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Basophils Are the Major Producers of IL-4 during Primary Helminth Infection

Nicholas van Panhuys,^{*,†,1} Melanie Prout,^{*,1} Elizabeth Forbes,^{*} Booki Min,[‡] William E Paul,[†] and Graham Le Gros^{*}

IL-4 production by leukocytes is a key regulatory event that occurs early in the type 2 immune response, which induces allergic reactions and mediates expulsion of parasites. CD4⁺ T cells and basophils are thought to be the key cell types that produce IL-4 during a type 2 response. In this study, we assessed the relative contribution of both CD4⁺ T cell- and basophil-IL-4 production during primary and secondary responses to *Nippostrongylus brasiliensis* using a murine IL-4-enhanced GFP reporter system. During infection, IL-4-producing basophils were detected systemically, and tissue recruitment occurred independent of IL-4/STAT6 signaling. We observed that basophil recruitment to a tissue environment was required for their full activation. Basophil induction in response to secondary infection exhibited accelerated kinetics in comparison with primary infection. However, total basophil numbers were not enhanced, as predicted by previous models of protective immunity. Overall, the induction and migration of IL-4-producing basophils into peripheral tissues was found to be a prominent characteristic of the primary but not memory responses to *N. brasiliensis* infection, in which CD4⁺ T cells were identified as the major source of IL-4. Whereas basophils were the major initial producers of IL-4, we determined that normal Th2 differentiation occurs independently of basophils, and depletion of basophils led to an enhancement of inflammatory cell recruitment to the site of infection. *The Journal of Immunology*, 2011, 186: 000–000.

Interleukin 4 has been widely described as the signature cytokine of the Th2 immune response in which it plays multiple roles during adaptive immune responses. As such, IL-4 is essential for in vitro Th2 induction (1), B cell class switching to IgE production (2), and cellular migration into peripheral tissues (2, 3). IL-4 is also considered important in the secondary response, as symptoms in patients with allergic disorders are believed, at least in part, to be due to the chronicity of IL-4-producing effector cells. However, identification of the major IL-4 source is still a matter of uncertainty.

IL-4 is produced not only by differentiated Th2 CD4⁺ T cells, but also by basophils (4), mast cells (5), eosinophils (6), and NKT cells (7, 8). Basophils, the least common of the blood leu-

kocytes, are increasingly being recognized as an important component of the Th2 immune response, as they are recruited to sites of inflammation during type 2 responses (9), where they are believed to influence immune responses by releasing immune mediators, such as histamine and leukotriene, as well as rapidly producing the cytokines IL-4, IL-13, and thymic stromal lymphopoietic protein (TSLP) (9–12). IL-4-producing basophils have been identified as a key feature of parasitic infections in both mice (4, 13) and humans (14, 15) and have been implicated as a significant effector cell subset in allergic inflammation (16, 17). Moreover, basophils have been demonstrated to produce early IL-4 during a memory T-dependent response using goat anti-mouse IgD Ab (18). Mechanistically, a key role for basophil-derived IL-4 and IL-6 was recently identified in Denzel et al. (19), whereby basophil depletion profoundly impaired B cell proliferation and Ig production, highlighting the role that basophils play in humoral memory responses. Further basophils have been suggested to play a key role in T cell activation as APC to CD4⁺ cells following papain-induced activation (12, 20) and *Trichuris muris* infection (21). More recently, the ability of basophils to both cross present Ag and activate CD8⁺ cells has been demonstrated (22).

The nematode *Nippostrongylus brasiliensis* induces a robust Th2 immune response in mice, characterized by the production of high levels of IgE, IL-4, and IL-13 as well as an influx of eosinophils and basophils into mucosal tissues (23). *N. brasiliensis* is a skin-penetrating blood-borne parasite that migrates through the lungs to the gut, before being expelled at approximately day 9 postinfection, in the process eliciting immune reactions at these sites. Upon reinfection with *N. brasiliensis*, a protective memory response is elicited, characterized by an earlier enhanced Th2 immune response, which disrupts migration and prevents worm establishment in the host (24, 25).

We have previously shown that basophils accumulate in tissues postinfection with *N. brasiliensis* and are a major source of IL-4 (4). In this study, we used the G4 transgenic reporter system in

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Abbreviations used in this article: Alum, aluminum hydroxide; BAL, bronchoalveolar lavage; i.n., intranasal; KLH, keyhole limpet hemocyanin; KO, knockout; L3, third stage infectious larvae; Md Ln, mediastinal lymph node; MFI, mean fluorescence intensity; Ms Ln, mesenteric lymph node; NES, *Nippostrongylus brasiliensis* excretory secretory product; p.i., postinoculation; TSLP, thymic stromal lymphopoietic protein.

which an enhanced GFP construct was inserted into the first exon of the IL-4 gene, allowing identification of cellular production of IL-4 (26). Basophils detected in the G4 model system displayed low side scatter, were GFP⁺, FcεR1⁺, CD49b^{bright}, ckit^{neg}, and Gr1^{neg}, and were furthermore identified as basophils by electron microscopy. Significant levels of these GFP⁺ basophils were found systemically in various peripheral tissues. Induction of basophilia was found to be STAT6 and IL-4 independent, T cell dependent, and appeared to be partially dependent on IL-3 (27). In this study, we sought to examine whether IL-4-producing basophils were also a feature of the memory Th2 response induced following secondary infection with *N. brasiliensis*. Using G4 mice in which expression of GFP faithfully reports production of IL-4, we were able to directly identify GFP/IL-4-producing cells ex vivo without the need for restimulation. As Gessner et al. (28) had previously determined that both activated CD4⁺ Th2 cells and basophils produce similar quantities of IL-4, this allowed for a direct assessment of IL-4 production by CD4⁺ T cells and basophils in both primary and secondary *N. brasiliensis* responses. We determined that both GFP⁺ basophils and GFP⁺ CD4⁺ T cells were induced more rapidly following secondary *N. brasiliensis* infection with a greater number of IL-4-producing CD4⁺ T cells detected in the lung following reinfection. However, an enhanced GFP⁺ basophil response was not elicited throughout the course of secondary infection. Thus, our results demonstrate that GFP⁺ basophils constitute the major cellular source of IL-4 during the primary response, whereas GFP⁺ CD4⁺ T cells were the major source of IL-4 during the secondary infection. In addition, migration of IL-4-producing basophils is a prominent feature of the primary, but not the secondary, Th2 immune response, whereas CD4⁺ T cell-derived IL-4 is a dominant feature of the secondary immune response in the lung.

Materials and Methods

Mice

Heterozygous GFP/IL-4 knockin (G4) (26) and STAT6^{-/-} (31) mice were provided by Dr. William Paul at the Laboratory of Immunology (National Institute of Allergy and Infectious Diseases, National Institutes of Health). The G4/IL-4 mice were bred, and homozygous G4/G4 progeny were selected from the F1 generation at 6 wk of age via PCR detection of the enhanced GFP transgene. G4/G4 mice were crossed to the STAT6^{-/-} mice to obtain STAT6^{-/-} G4/G4 mice. Mice were bred and maintained by the Malaghan Institute of Medical Research animal facility in Wellington, New Zealand. All animal procedures were approved by the Victoria University of Wellington Animal Ethics committee. Six- to 12-wk-old mice were used for all experiments and within experiments were matched for age and sex.

Model systems

For *N. brasiliensis* infection, mice were inoculated with 600 third stage infectious larvae (L3) of *N. brasiliensis* by s.c. injection in the nape to generate a primary immune response. To induce a secondary response, mice infected 30 d previously as above were inoculated s.c. with an additional 600 L3. Where indicated, mice were depleted of basophils following i.v. injection of 150 µg anti-FcεR1 (MAR-1) 48 h prior to infection with *N. brasiliensis*. *N. brasiliensis* excretory secretory product (NES) was prepared as previously described (30); 2 µg L3 NES was administered via i.p. injection in adsorbed aluminum hydroxide (Alum) at days 0 and 14 followed by intranasal (i.n.) challenge at day 21 with either 100 µg NES in PBS or PBS alone. OVA 2 µg was adsorbed in Alum and administered i.p. at days 0 and 14 followed by i.n. challenge at day 21 with either 100 µg OVA in PBS or PBS alone. Additionally, 4 µg keyhole limpet hemocyanin (KLH) was adsorbed in Alum and administered i.p. at day 0 followed by i.n. challenge at day 7 with either 100 µg KLH in PBS or PBS alone. A total of 1 hemagglutinating unit influenza in PBS or PBS alone was administered via i.n. transfer.

Isolation and preparation of cells

Mice were tail bled and RBC lysed with ammonium chloride treatment before counting to determine the total number of lymphocytes per milliliter

of blood. Perfused lung was minced and incubated for 1 h in culture media (IMDM; Life Technologies) supplemented with 5% FCS (Life Technologies), 2 mM GlutaMAX (Life Technologies), 1% penicillin-streptomycin (Life Technologies), and 5×10^5 M 2-ME (Life Technologies) containing 2.4 mg/ml collagenase type I (Invitrogen) and 0.1% DNase I (Roche). A single-cell suspension was layered over a 30/70% Percoll (Amersham Biosciences) gradient and centrifuged at 2000 rpm for 20 min at room temperature. Cells at the interface were recovered and washed before counting. Livers were perfused and mechanically disrupted to single-cell suspensions, followed by resuspension in 30% Percoll and centrifugation at 2000 rpm for 10 min at room temperature. RBC were subsequently lysed, and the resulting mononuclear cells were counted. Single-cell suspension of the mesenteric lymph nodes (Ms Ln) and mediastinal lymph nodes (Md Ln) were obtained and analyzed by flow cytometry.

Flow cytometry

Processed cells were stained for cell-surface markers to identify different cell types and their GFP expression. PE-labeled anti-FcεR1a (MAR-1) Ab was purchased from eBioscience. Anti-CD4-APC, CD45-APC, and PanNK-PE (DX5) Abs were purchased from BD Pharmingen (San Diego, CA). Data were acquired using either a FACSort or FACSCalibur (BD Biosciences) and analyzed with a FlowJo software (Tree Star).

IL-4 quantitation

BALB/c animals were infected with 600 L3 *N. brasiliensis* s.c. At day 9 postinfection, lungs were perfused, and lung tissue was dissociated through cell strainers. Single-cell suspensions were stimulated with either Dynabeads Mouse T-Activator CD3/CD28 (Invitrogen; 1:1 ratio) plus 30 U/ml IL-2 or 10 µg/ml anti-mouse IgE (6HD5) plus IL-3 (1.2 ng/ml $\times$ 63 IL-3) for 6 h. Cells were plated at 2×10^6 cells in 200 µl with the addition of 20 µg/ml anti-mouse CD124 (IL-4Rα; BD Pharmingen) for IL-4 ELISA analysis and 1×10^5 in 150 µl for the IL-4 ELISPOT. IL-4 ELISPOT was performed according to the manufacturer's instructions (Mabtech MacLeod, Victoria, Australia), and ELISA determined supernatant IL-4. Briefly, plates were coated overnight at 4°C with 100 µl anti-mouse IL-4 Ab (11B11; 2 µg/ml) and then blocked with 200 µl 10% FBS (FBS diluted in sterile PBS) before adding serial dilutions of supernatant samples (100 µl/well). Samples were incubated for 2 h, and biotinylated anti-mouse IL-4 Ab was added (BVD6-24G2; BD Pharmingen, 2 µg/ml, 100 µl/well). After 2 h of incubation, streptavidin-HRP (1:1000 dilution; GE Healthcare, Buckinghamshire, U.K.) was added. Prior to the initiation of each step, plates were washed with 0.05% Tween-20 in PBS. Finally, after 1-h incubation, 100 µl substrate (TMB Substrate Reagent Set; BD OptEIA, San Diego, CA) was added. Colorimetric reaction was stopped with 1 M H₂SO₄ and quantified by measuring OD with an ELISA plate reader at 450 nm.

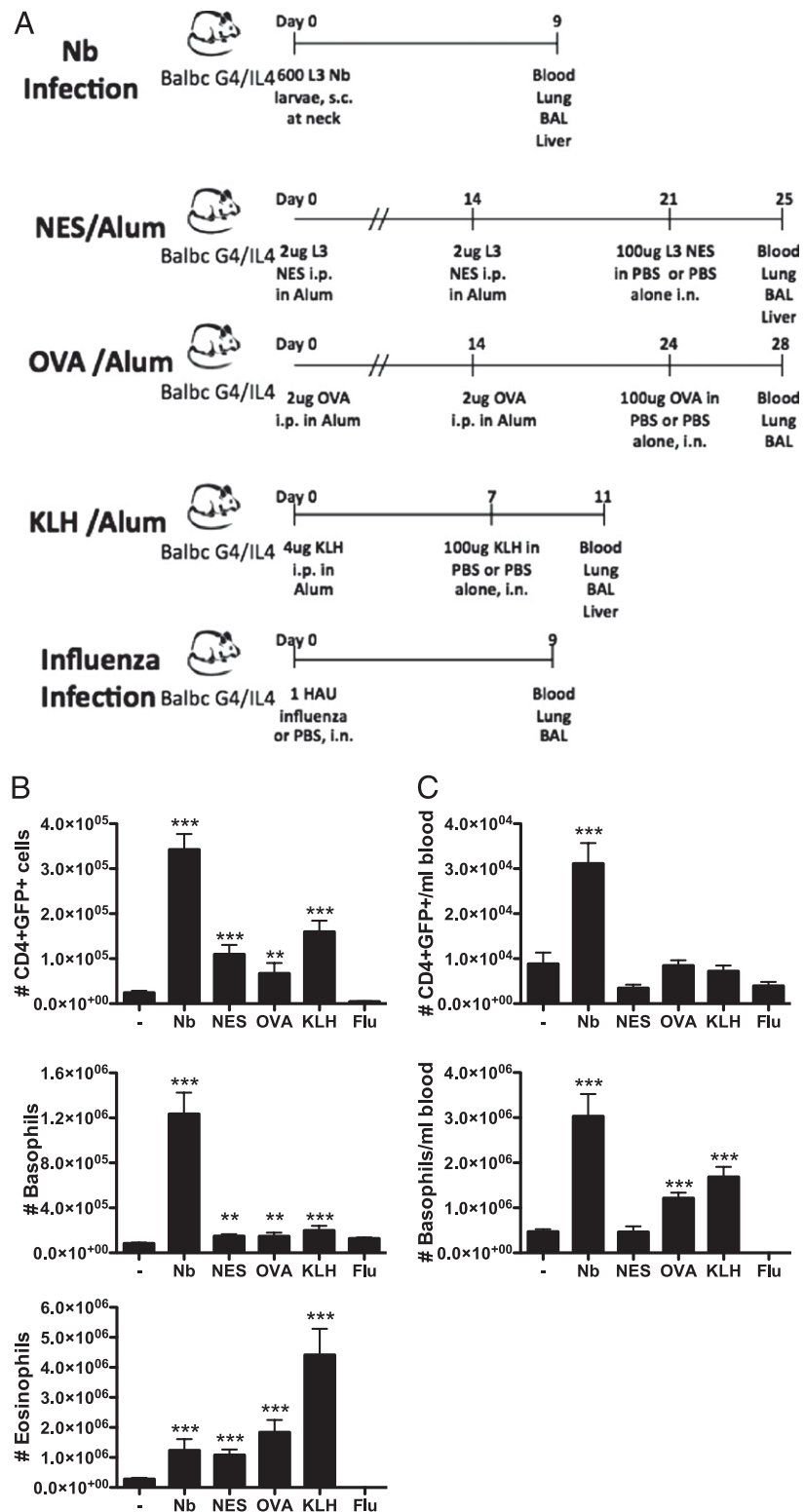
Results

Basophil induction and recruitment during type 2 immune responses

Although basophilia has been widely described as a key feature of the Th2-associated airway inflammatory response, little effort has previously been made to differentiate between the role that basophils play in parasitic infections and asthma/allergy-type responses. In this study, we investigated the comparative induction and recruitment of basophils using several common models of type 2 lung inflammation (Fig. 1A). *N. brasiliensis* infection was used as a classical model of helminthic response and was compared with the allergic-type responses initiated after protein/adjuvant prime challenge with KLH or OVA in Alum. NES in Alum was included to determine whether a synergistic response between helminth-associated factors and Alum could be elicited. Additionally, animals were infected with influenza to examine whether basophil recruitment occurs under conditions of type 1 inflammation.

To analyze the role of basophils in immune responses, lung inflammatory infiltrate was analyzed at the peak of responses (Fig. 1B), and, as expected following *N. brasiliensis*, KLH, OVA, and NES challenge, a significant type 2 response was induced with increased numbers of CD4⁺GFP⁺ cells and eosinophils being recruited to the lung, whereas influenza infection led to a decrease

FIGURE 1. Comparison of basophil induction in murine immune response models. A, Comparison of different mouse immune response models. Total numbers of CD4⁺GFP⁺, basophils, and eosinophils were measured in the lungs (B) and total numbers of CD4⁺GFP⁺, basophils in BALB/c G4/IL-4 mice day 9 following *N. brasiliensis* infection (C), day 4 post i.n. challenge with NES, OVA, or KLH Ag, or day 9 following i.n. influenza (flu) infection. — shows the level of cells present in uninfected or PBS-challenged control mice. NES and KLH data are representative of single 3-mice experiments, whereas OVA is an average of 6 mice from two experiments and *N. brasiliensis* of 22 mice from seven experiments. ***p* ≤ 0.001; ****p* ≤ 0.0001; no asterisk, *p* > 0.05, relative to naive animal with Student *t* test.



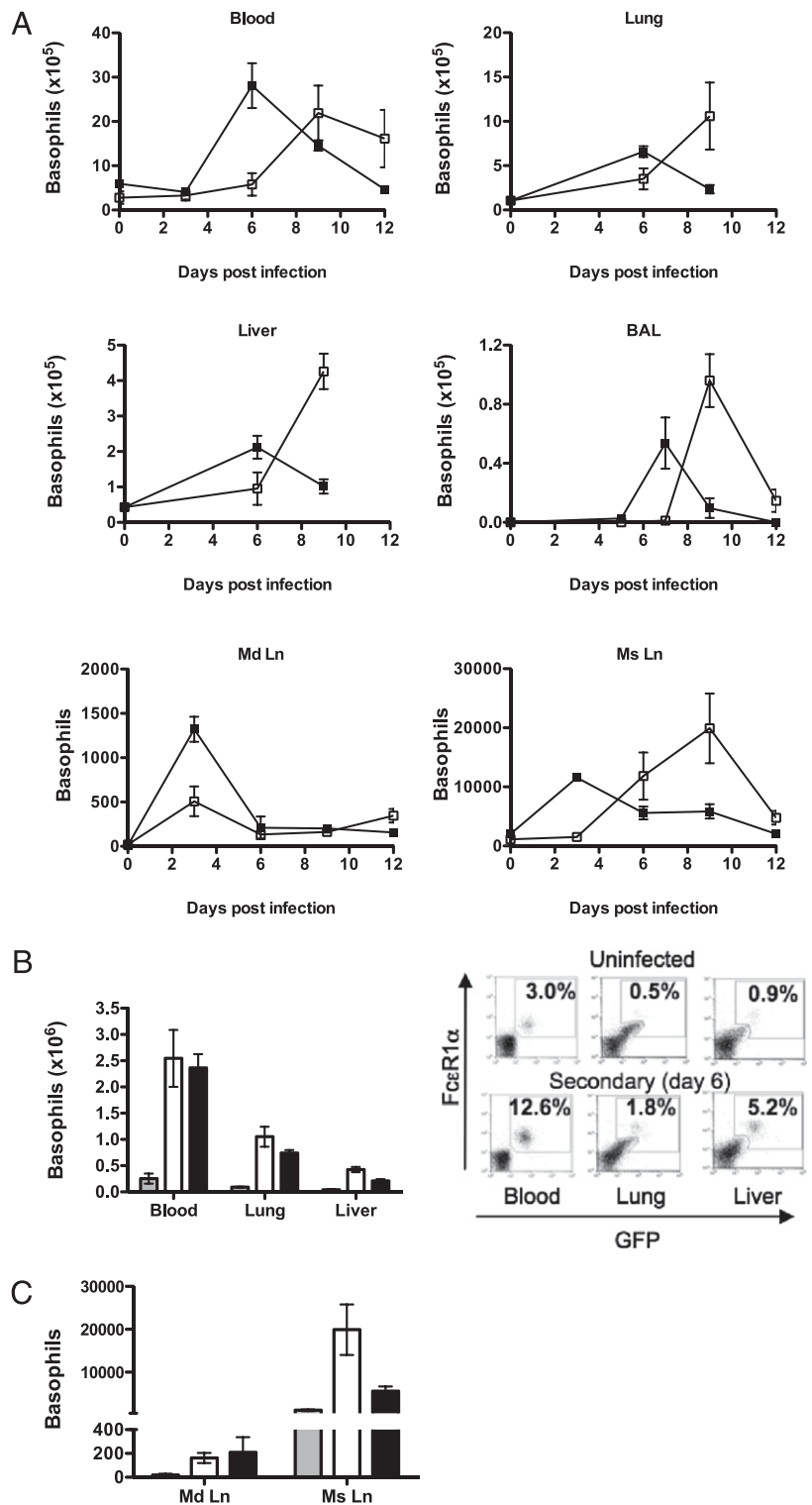
in CD4⁺GFP⁺ and eosinophil numbers, and lung basophil numbers were not substantially altered. Interestingly, although CD4⁺GFP⁺ cells and eosinophils were induced and recruited to the lung in large numbers by both allergic (protein/Alum) models and *N. brasiliensis* infection, significant lung basophilia was only detected following *N. brasiliensis* infection. *N. brasiliensis* infection also induced a significant basophilia in the lung, with a 15-fold increase in numbers above background (Fig. 1B). This increase was due to both a 2.7-fold increase in total cells in the lung as well

as an increase in the proportion of basophils from 0.9% of CD45⁺ cells in naive mice to 4.8% at the peak of infection. Following both OVA and KLH treatment, an increase in blood basophilia was observed, whereas CD4⁺GFP⁺ levels remained similar to numbers observed in control animals. However, following both NES treatment and influenza infection, a decrease in circulating CD4⁺GFP⁺ cells was observed, along with a decrease in circulating basophils following influenza treatment. During influenza infection, increased levels of circulating CD4⁺GFP⁺ cells were

observed (Fig. 1C) coincident with a pronounced infiltration of CD8⁺CD69⁺ cells to the lung (data not shown). Considering the host phase of the *N. brasiliensis* life cycle involves migration through multiple organs and the induction of a multitissue immune response, it is reasonable we find a comparatively larger basophil response than that induced for protein/adjuvant challenge models. It is additionally interesting to note that *N. brasiliensis* infection led to the recruitment of similar numbers of basophils and eosinophils to the lung ($\sim 1.2 \times 10^6$ cells/lung), whereas the allergic airway models resulted in significant airway eosinophilia,

with only a moderate increase in basophil recruitment. Even in the case of KLH/Alum, which led to a 16-fold increase of lung eosinophils, lung basophils were not markedly increased. The comparison of a primary helminth infection with allergen challenge models is not exactly parallel because mice sensitized with KLH/Alum and OVA/Alum both had specific IgE present (31) and loaded on their basophils at the time of i.n. allergen challenge, whereas no specific IgE was present at the time of primary helminth infection. Thus, we evaluated blood basophilia during KLH/Alum and OVA/Alum sensitization and determined that initial

FIGURE 2. Timing and magnitude of basophil induction in response to a primary and secondary *N. brasiliensis* infection. The total numbers of non-CD4 FcεR1α⁺ GFP⁺ basophils were measured in the blood, lung, BAL, Md Ln, and Ms Ln of *G4/G4* mice at various time points after primary (clear) and secondary (black) *N. brasiliensis* infections (A). B and C, Comparison of GFP⁺ basophil numbers in uninfected animals (gray) at the peak of primary (day 9, clear) and secondary (day 6, black) responses. GFP basophil numbers shown are per milliliter of blood. Blood data are the average of nine mice from three independent experiments; lung data are from six mice from two experiments and liver, Md Ln, and Ms Ln three mice per time point from a single experiment.



priming with KLH/Alum and OVA/Alum led to an increase in the level of circulating basophils (Supplemental Fig. 1A), whereas the CD4⁺GFP⁺ cell levels remained unchanged (Supplemental Fig. 1B). Hence, these results indicate that helminths such as *N. brasiliensis* may secrete factors that specifically induce the recruitment of basophils to peripheral tissues.

Kinetics and magnitude of basophil induction in response to a primary and secondary *N. brasiliensis* infection

As *N. brasiliensis*-induced basophilia was the most pronounced of the models tested, we next sought to examine whether IL-4–producing basophils were also a prominent feature of the memory Th2 response induced following secondary infection with *N. brasiliensis*. We have previously reported that both the enhanced basophil production and subsequent peripheral tissue accumulation following primary *N. brasiliensis* infection are IL-4–independent processes (32). To compare basophil responses during primary and secondary *N. brasiliensis* infection, groups of IL-4–deficient G4/G4 mice were infected with 600 L3 *N. brasiliensis* and, where indicated, reinfected with 600 L3 *N. brasiliensis* 30 d after the primary infection. Blood, liver, lung, bronchoalveolar lavage (BAL), Md Ln, and Ms Ln cells (Fig. 2A) were isolated at the indicated time points and the numbers of GFP⁺ CD4⁺FcεRIα⁺ basophils followed by flow cytometric analysis. Few GFP⁺ basophils were found in the tissues of naive animals: 2.5×10^5 /ml blood, 9×10^4 /lung, and 4×10^4 /liver, comprising on average 3.0, 0.6, and 1% of total blood, lung, and liver cells, respectively. Following infection, basophils were found to be significantly elevated, 10- to 12-fold at the peak of the primary response (day 9), rapidly decreasing thereafter (Fig. 2A) (4). Following secondary *N. brasiliensis* infection, mice generated a basophilic response in the blood and lung, which peaked at day 6 postinfection, 3 d earlier than the peak response observed following primary infection (Fig. 2A). Surprisingly, the magnitude of the secondary basophil response was not found to be greater than that observed during the primary response (Fig. 2B). Basophil numbers in the liver during both primary and the secondary infection showed identical kinetics to that observed in the lung. Additionally, basophilic infiltration into the BAL followed a similar pattern (Fig. 2A). It was recently reported that blood basophils are recruited into the draining lymph nodes following allergen challenge (33); the reported recruitment was transient in nature, peaked at day 3 post papain challenge, and rapidly disappeared from lymphoid sites thereafter (33). It was also demonstrated that the recruited basophils produce IL-4 and TSLP, both of which appear to contribute to Th2 differentiation. In support of this, depletion of basophils during papain challenge abolished the development of IL-4–producing Th2 cells (33). Thus, to determine whether basophils are also recruited into the draining lymph nodes during *N. brasiliensis* infection, basophils were measured in both the Md and the Ms Ln following infection. Similar to papain challenge, basophil numbers also peaked in the Md Ln at day 3 following both primary and secondary infection (Fig. 2A). Unlike other tissues tested, basophil recruitment to the Md Ln after secondary infection was significantly higher than observed following primary infection. Basophil recruitment in the Ms Ln peaked at day 9 post primary infection, and recruitment to the Ms Ln after secondary infection peaked at day 3 postinfection but was reduced in comparison with primary infection (Fig. 2C). Basophil recruitment was only observed in lymph nodes draining the sites of inflammation and was not observed in nondraining inguinal lymph nodes (data not shown), suggesting that the recruitment is induced by Ag drainage and possibly by the local induction of immune responses. Taken together, these results demonstrate that

basophil responses following secondary *N. brasiliensis* infection are induced more rapidly in comparison with primary infection. However, no significant increase in the magnitude of the basophil response during secondary infection was observed except in the draining Md Ln.

Basophils become fully activated upon migration into tissues

Basophil activation was assessed via measurement of GFP mean fluorescence intensity (MFI) in G4/G4 mice as a measure of potential for the production of IL-4 and was compared following primary infection in blood, Ms Ln, and BAL samples (Fig. 3A, Supplemental Fig. 2). GFP intensity was not found to be significantly increased in circulating basophils following *N. brasiliensis* infection (Fig. 3C). Upon migration into either the airways (BAL) or Ms Ln, basophils were found to have an increased GFP MFI (Fig. 3C), indicating that although basophils are not tissue resident, localization to tissues leads to an increase in effector function. To determine whether the circulating basophils observed following infection comprise a recently activated immature subset that are unable to respond to activation stimuli, MAR-1 was administered via i.v. injection. As MAR-1 has previously been described as a basophil-depleting (12) agent, we assessed blood basophilia at 12 h after MAR-1 administration and determined that a significant proportion of basophils were still present in peripheral blood. However, the percentage of basophils observed had only decreased slightly from 0.9% (± 0.1) to 0.7% (± 0.2) of CD45⁺ cells present in peripheral blood (Fig. 3B). Additionally the majority of basophils present (>75%) displayed an increased GFP MFI (Fig. 3D), indicating they were able to be fully activated in response to the MAR-1–induced cross-linking of surface FcεRI and constitute a mature basophilic population. Thus, the majority of basophils that have recently emigrated from the bone marrow are sufficiently matured such that they rapidly increase production of IL-4 in response to activation stimuli, such as FcεRI cross-linking. However, during the course of a natural infection,

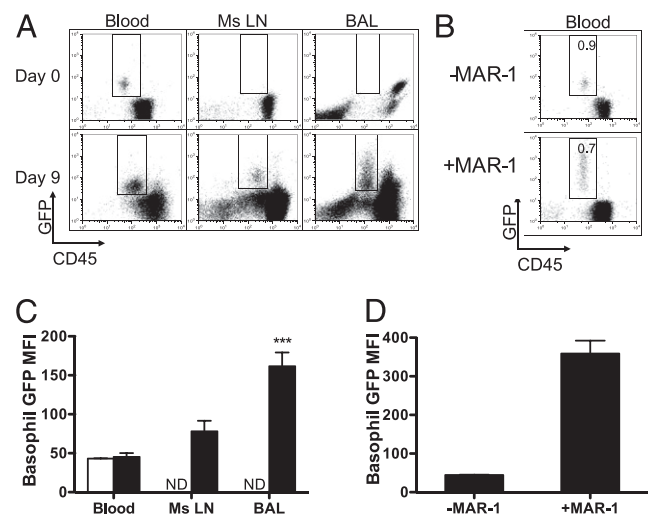


FIGURE 3. Basophil GFP MFI in tissues. **A**, FACS plots of non-CD4 cells from uninfected, day 9 blood, BAL, and Ms Ln of primary *N. brasiliensis*-infected G4/G4 mice, showing FcεRIα⁺GFP⁺ basophils. **B**, FACS plot of peripheral blood 12 h following i.v. administration of MAR-1. Indicated percentages represent the proportion of GFP⁺ basophils among total CD45⁺ cells. **C**, MFI of basophils present in blood, BAL, and Ms Ln at days 0 (clear) and 9 (black) postinfection. **D**, MFI of basophils present in peripheral blood at day 1 ± MAR-1. Data points shown indicate mean $\pm$ SE from three individual animals from two experiments. *** $p \leq 0.0001$; no asterisk, $p > 0.05$, relative to day 9 blood basophils with Student *t* test. ND, none detected.

basophils require additional activation to become fully activated, as migration into a tertiary tissue environment, such as the lung, was required to induce fully enhanced IL-4 production (Fig. 3A, 3C, Supplemental Fig. 2) as opposed to that observed in the blood or lymph nodes. Thus, it is possible that upon migration into the tissue environment, basophils receive a secondary signal that allows them to fully mature or that the level of parasite Ag present in the tissue is sufficiently high compared with that encountered in the blood or lymphoid compartments, such that FcERI cross-linking associated maturation occurs. Further investigations are required to assess these possible mechanisms.

*The number of GFP-expressing basophils induced in response to a secondary *N. brasiliensis* infection is not dependent on IL-4 or STAT6*

We have previously demonstrated that STAT6 signaling is not required for basophil accumulation or GFP (IL-4) expression during the course of primary *N. brasiliensis* infection (4). To examine the role of STAT6 during secondary infection, groups of *N. brasiliensis*-infected G4/IL-4, G4/G4, and G4/G4 STAT6 knock-out (KO) mice were reinfected with *N. brasiliensis* 30 d post-primary infection as described above, and basophil numbers were examined at days 6 and 9 following secondary infection. As shown in Fig. 4A, the levels of basophil induction following infection in the lung were comparable among G4/IL-4, G4/G4, and G4/G4 STAT6 KO groups. Total basophil numbers found in the lung, liver, and blood were also indistinguishable among all three groups, suggesting that IL-4 and STAT6 signaling has no role in the induction of basophilia during *N. brasiliensis* infection (Fig. 4B). Furthermore, basophil recruitment into the Md Ln and Ms Ln following the secondary infection with *N. brasiliensis* was not affected by the absence of IL-4 or STAT6 (Fig. 4C). Indeed, when blood basophils were examined in G4/IL-4 STAT6 KO and G4/G4 STAT6 KO mice throughout the 50-d period during primary and secondary *N. brasiliensis* infection, neither the presence nor absence of IL-4 in the complete absence of STAT6 altered the kinetics of circulating basophils (Fig. 4D). These results demonstrate that, as is the case with primary infection, the induction and migration of GFP-producing basophils during a secondary response to *N. brasiliensis* is not dependent on STAT6, further suggesting that other cytokines that use the STAT6 signaling

pathway, such as IL-13, are not involved in the induction of basophilic responses.

*The timing and magnitude of CD4⁺ T cell and eosinophil induction in response to a primary and secondary *N. brasiliensis* infection*

CD4⁺ T cells and eosinophils are also important sources of IL-4 in Th2 immune responses (13, 34, 35). In this study, we examined the involvement of such GFP/IL-4-producing cell types in our primary and secondary *N. brasiliensis* models. Induction of GFP-expressing CD4⁺ T cells after secondary *N. brasiliensis* infection closely followed the faster kinetics of GFP⁺ basophil induction, with peak GFP expression by CD4⁺ cells occurring at day 6 postinoculation (p.i.) in both blood and lung (Fig. 5A). In contrast to the basophilic response in which secondary infection did not increase the magnitude of the response, ~3-fold more GFP⁺ CD4⁺ T cells were detected in the lung following secondary infection.

Others have previously observed a significant number of GFP⁺ eosinophils in the lungs of *N. brasiliensis*-infected 4get mice (13, 34). Eosinophils were defined in this study as the population of lung cells that exhibited high side scatter, low forward scatter, and lacked expression of the FcεR1 and CD4. This eosinophilic phenotype was confirmed using standard cytochemical and histological criteria following fluorescence-assisted cell sorting (data not shown). Analysis of BAL eosinophilia revealed a significant proportion of both G4/IL-4 and G4/G4 eosinophils expressed GFP, 8.6 and 7.4%, respectively (Fig. 5C). GFP⁺ eosinophils were induced with faster kinetics and were detected in greater numbers following secondary infection in comparison with primary infection (Fig. 5B), comprising 1% and 0.7% of total cells at the peak of the secondary and primary response, respectively. GFP⁺ eosinophils were not detected in the blood or lymph nodes (data not shown). Therefore, both the kinetics and magnitude of the GFP⁺ CD4⁺ and GFP⁺ eosinophil response is enhanced upon secondary infection.

*The differential contribution to IL-4 production by basophils, CD4⁺ T cells, and eosinophils in response to primary and secondary *N. brasiliensis* infection*

We have shown that GFP-expressing basophils, CD4⁺ T cells, and, to a lesser extent, eosinophils are induced in both the primary and secondary *N. brasiliensis*-infected lung. Previously, Gessner et al.

FIGURE 4. Number of GFP-producing basophils in response to a secondary *N. brasiliensis* infection is not dependent on IL-4 or STAT6. The total number of GFP-expressing basophils was detected following a secondary *N. brasiliensis* infection in G4/IL-4 (gray), G4/G4 (clear), and G4/G4 × STAT6 ko (black) mice. A, Kinetics of GFP basophil induction in the lung. Total number of GFP basophils in the lung, liver, blood (B), Md Ln, and Ms Ln (C) day 6 postinfection. D, Kinetics of GFP basophil induction in the BAL following primary (day 0) and secondary (day 30) infection with *N. brasiliensis* in G4/IL-4 × STAT6 ko (gray) and G4/G4 × STAT6 ko (black) mice. Data points shown indicate mean ± SE from three individual animals from two experiments.

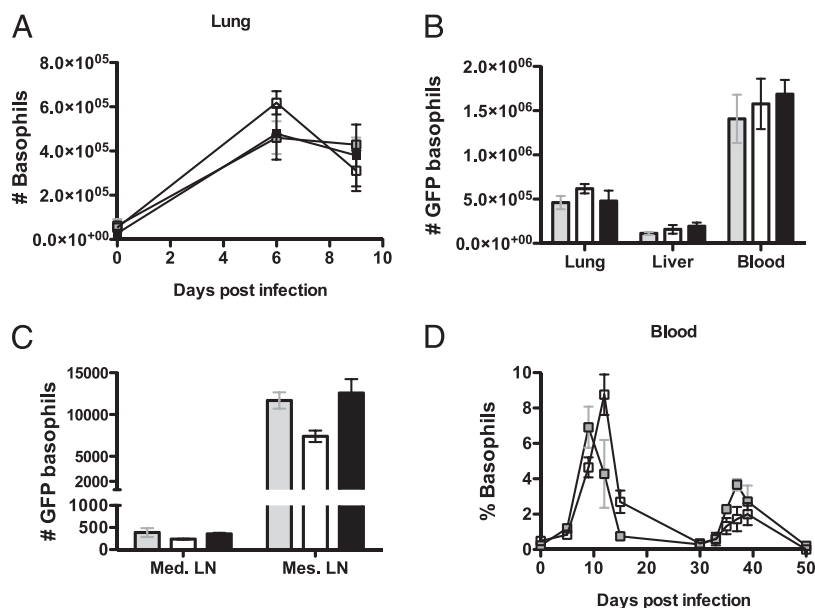
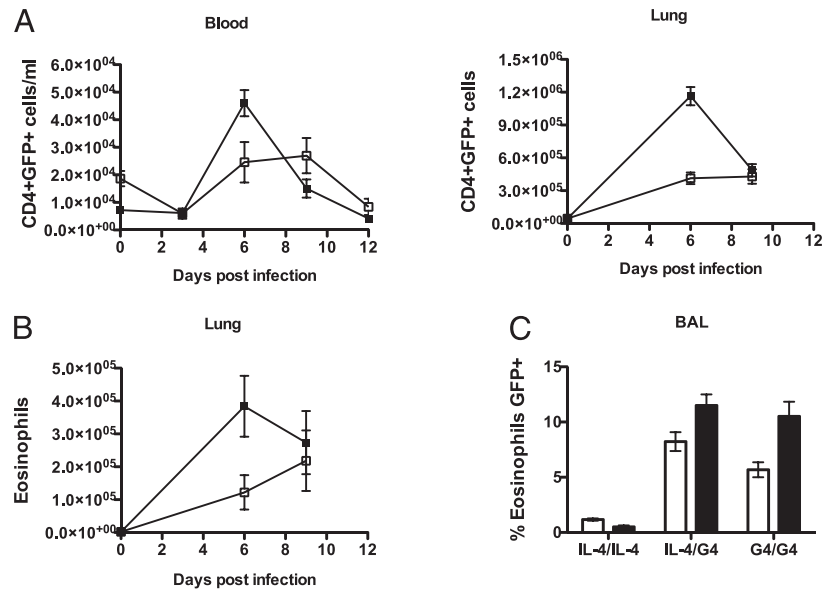


FIGURE 5. Timing and magnitude of CD4 T cells and eosinophil induction in response to primary and secondary *N. brasiliensis* infection. The total number of GFP-expressing CD4⁺ T cells (A) and GFP-expressing eosinophils (B) was measured in the blood and lung of *G4/G4* mice at various time points after primary (clear) and secondary (black) *N. brasiliensis* infections. C, Proportion of GFP-expressing eosinophils in BAL at the peak of primary (day 9, clear) and secondary (day 6, black) responses. Blood, lung, and BAL fluid was collected and expression of CD4 and GFP by SSC^{Hi} and SSC^{Lo} cells was examined. SSC^{Hi} cells had previously been identified as >95% eosinophils following fluorescence-assisted cell sorting and identification by Difquick staining according to using standard morphological and cytochemical criteria. Data points shown indicate mean $\pm$ SE from three individual animals from two experiments.



(28) have reported that Th2 cells and basophils isolated from blood produced similar amounts of IL-4 on a per-cell basis following activation and that eosinophils produced ~0.6-fold as much IL-4 per cell. However, as we previously observed that lung-associated basophils are more highly activated, we felt it important to directly compare the amount of IL-4 produced by basophils and Th2 cells that had been specifically recruited to the lung. Thus, at day 9 postinfection with live *N. brasiliensis*, whole lungs were processed to a single-cell suspension, Th2 cells were activated with T cell activator anti-mouse CD3/CD28 beads, and basophils activated with anti-mouse IgE (6HD5) for 6 h in the presence of anti-IL-4R α to prevent protein uptake (Fig. 6A). Specific numbers of IL-4 producers were measured by ELISPOT, and ELISA determined total IL-4, thus a direct comparison of IL-4 production per cell was able to be determined ex vivo. Our results indicated that there was no statistically significant difference in the production of IL-4 by basophils (0.13 ± 0.07 U/cell) and Th2 cells (0.08 ± 0.05 U/cell). To determine the relative contribution of these cell types to total IL-4 production, we compared the number of GFP-expressing basophils, CD4⁺ cells and eosinophils in the lung day 9 following a primary and day 6 following a secondary *N. brasiliensis* infection (Fig. 6B). The majority of GFP-producing cells during the primary response were basophils (65%), with CD4⁺ T cells (25%) and eosinophils (10%) comprising the remainder of the GFP⁺ population. Consistent with the

notion that reinfection induces a stronger immune response, secondary infection resulted in 1.5-fold more GFP⁺ cells in the lung than did the primary. Although the magnitude of the GFP⁺ CD4⁺ T cell and GFP⁺ eosinophil response tripled, indicating that CD4⁺ T cells were the main source of IL-4 during a recall response, there were in fact 0.7-fold fewer GFP⁺ basophils induced during secondary infection, implying that although GFP⁺ basophils are a key feature of the secondary response to *N. brasiliensis*, they are not subject to the enhanced response that is characteristic of memory responses associated with the adaptive immune system. Overall, this data indicates that basophils are the main source of IL-4 in the primary lung response to *N. brasiliensis*, whereas CD4⁺ T cells are the predominant IL-4-producing cells during secondary infection.

Basophils do not influence Th2 differentiation in response to N. brasiliensis infection

As previous papers (20, 21, 36) have indicated that basophils may play a significant role in the presentation of Ag to CD4⁺ cells and the induction of Th2 priming, we sought to ascertain whether basophils were required for Th2 induction using the dead *N. brasiliensis* model of infection (23), which allowed us to study a localized response to *N. brasiliensis* independent of secondary features of inflammation, which may be induced by the migration of *N. brasiliensis* through host tissues. IL-4/G4 animals were

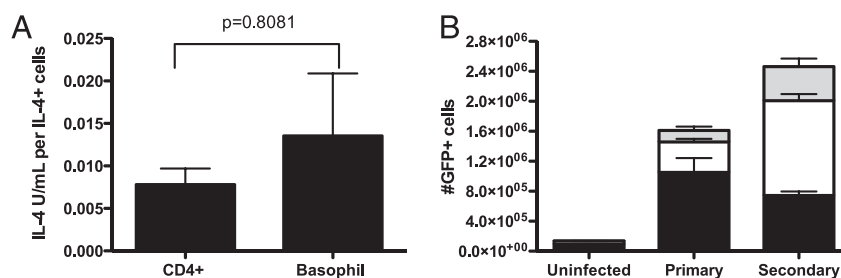


FIGURE 6. Differential contribution to IL-4 production by basophils, CD4 T cells, and eosinophils in a primary and secondary *N. brasiliensis* infection. A, BALB/c animals were infected s.c. with 600 L3 *N. brasiliensis*, and at day 9 postinfection, lung-resident CD4⁺ cells were stimulated with anti-mouse CD3/CD28 beads and basophils stimulated with anti-mouse IgE. IL-4 production was quantitated by ELISA and the number of cells producing IL-4 determined by ELISPOT. IL-4 production on a per-cell basis was calculated as units of IL-4 produced per IL-4-producing cell. Data points shown indicate mean $\pm$ SEM from three replicates from two individual experiments. B, The total number of GFP⁺ basophils, GFP⁺ CD4 cells, and GFP⁺ eosinophils were detected in lungs of *G4/G4* mice 9 d following a primary and 6 d following a secondary *N. brasiliensis* infection and compared with levels seen in uninfected mice. Data points shown indicate mean $\pm$ SE from three individual animals from two experiments.

depleted of basophils following i.v. administration of MAR-1 at day -2 (Fig. 7A); following this, depleted and nondepleted animals were infected intradermally with dead *N. brasiliensis* and the development of a localized Th2 response determined. At the peak of response (day 7 p.i.), there was no discernable difference in the total numbers of CD45⁺, CD4⁺, or CD4⁺GFP⁺ cells present in the draining lymph nodes of infected animals. Following the administration of MAR-1, background levels of basophils were detected in both naive and depleted animals (~20–30 per lymph node), whereas a 50–60-fold increase in the number of basophils was detectable in PBS-treated nondepleted animals following infection (Fig. 7B). These data suggest that the recruitment of basophils to the lymph node does not have a significant effect on the induction of Th2 differentiation. Previous studies (12, 36) using papain as an adjuvant have indicated that basophil-derived IL-4 may be important for the induction of Th2 differentiation. To determine whether IL-4 was required for Th2 differentiation in this system, we transferred either +G4 or G4/G4 Rag-deficient CD4⁺ cells specific for PCC (5CC7) into either wild-type B10.A or IL-4-deficient G4/G4 hosts and primed them with papain and PCC. At day 4 p.i., there was no significant difference observed in the total number of cells or number of CD4⁺ cells present in draining lymph nodes (Supplemental Fig. 3). In the absence of IL-4, Th2 differentiation was not found to be impaired as measured by the induction of GFP expression by CD4⁺ cells present (Supplemental Fig. 3).

We additionally examined the effect of basophil depletion on cellular infiltrate to the inflamed dermis and determined that treatment with MAR-1 actually increased the total number of CD45⁺, CD4⁺, CD4⁺GFP⁺ cells (Fig. 7C), and eosinophils (Fig. 7D) present. Following MAR-1 treatment, background levels of basophils were present at levels comparable to naive controls (Fig. 7C), whereas significant numbers of basophils were recruited to the dermis following treatment with PBS, thus indicating that basophils may actually be playing a role in regulating the recruitment of effector cells to the dermis during type 2 inflammation.

Discussion

The role of basophil-derived IL-4 has so far remained elusive, in part due to difficulties in basophil identification and the lack of appropriate KO animal models. The use of G4 mice has enabled us to directly investigate the involvement of IL-4-expressing basophils in response to infection with *N. brasiliensis*, without the need for ex vivo restimulation. Following both primary and secondary *N. brasiliensis* infection, IL-4-expressing basophils were found systemically at various tissue sites including the lung, liver, lymph nodes, and blood. Basophils were also present in mice lacking IL-4 and STAT6, indicating their induction and migration to these sites does not require IL-4 or STAT6 signaling. Alternatively, as basophils have been observed to enhance humoral immunity through the production IL-6 (19), it is possible that basophils recruited to the lymph node are required for enhanced levels of IgG2a, IgG2b, and IgG3 observed in response to *N. brasiliensis* in the absence of IL-4 and may form an alternate positive-feedback loop to increase basophil production in the absence of specific IgE or IgG1.

As is characteristic of a memory response, IL-4-expressing basophils were induced more rapidly upon secondary infection, peaking at day 6 following infection, 3 d prior to the primary peak at day 9. Surprisingly, the magnitude of the secondary GFP⁺ basophil response was not enhanced and was even reduced in some tissues. Other IL-4-producing subsets were also examined, and both GFP⁺ CD4⁺ T cells and GFP⁺ eosinophils exhibited faster secondary kinetics, peaking on day 6 postinfection. In contrast, the

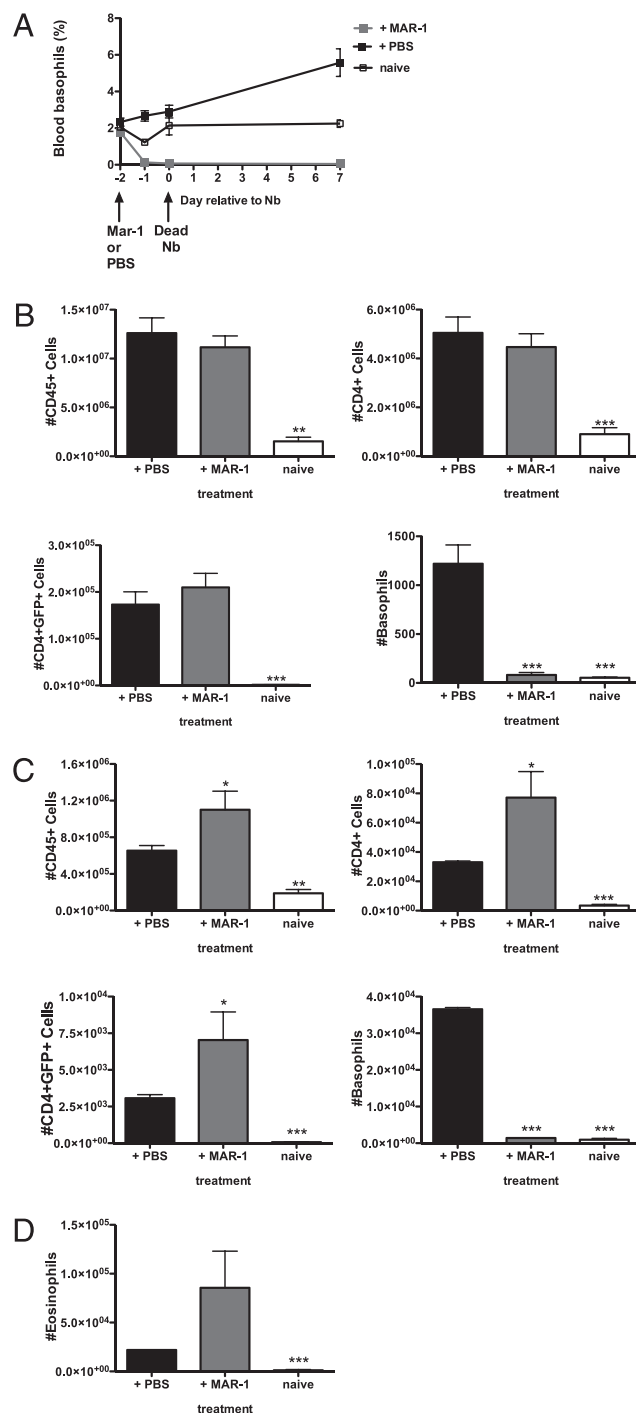


FIGURE 7. Basophil and the initiation of type 2 immune responses. IL-4/G4 animals were administered either MAR-1 or PBS via i.v. injection 48 h prior to intradermal infection with 600 dead *N. brasiliensis*. A, Time course of basophils present in the blood as a percentage of CD45⁺ cells present. Total numbers of CD45⁺, CD4⁺, and CD4⁺GFP⁺ cells present were determined by flow cytometry in the draining lymph node (B) and ear (C) at 7 d postinfection. D, Total number of GFP⁺ eosinophils present in ear dermis. Data points shown indicate mean $\pm$ SE from three individual animals from two experiments. * $p \leq 0.01$; ** $p \leq 0.001$; *** $p \leq 0.0001$, no asterisk, $p > 0.05$, relative to infection plus PBS with Student *t* test.

numbers of basophils found in the lung were increased 3-fold at the peak of the primary response. Interestingly, when the various contributors to overall IL-4 production are compared, it is evident that basophils are the main cellular source of IL-4 during the primary response, whereas CD4⁺ T cells are the major source of

IL-4 during memory responses. Additionally, we extend the findings of Sokol et al. (33) concerning the recruitment of basophils to lymph nodes in an allergen-based Th2 model by demonstrating that the kinetics of basophilic accumulation following parasitic infection in primary and secondary infection generally precedes total lymphoid expansion and Th2 induction via a transient recruitment to draining lymphoid organs. Basophil-dependent Ag presentation and production of both IL-4 and TSLP have been identified as potential enhancers of Th2 differentiation (20, 21, 36). However, we have previously observed that in vivo Th2 differentiation occurs independently of IL-4/STAT6 (23), and we have additionally noted in this paper that maximal basophil IL-4 production as measured by GFP does not occur in the lymphoid compartment, but was only observed upon recruitment of basophils to tissue sites such as the liver, lung, and BAL. This would indicate that basophils most likely exert the majority of their IL-4-dependent effects in peripheral tissues. Our finding that basophil depletion did not alter Th2 differentiation in the lymphoid compartment, but instead led to an increase in the recruitment of type 2 effector cells to peripheral tissues, further supports this view (Fig. 7D). As our data indicate that basophils are activated in response to MAR-1 injection (Fig. 3B) prior to their depletion (Fig. 7A), it is possible that administration of MAR-1 may be responsible for the increase in skin inflammation observed. However, we noted that basophil depletion was almost complete at day -1 prior to *N. brasiliensis* infection, with the percentage of blood basophils decreasing from 1.9% in naive animals to 0.1% with a further reduction in blood basophilia to 0.05% by the time of infection, thus indicating the virtual absence of basophils, activated or not, at the time of infection (Fig. 7A). In addition, it has previously been noted that the in vivo $t_{1/2}$ of cytokines is very short (37–39), with IL-4 having a reported $t_{1/2}$ of 5–30 min (40). Thus, any cytokines released through the activation of basophils via MAR-1 would likely be cleared prior to infection with *N. brasiliensis* and the initiation of the Th2 response.

Previous studies (12, 21, 36) noting that basophils may play a role in Th2 development have relied on in vitro models of Th2 differentiation in which the requirement for IL-4 is well described (1, 31, 32). Further, a recent study by Massacand et al. (41) has indicated that the absence of TSLP signaling had no impact on Th2 memory responses or the development of a protective immune response to *N. brasiliensis*, thus calling into question the previously perceived role for basophil-produced TSLP in Th2 differentiation.

Overall, these data indicate that basophils represent a significant source of IL-4 in both primary and secondary responses to parasites and allergens, whereas the production of CD4⁺ T cell-derived IL-4 is greatly increased in the secondary response as compared with basophilic IL-4. Basophil-derived IL-4, though not required for Th2 differentiation, is highly influential in amplifying both peripheral and humoral aspects of the broader type 2 responses.

Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.

References

- Le Gros, G., S. Z. Ben-Sasson, R. Seder, F. D. Finkelman, and W. E. Paul. 1990. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. *J. Exp. Med.* 172: 921–929.
- Finkelman, F. D., J. Holmes, I. M. Katona, J. F. Urban, Jr., M. P. Beckmann, L. S. Park, K. A. Schooley, R. L. Coffman, T. R. Mosmann, and W. E. Paul. 1990. Lymphokine control of in vivo immunoglobulin isotype selection. *Annu. Rev. Immunol.* 8: 303–333.
- Ohnmacht, C., and D. Voehringer. 2009. Basophil effector function and homeostasis during helminth infection. *Blood* 113: 2816–2825.
- Min, B., M. Prout, J. Hu-Li, J. Zhu, D. Jankovic, E. S. Morgan, J. F. Urban, Jr., A. M. Dvorak, F. D. Finkelman, G. LeGros, and W. E. Paul. 2004. Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J. Exp. Med.* 200: 507–517.
- Gregory, G. D., S. S. Raju, S. Winandy, and M. A. Brown. 2006. Mast cell IL-4 expression is regulated by Ikaros and influences encephalitogenic Th1 responses in EAE. *J. Clin. Invest.* 116: 1327–1336.
- Voehringer, D., T. A. Reese, X. Huang, K. Shinkai, and R. M. Locksley. 2006. Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. *J. Exp. Med.* 203: 1435–1446.
- Yoshimoto, T., and W. E. Paul. 1994. CD4pos, NK1.1pos T cells promptly produce interleukin 4 in response to in vivo challenge with anti-CD3. *J. Exp. Med.* 179: 1285–1295.
- Godfrey, D. L., and M. Kronenberg. 2004. Going both ways: immune regulation via CD1d-dependent NKT cells. *J. Clin. Invest.* 114: 1379–1388.
- Wedemeyer, J., M. Tsai, and S. J. Galli. 2000. Roles of mast cells and basophils in innate and acquired immunity. *Curr. Opin. Immunol.* 12: 624–631.
- Miyajima, I., D. Dombrowicz, T. R. Martin, J. V. Ravetch, J. P. Kinet, and S. J. Galli. 1997. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J. Clin. Invest.* 99: 901–914.
- Kawakami, T., and S. J. Galli. 2002. Regulation of mast-cell and basophil function and survival by IgE. *Nat. Rev. Immunol.* 2: 773–786.
- Sokol, C. L., G. M. Barton, A. G. Farr, and R. Medzhitov. 2008. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat. Immunol.* 9: 310–318.
- Voehringer, D., K. Shinkai, and R. M. Locksley. 2004. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* 20: 267–277.
- Mitre, E., R. T. Taylor, J. Kubofcik, and T. B. Nutman. 2004. Parasite antigen-driven basophils are a major source of IL-4 in human filarial infections. *J. Immunol.* 172: 2439–2445.
- Falcone, F. H., C. A. Dahinden, B. F. Gibbs, T. Noll, U. Amon, H. Hebestreit, O. Abrahamsen, J. Klauke, M. Schlaak, and H. Haas. 1996. Human basophils release interleukin-4 after stimulation with *Schistosoma mansoni* egg antigen. *Eur. J. Immunol.* 26: 1147–1155.
- Mukai, K., K. Matsuoka, C. Taya, H. Suzuki, H. Yokozeki, K. Nishioka, K. Hirokawa, M. Etori, M. Yamashita, T. Kubota, et al. 2005. Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. *Immunity* 23: 191–202.
- Luccioli, S., D. T. Brody, S. Hasan, A. Keane-Myers, C. Prussin, and D. Metcalfe. 2002. IgE(+), Kit(-), I-A/I-E(-) myeloid cells are the initial source of IL-4 after antigen challenge in a mouse model of allergic pulmonary inflammation. *J. Allergy Clin. Immunol.* 110: 117–124.
- Khodoun, M. V., T. Orekhova, C. Potter, S. Morris, and F. D. Finkelman. 2004. Basophils initiate IL-4 production during a memory T-dependent response. *J. Exp. Med.* 200: 857–870.
- Denzel, A., U. A. Maus, M. Rodriguez Gomez, C. Moll, M. Niedermeier, C. Winter, R. Maus, S. Hollingshead, D. E. Briles, L. A. Kunz-Schughart, et al. 2008. Basophils enhance immunological memory responses. *Nat. Immunol.* 9: 733–742.
- Sokol, C. L., N. Q. Chu, S. Yu, S. A. Nish, T. M. Laufer, and R. Medzhitov. 2009. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat. Immunol.* 10: 713–720.
- Perrigou, J. G., S. A. Saenz, M. C. Siracusa, E. J. Allenspach, B. C. Taylor, P. R. Giacomin, M. G. Nair, Y. Du, C. Zaph, N. van Rooijen, et al. 2009. MHC class II-dependent basophil-CD4⁺ T cell interactions promote T(H)2 cytokine-dependent immunity. *Nat. Immunol.* 10: 697–705.
- Kim, S., T. Shen, and B. Min. 2009. Basophils can directly present or cross-present antigen to CD8 lymphocytes and alter CD8 T cell differentiation into IL-10-producing phenotypes. *J. Immunol.* 183: 3033–3039.
- van Panhuys, N., S. C. Tang, M. Prout, M. Camberis, D. Scarlett, J. Roberts, J. Hu-Li, W. E. Paul, and G. Le Gros. 2008. In vivo studies fail to reveal a role for IL-4 or STAT6 signaling in Th2 lymphocyte differentiation. *Proc. Natl. Acad. Sci. USA* 105: 12423–12428.
- Jacobson, R. H., N. D. Reed, and D. D. Manning. 1977. Expulsion of *Nippostrongylus brasiliensis* from mice lacking antibody production potential. *Immunology* 32: 867–874.
- Taliaferro, W. 1939. The Cellular Reactions in the Skin, Lungs and Intestines of Normal and Immune Rats After Infection with *Nippostrongylus Muris*. *J. Infect. Dis.* 64: 157–192.
- Hu-Li, J., C. Pannetier, L. Guo, M. Löhning, H. Gu, C. Watson, M. Assenmacher, A. Radbruch, and W. E. Paul. 2001. Regulation of expression of IL-4 alleles: analysis using a chimeric GFP/IL-4 gene. *Immunity* 14: 1–11.
- Shen, T., S. Kim, J. S. Do, L. Wang, C. Lantz, J. F. Urban, G. Le Gros, and B. Min. 2008. T cell-derived IL-3 plays key role in parasite infection-induced basophil production but is dispensable for in vivo basophil survival. *Int. Immunol.* 20: 1201–1209.

28. Gessner, A., K. Mohrs, and M. Mohrs. 2005. Mast cells, basophils, and eosinophils acquire constitutive IL-4 and IL-13 transcripts during lineage differentiation that are sufficient for rapid cytokine production. *J. Immunol.* 174: 1063–1072.
29. Kaplan, M. H., U. Schindler, S. T. Smiley, and M. J. Grusby. 1996. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity* 4: 313–319.
30. Camberis, M., G. Le Gros, and J. Urban, Jr. 2003. Animal model of *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*. *Curr. Protoc. Immunol.* Chapter 19: Unit 19.12.
31. Le Gros, G., N. Schultze, S. Walti, K. Einsle, F. Finkelman, M. H. Kosco-Vilbois, and C. Heusser. 1996. The development of IgE+ memory B cells following primary IgE immune responses. *Eur. J. Immunol.* 26: 3042–3047.
32. Cote-Sierra, J., G. Foucras, L. Guo, L. Chiodetti, H. A. Young, J. Hu-Li, J. Zhu, and W. E. Paul. 2004. Interleukin 2 plays a central role in Th2 differentiation. *Proc. Natl. Acad. Sci. USA* 101: 3880–3885.
33. Sokol, C. L., G. M. Barton, A. G. Farr, and R. Medzhitov. 2008. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat. Immunol.* 9: 310–318.
34. Shinkai, K., M. Mohrs, and R. M. Locksley. 2002. Helper T cells regulate type-2 innate immunity in vivo. *Nature* 420: 825–829.
35. Chen, L., K. A. Grabowski, J. P. Xin, J. Coleman, Z. Huang, B. Espiritu, S. Alkan, H. B. Xie, Y. Zhu, F. A. White, et al. 2004. IL-4 induces differentiation and expansion of Th2 cytokine-producing eosinophils. *J. Immunol.* 172: 2059–2066.
36. Yoshimoto, T., K. Yasuda, H. Tanaka, M. Nakahira, Y. Imai, Y. Fujimori, and K. Nakanishi. 2009. Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. *Nat. Immunol.* 10: 706–712.
37. Finkelman, F. D., K. B. Madden, S. C. Morris, J. M. Holmes, N. Boiani, I. M. Katona, and C. R. Maliszewski. 1993. Anti-cytokine antibodies as carrier proteins. Prolongation of in vivo effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. *J. Immunol.* 151: 1235–1244.
38. Lotze, M. T., L. W. Frana, S. O. Sharrow, R. J. Robb, and S. A. Rosenberg. 1985. In vivo administration of purified human interleukin 2. I. Half-life and immunologic effects of the Jurkat cell line-derived interleukin 2. *J. Immunol.* 134: 157–166.
39. Sherman, M. L., D. R. Spriggs, K. A. Arthur, K. Imamura, E. Frei, III, and D. W. Kufe. 1988. Recombinant human tumor necrosis factor administered as a five-day continuous infusion in cancer patients: phase I toxicity and effects on lipid metabolism. *J. Clin. Oncol.* 6: 344–350.
40. Ma, Y., H. E. Hurst, and R. Fernandez-Botran. 1996. Soluble cytokine receptors as carrier proteins: effects of soluble interleukin-4 receptors on the pharmacokinetics of murine interleukin-4. *J. Pharmacol. Exp. Ther.* 279: 340–350.
41. Massacand, J. C., R. C. Stettler, R. Meier, N. E. Humphreys, R. K. Grencis, B. J. Marsland, and N. L. Harris. 2009. Helminth products bypass the need for TSLP in Th2 immune responses by directly modulating dendritic cell function. *Proc. Natl. Acad. Sci. USA* 106: 13968–13973.

Supplementary Figure 1: Basophil and CD4+GFP+ levels during allergen priming.

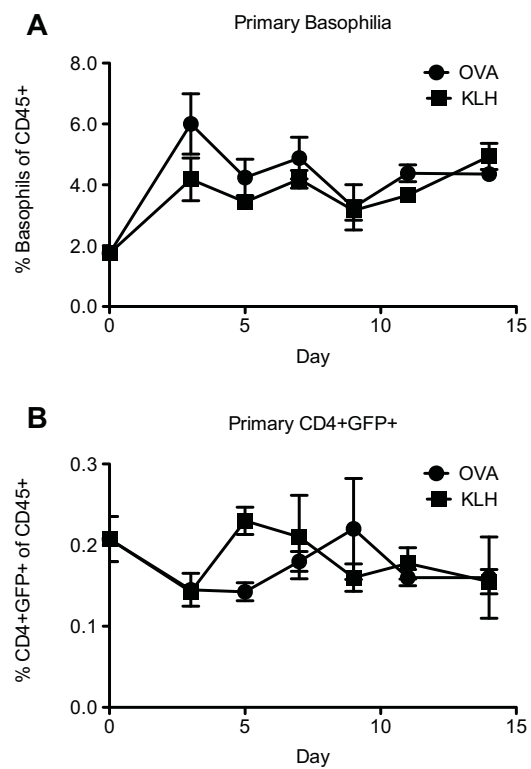
Following the intra peritoneal administration 2µg OVA in Alum or 4µg KLH in Alum on the days indicated the percentage of (A) basophils and (B) CD4+GFP+ cells present in blood was determined by flow cytometry.

Supplementary Figure 2: Basophils are further activated at the site of inflammation.

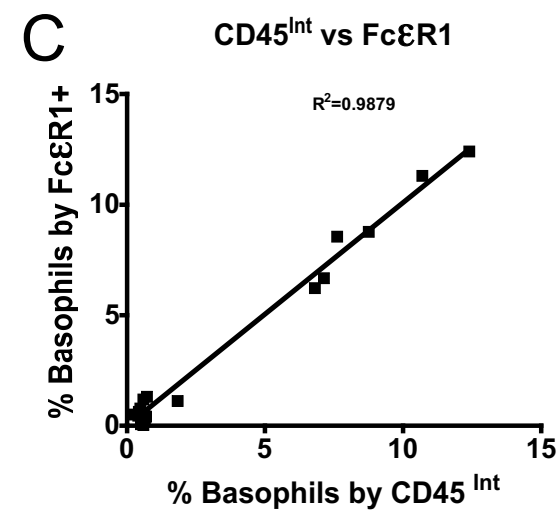
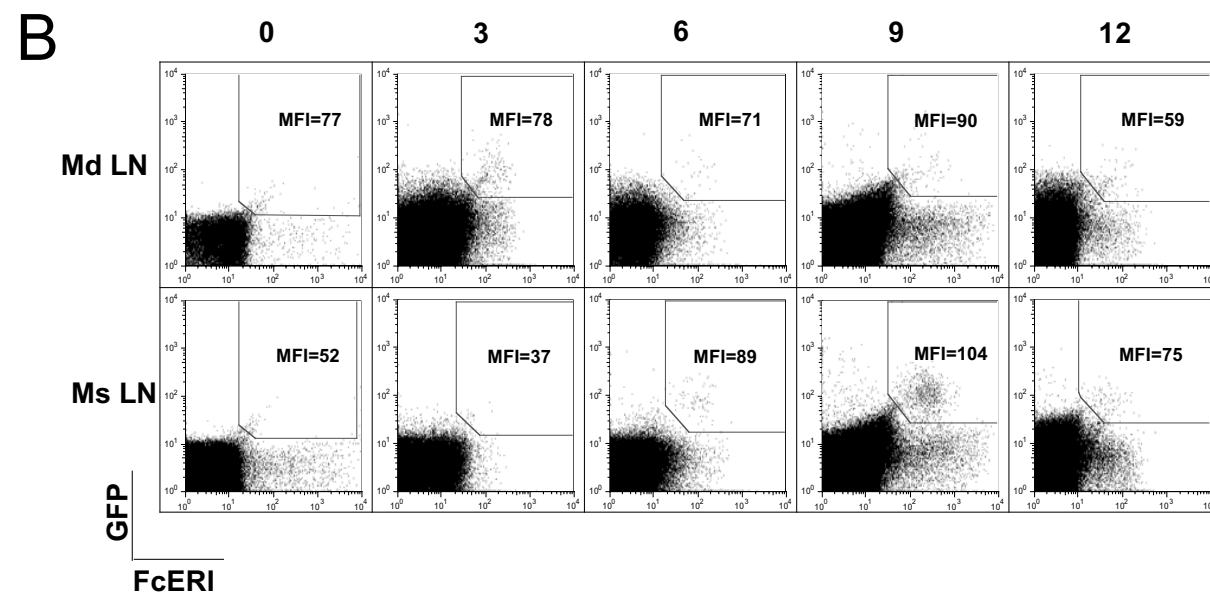
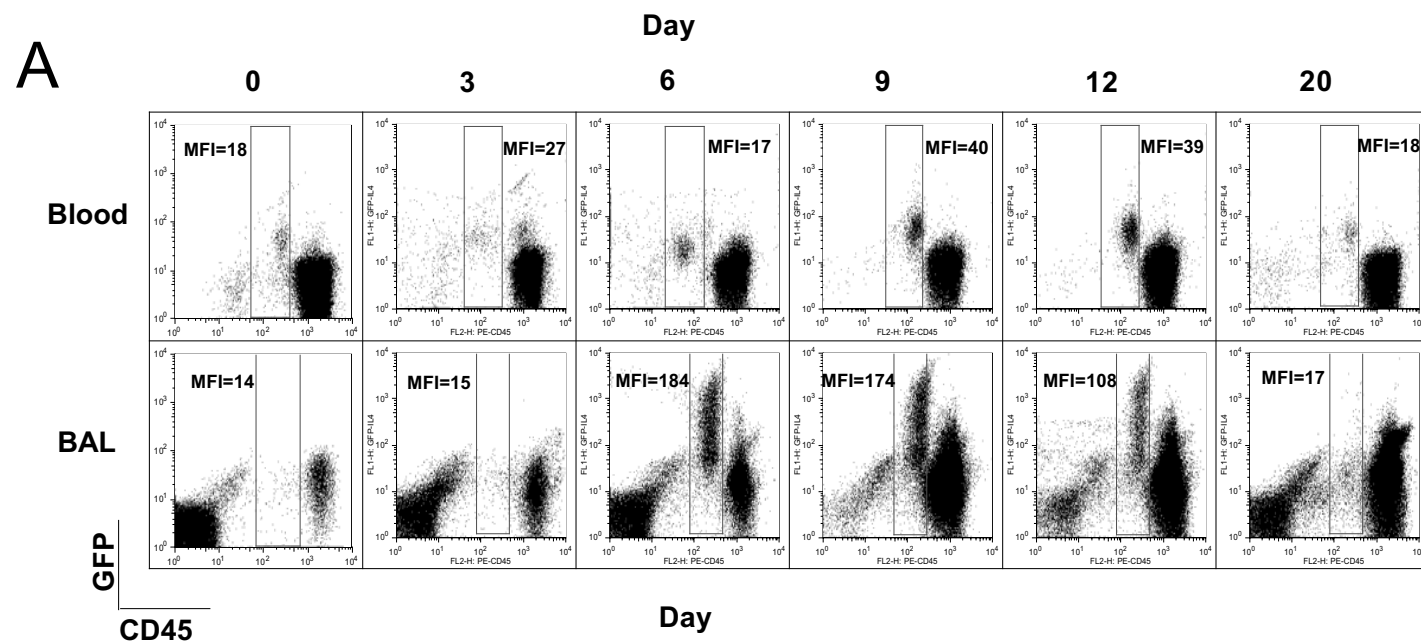
Basophils were isolated from the blood, BAL, mediastinal and mesenteric lymph nodes at days indicated following intra dermal infection with Nb from G4/G4 mice. The GFP MFI of basophils present was determined from basophils defined as (A) CD45-Int GFP+ for BAL and blood basophils and as (B) FcERI+GFP+CD4- for Md and Ms lymph node basophils. (C) Comparison of CD45-Int GFP+ and FcERI+GFP+ methods of basophils identification from BAL and Blood samples. Data shown is a representative plot from 3 animals used per time point.

Supplementary Figure 3: TH2 Priming with Papain is Independent of IL-4.

1x10⁶ CD4+ T cells from +/-G4 or G4/G4 5CC7 Rag-/- animals were adoptively transferred into either B10.A wild type or B10.A G4/G4 hosts. Following adoptive transfer animals were primed subcutaneously in the footpad with 50µg pPCC and 50µg Papain in PBS. At day 4 following administration both draining (dLN) and non-draining (n.dLN) lymph nodes were examined. Cells were processed to a single cell suspension and total cell numbers determined via trypan blue exclusion. Cells were stained with CD45, CD4, DX5 and Vα11 and cytometric analysis conducted to determine (A) frequency of TH2 development in PCC specific cells (CD4+Vα11+GFP+) and (B) total number of CD45+, CD4+DX5- and CD4+DX5-Vα11+GFP+ cells present.

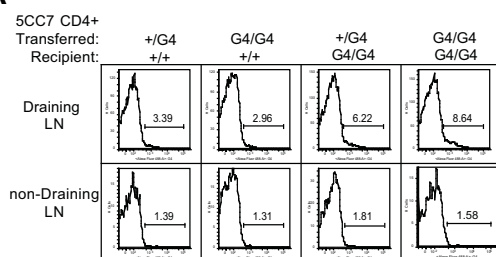


Supplementary Figure 1

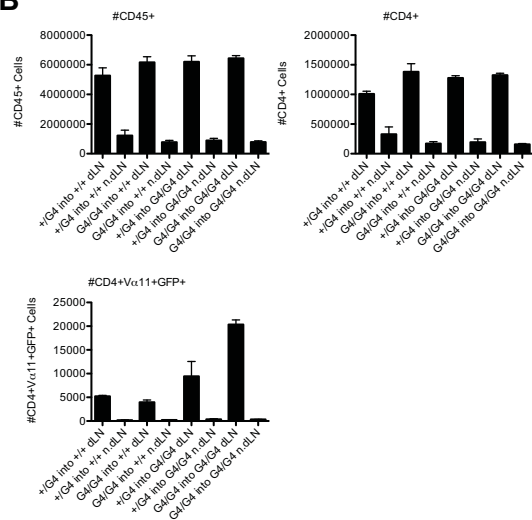


Supplementary Figure 2

A



B



Supplementary Figure 3

Supplementary Figure 1: Basophil and CD4+GFP+ levels during allergen priming.

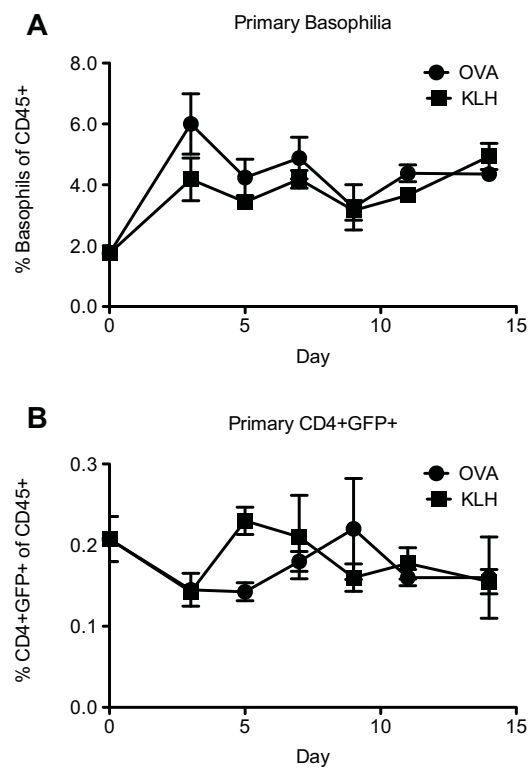
Following the intra peritoneal administration 2µg OVA in Alum or 4µg KLH in Alum on the days indicated the percentage of (A) basophils and (B) CD4+GFP+ cells present in blood was determined by flow cytometry.

Supplementary Figure 2: Basophils are further activated at the site of inflammation.

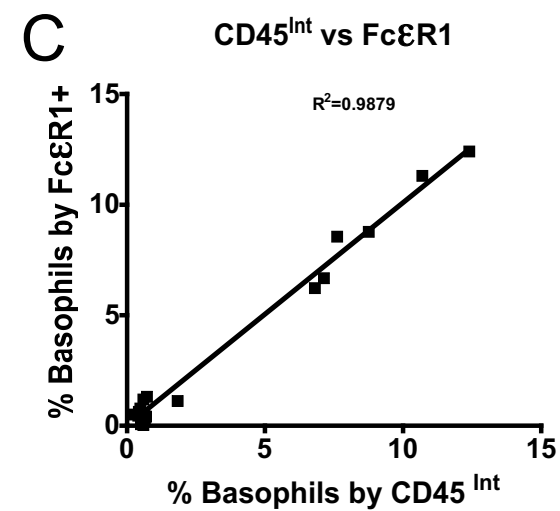
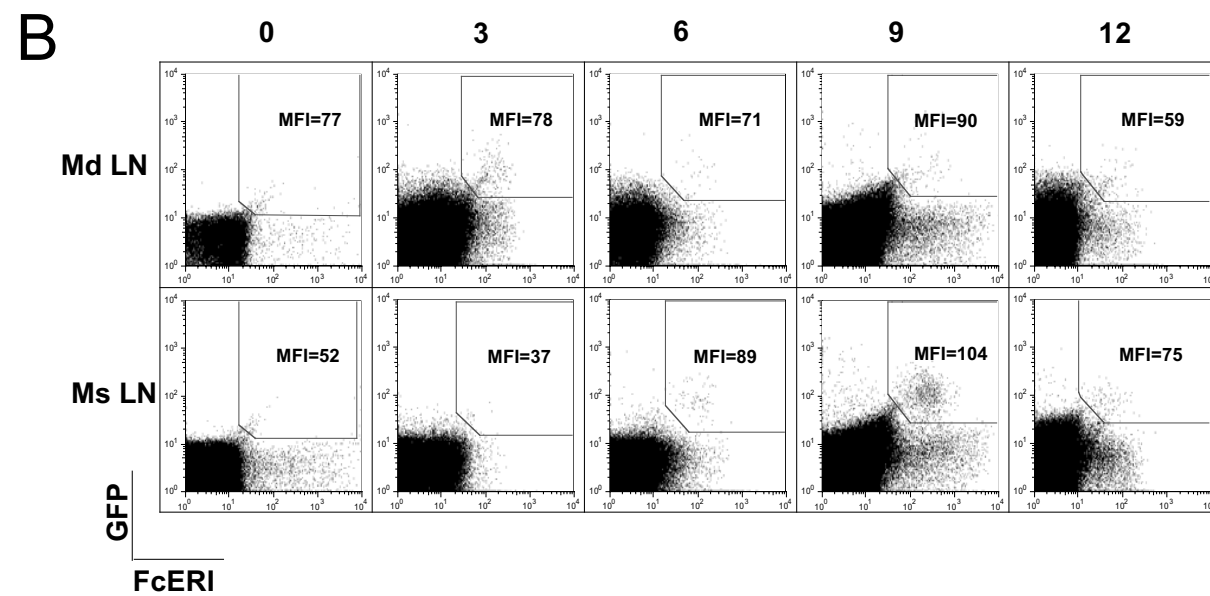
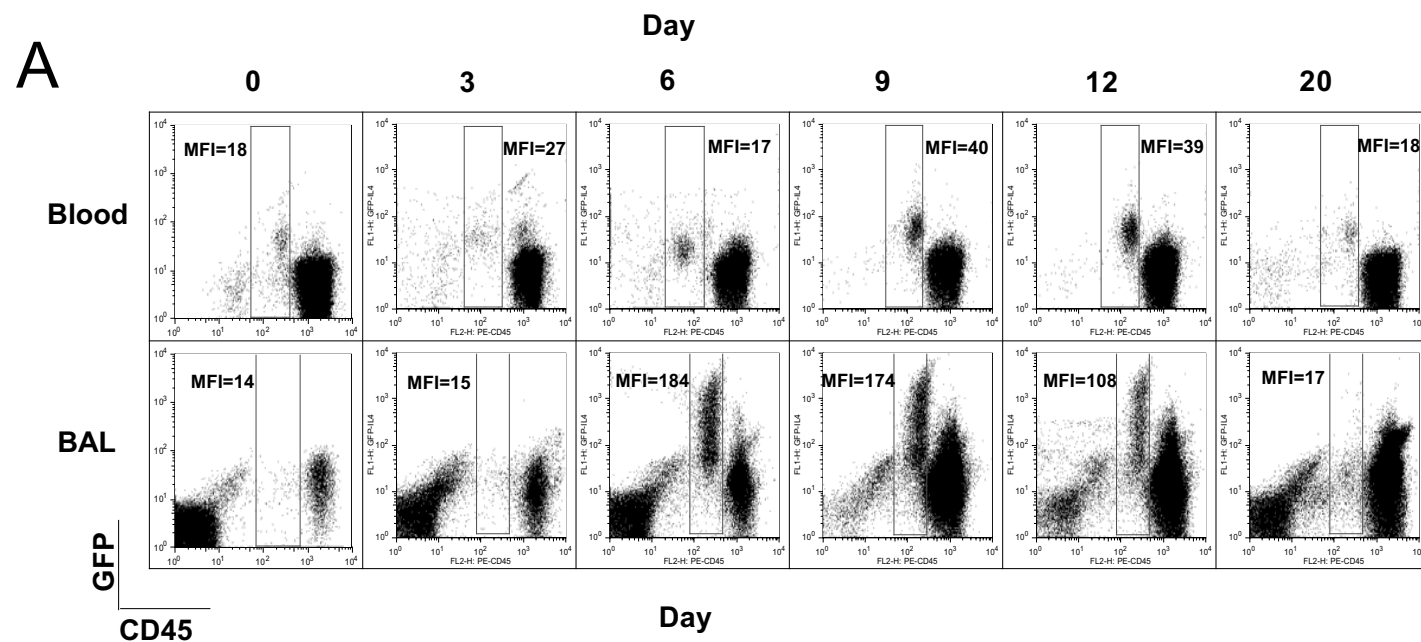
Basophils were isolated from the blood, BAL, mediastinal and mesenteric lymph nodes at days indicated following intra dermal infection with Nb from G4/G4 mice. The GFP MFI of basophils present was determined from basophils defined as (A) CD45-Int GFP+ for BAL and blood basophils and as (B) FcERI+GFP+CD4- for Md and Ms lymph node basophils. (C) Comparison of CD45-Int GFP+ and FcERI+GFP+ methods of basophils identification from BAL and Blood samples. Data shown is a representative plot from 3 animals used per time point.

Supplementary Figure 3: TH2 Priming with Papain is Independent of IL-4.

1x10⁶ CD4+ T cells from +/-G4 or G4/G4 5CC7 Rag-/- animals were adoptively transferred into either B10.A wild type or B10.A G4/G4 hosts. Following adoptive transfer animals were primed subcutaneously in the footpad with 50µg pPCC and 50µg Papain in PBS. At day 4 following administration both draining (dLN) and non-draining (n.dLN) lymph nodes were examined. Cells were processed to a single cell suspension and total cell numbers determined via trypan blue exclusion. Cells were stained with CD45, CD4, DX5 and Vα11 and cytometric analysis conducted to determine (A) frequency of TH2 development in PCC specific cells (CD4+Vα11+GFP+) and (B) total number of CD45+, CD4+DX5- and CD4+DX5-Vα11+GFP+ cells present.

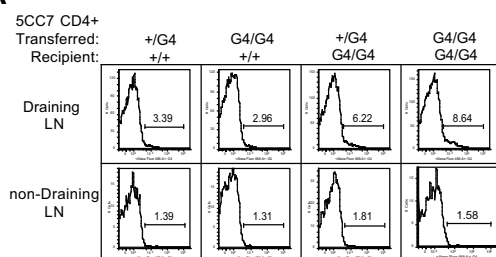


Supplementary Figure 1

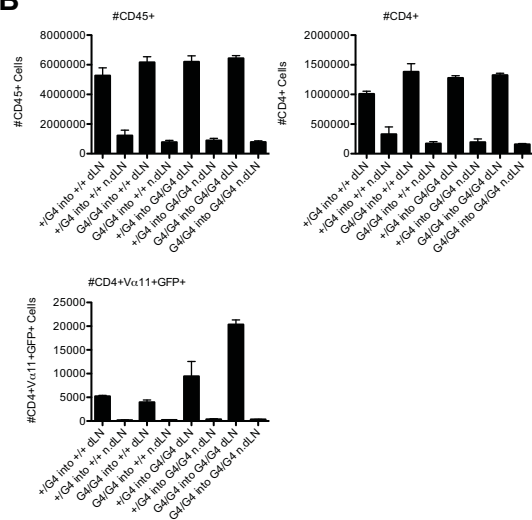


Supplementary Figure 2

A



B



Supplementary Figure 3