺ T cells, CD4⁺CD25⁺ T regulatory cells, CD8⁺ T cells, B cells, monocytes, and NK cells (49). Our data expand the known suppressive capabilities of IL-10 because, to our knowledge, this is the first study to find that IL-10 plays a role in basophil suppression. It is important to note that although IL-10 is an important cause of reduced basophil responses to IgE-mediated activation in the *L. sigmodontis* model, it is probably not the only mecha-

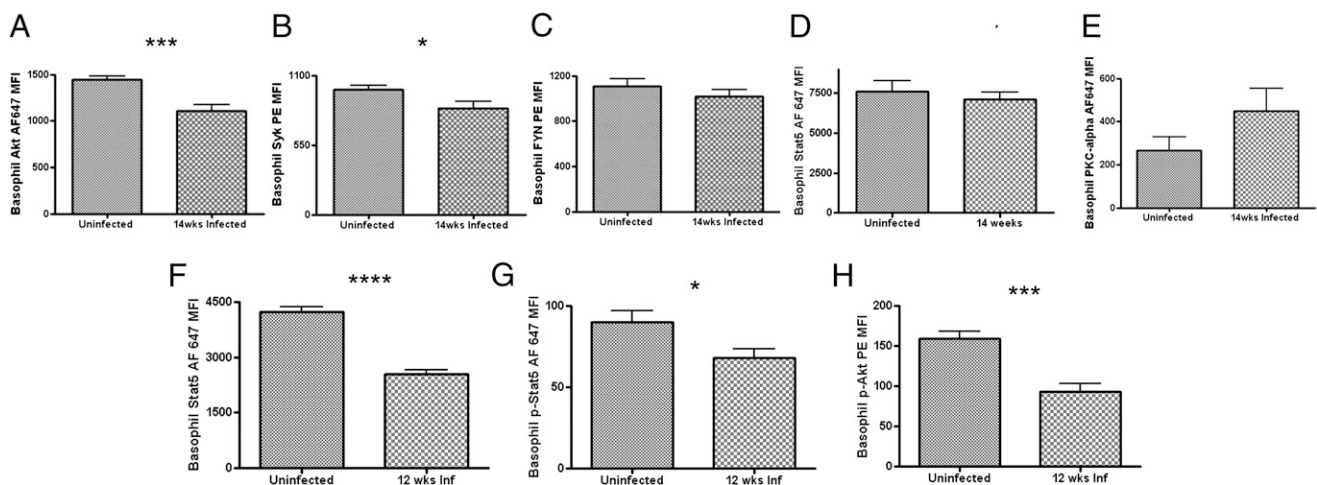


FIGURE 9. Alterations in signal transduction molecules in basophils of mice chronically infected with *L. sigmodontis*. Expression levels of key signaling molecules were assessed by flow cytometry. After identifying basophils as CD4⁺B220⁺IgE⁺ peripheral blood cells, baseline Akt (A), Syk (B), FYN (C), STAT5 (D), and PKC- α (E) MFI levels were measured from chronically infected BALB/c mice and age-matched uninfected BALB/c controls. Basophils from chronically infected mice and age-matched controls were also stimulated for 10 min with 0.0078 $\mu\text{g/ml}$ anti-IgE and then MFI levels of STAT5 (F), phospho-STAT5 (G), and phospho-Akt (H) measured by flow cytometry. Each data point represents the mean of at least four independent experiments (* $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$).

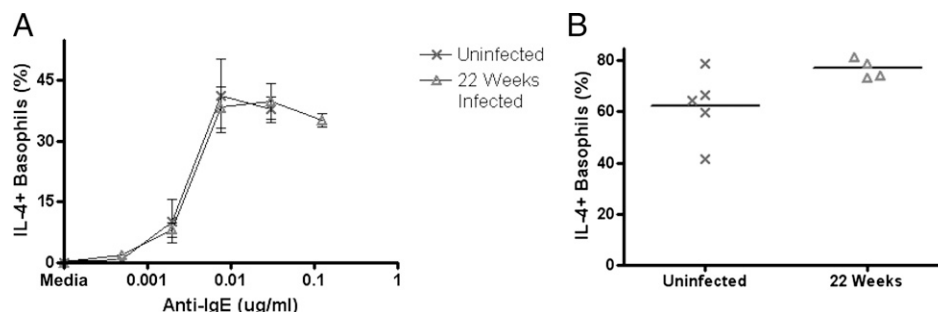


FIGURE 10. Basophil responsiveness in BALB/c mice after clearance of *L. sigmodontis* infection. Basophils from mice that were infected 22 wk prior, but no longer actively infected ($n = 4$) and uninfected control mice ($n = 5$) were stimulated with several concentrations of anti-IgE (**A**) or $1 \mu\text{g/ml}$ ionomycin (**B**). To assess activation, percentages of IL-4⁺ basophils were measured using flow cytometry after subtracting media stimulation levels.

nism suppressing basophil activation toward helminths because some decreased responsiveness to *LsAg* developed in chronically infected IL-10-deficient mice despite increases in specific IgE.

Recently, ES-62, a secreted phosphorylcholine-containing E/S product of the filarial worm *Acanthocheilonema viteae*, was shown to directly inhibit mast cell function in vitro by reducing intracellular levels of PKC- α , a molecule implicated in a non-canonical IgE-signaling pathway (40). Whereas we observed that E/S products from *L. sigmodontis* microfilariae inhibit basophil responsiveness in vitro, direct injection of microfilariae into mice did not result in basophil suppression, and intracellular levels of PKC- α were not diminished in basophils of chronically infected animals. These findings suggest that although E/S products of *L. sigmodontis* microfilariae have the capability to downmodulate basophil function, they likely do not do so in vivo during chronic infection.

Another mechanism that did not play a large role in basophil suppression was repeated IgE-mediated stimulation. Whereas IgE-mediated activation can cause negative feedback signals that suppress subsequent IgE-mediated signaling (36), this process is not required for basophil suppression during filaria infection because basophil suppression developed in chronically infected Ab-deficient mice.

Interestingly, suppression of basophil function required the presence of living worms as basophil responsiveness returned to baseline after all worms had died. This requirement for active worm infection is consistent with studies in human filariasis in which decreased cellular proliferation and inflammatory cytokine responses to parasite Ags reverse after therapy (50, 51).

The finding that chronic helminth infections suppress basophil responsiveness has important clinical implications. Recently, basophils have become increasingly recognized as being important mediators of allergic disease. In terms of acute effector function, basophils release histamine and leukotriene C4 after becoming activated. These molecules induce classic allergy symptoms by increasing vascular permeability, mucus secretion, and smooth muscle contraction (52, 53). Whereas the contribution basophils make to acute inflammatory responses most likely varies between different diseases, a recent clinical study demonstrated that basophils may be responsible for the majority of allergic symptoms that occur after intranasal allergen challenge of individuals with cat allergy (54). Similarly, two murine studies have identified basophils as the principal effector cells of chronic allergic inflammation (18, 22). One of the mysteries regarding helminth infections and allergy has been the observation that, in contrast to its utility in developed countries, allergen-specific IgE has poor predictive value for allergic disease in developing countries with high rates of helminth disease (55). Our discovery that

chronic helminth infections can suppress IgE-mediated activation of basophils provides an explanation for this finding. Given the many differences between human and mouse Fc ϵ RI expression and basophil properties (56, 57), it will be important to assess whether helminths exert a similar phenomenon in people.

In addition to contributing to acute allergic inflammation, basophils also play prominent roles in driving the type 2 responses responsible for allergic diseases. Whereas recent studies have demonstrated that basophils play little, if any, role in initiating type 2 immune responses (21, 58), a number of studies have shown that basophils can amplify type 2 responses that are already present (21–23, 25, 26, 38, 59). Thus, suppression of basophil responsiveness may be a principal mechanism by which chronic helminth infections protect against development of allergic diseases. Interestingly, some animal studies have implicated IL-10 as being important for helminth-mediated protection against allergic disease (59, 60). Our finding that basophil suppression in chronic *L. sigmodontis* infection is due to IL-10 provides a downstream mechanism explaining how helminth-induced IL-10 can block allergic inflammation.

Changes in basophil responsiveness may also have important implications for susceptibility to helminths, as there is increasing evidence that basophils play a role in protective immunity against some of these infections (61, 62). Although basophils do not protect against primary murine filariasis (26), basophil depletion or deficiency results in impaired expulsion of the intestinal nematode *Trichuris muris* (24) and inhibits protection against reinfection by the hookworm *Nippostrongylus brasiliensis* (22, 23). Thus, it is possible that in certain helminth infections reduced basophil responsiveness may play a role in enabling parasite survival.

Finally, it is interesting to note that basophils have recently been implicated as contributing to the pathogenesis of lupus nephritis. In that study, basophils were shown to contribute to disease by amplifying production of autoantibodies in an IL-4- and IgE-dependent manner (63). As with allergy, worm infections are associated with protection against autoimmune disease in both human studies and animal models. If basophils play a role in mediating pathology in certain autoimmune diseases, then it is possible that basophil suppression could be one of the mechanisms by which helminths protect against autoimmunity.

In summary, this study demonstrates that chronic helminth infections reduce basophil responsiveness in an IL-10-dependent manner through reduction of key IgE signaling molecules. Given the prominent roles basophils play in the development and effector phases of type 2 responses, the protective role they may have against certain helminth infections, and their possible contribution to autoimmune disease pathogenesis, this finding has broad

implications for understanding how helminth infections alter disease manifestations in people.

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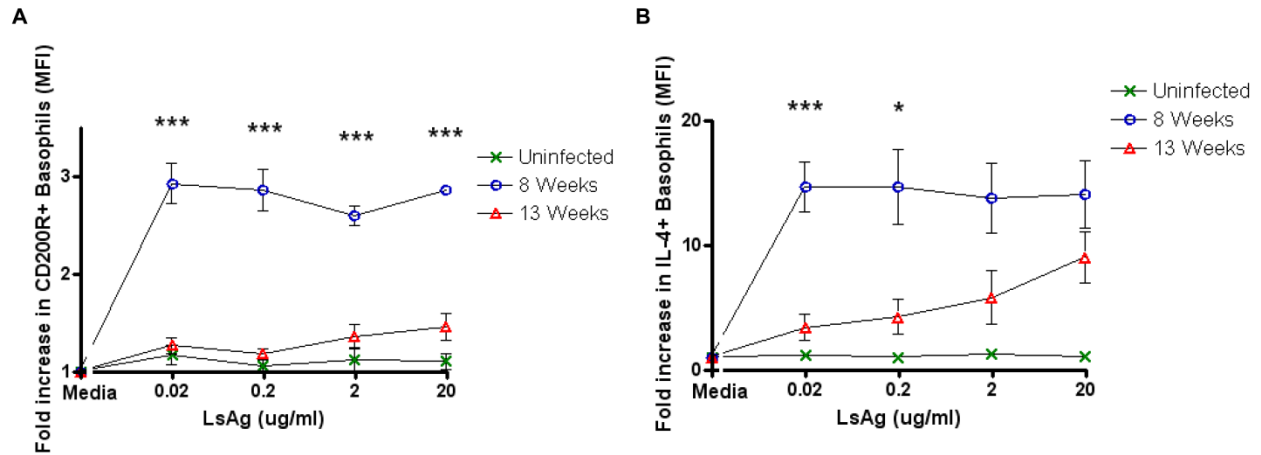
Disclosures

The authors have no financial conflicts of interest.

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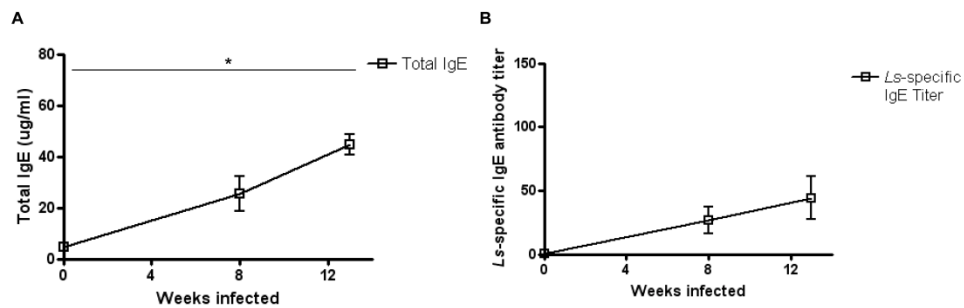
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SUPPLEMENTAL FIGURE 1: Responsiveness of basophils from *L. sigmodontis*-infected mice to *LsAg* stimulation (MFI measurements).

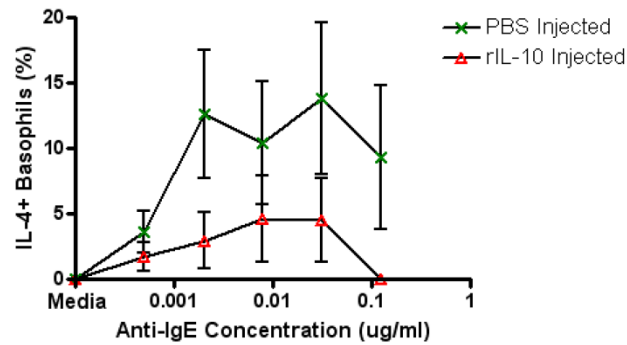
MFI of basophils that stain positively for CD200R (A) and IL-4 (B) after stimulation with several concentrations of *LsAg* as measured by flow cytometry. Fold increases in MFI levels were obtained by dividing the MFI levels of antigen-stimulated basophils by those of media stimulated cells. Each data point represents the mean of at least three independent experiments. (* $p < 0.05$, *** $p < 0.001$).



SUPPLEMENTAL FIGURE 2: Total and *LsAg*-specific IgE levels in IL-10-deficient mice.

Levels of total IgE (A) and *LsAg*-specific IgE (B) were measured in the plasma of uninfected ($n = 3$), 8 week ($n = 5$), and 13 week ($n = 5$) *L. sigmodontis*-infected IL-10-deficient mice.

A



SUPPLEMENTAL FIGURE 3: IL-10 administration suppresses basophil responsiveness to IgE-mediated activation.

Balb/c mice were administered 2 μ g of IL-10 in 100 μ l of PBS or PBS alone i.p. twice daily for 5 days. Mice were then euthanized and basophil activation in response to increasing concentrations of anti-IgE assessed by flow cytometric detection of basophil IL-4 expression after subtraction of media stimulation levels.