Eosinophil Deficiency Compromises Parasite Survival in Chronic Nematode Infection[1]

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Immune responses elicited by parasitic worms share many features with those of chronic allergy. Eosinophils contribute to the inflammation that occurs in both types of disease, and helminths can be damaged or killed by toxic products released by eosinophils in vitro. Such observations inform the widely held view that eosinophils protect the host against parasitic worms. The mouse is a natural host for *Trichinella spiralis*, a worm that establishes chronic infection in skeletal muscle. We tested the influence of eosinophils on *T. spiralis* infection in two mouse strains in which the eosinophil lineage is ablated. Eosinophils were prominent in infiltrates surrounding infected muscle cells of wild-type mice; however, in the absence of eosinophils *T. spiralis* muscle larvae died in large numbers. Parasite death correlated with enhanced IFN-γ and decreased IL-4 production. Larval survival improved when mice were treated with inhibitors of inducible NO synthase, implicating the NO pathway in parasite clearance. Thus, the long-standing paradigm of eosinophil toxicity in nematode infection requires reevaluation, as our results suggest that eosinophils may influence the immune response in a manner that would sustain chronic infection and insure worm survival in the host population. Such a mechanism may be deployed by other parasitic worms that depend upon chronic infection for survival. *The Journal of Immunology,* 2009, 182: 1577–1583.

Eosinophils are prominent in the inflammatory processes associated with allergy and helminth infection. Effector functions of eosinophils have been attributed largely to their granular contents, which include a collection of cytotoxic cationic proteins (eosinophil peroxidase, major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin) (1). However, studies in targeted gene knockout mice have shown that granule proteins do not contribute to disease in a model of allergic asthma (2). In contrast, degranulation of eosinophils onto the surfaces of helminth parasites in vitro injures the worm (3, 4). Eosinophil toxicity for helminths in vivo has been demonstrated most convincingly in model systems in which mice are implanted with nematodes encased in diffusion chambers (5, 6), although evidence supporting a protective role for eosinophils in immunity to helminths during primary, natural infections is not compelling (6–8). This limitation may be attributed to methodologies that have been available for blocking eosinophil function. Depletion, deficiency, or overproduction of IL-5 (9, 10) or CCR3 deficiency (11) affect production or recruitment of eosinophils but do not ablate the lineage.

For pathogens to establish chronic infection, they must develop the means either to evade or to regulate the immune response. Helminths are particularly adept at establishing a long-term relationship with their hosts, and we are only beginning to understand how they manipulate the immune system. Blood and tissue eosinophilia are prominent features of the response to worm infection. In this study, we describe the functional consequences of eosinophil deficiency during primary infection with a nematode that is a natural parasite of humans and rodents. The complex life cycle of *Trichinella spiralis* affords opportunities to investigate the influence of eosinophils during both intestinal and systemic infection. In mice, first stage *T. spiralis* larvae (L_1) initiate infection in the intestine. Within 4 days, fecund adults release newborn larvae (NBL)[4] that enter the general circulation (12) and extravasate in skeletal muscle (13). The diaphragm and tongue are preferred sites of infection. Each larva invades a single myotube and, over the course of 20 days, matures to become an infectious L_1 (14), thereby completing the life cycle. Infected muscle cells are known as nurse cells (15) and they provide a long-term intracellular habitat for the larva. Infected cells do not escape the notice of the immune system but rather induce a pronounced myositis that is dramatically down-modulated with time (16).

Although there is convincing evidence that eosinophils can kill *T. spiralis* larvae in vitro (3, 4), evidence in support of a role for eosinophils in host defense against *T. spiralis* in vivo is contradictory. Little or no alteration in parasite survival during a primary infection has been found in IL-5-deficient mice, IL-5 transgenic mice, or in mice depleted of eosinophils with specific Abs (9, 10, 17). It has been shown that CCR3-deficient mice fail to recruit eosinophils to nurse cells, and this finding was correlated with a

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[4] Abbreviations used in this paper: NBL, newborn larvae; LPF, low power field; iNOS, inducible NO synthase; AMG, aminoguanidine; dpoi, days post-oral infection; dpi, days postinfection; CLN, cervical lymph node; MLN, mesenteric lymph node; L-NIL, N^6(2-iminoethyl) L-lysine dihydrochloride.

reduction in the number of necrotic nurse cells observed in histologic sections of tongue (11).

In this study we describe the outcome of *T. spiralis* infection in two models of eosinophil ablation, Δdbl-GATA$^{-/-}$ (18) and PHIL (19). Eosinophil lineage development is blocked in Δdbl-GATA$^{-/-}$ mice that bear a deletion of the high-affinity double GATA site in the GATA-1 promoter (18). In contrast, PHIL mice were engineered to incorporate a coding sequence for diphtheria toxin A chain in the eosinophil peroxidase locus (19). Although both systems are characterized by eosinophil deficiency, some differences in responses have been noted. For example, when tested in a model of allergic respiratory inflammation, PHIL mice demonstrated reduced airway hyperresponsiveness (19), whereas Δdbl-GATA$^{-/-}$ mice showed reduced collagen deposition and tissue remodeling (18). More recent work revealed that the reported difference in phenotype is attributable to differences in genetic background. When the Δdbl-GATA mutant was backcrossed onto a C57BL/6 background, animals demonstrated reduced airway hyperresponsiveness similar to PHIL mice on the same genetic background (20).

Using these models of eosinophil deficiency, our studies with *T. spiralis* provide evidence that it may be the parasite, rather than the host, that relies upon eosinophils for survival.

Materials and Methods

Rats and mice

Adult Albino Oxford strain rats were produced and maintained in the James A. Baker Institute Vivarium (Ithaca, NY). IL-10$^{-/-}$ mice (C57BL/10 background), PHIL mice, and IL-10$^{-/-}$/PHIL mice (C57BL/6 background) were bred at Cornell Transgenic Mouse Core Facility and offspring were transferred to the James A. Baker Institute. Transgenic PHIL mice were identified as previously described (19). The sequences used to confirm the IL-10$^{-/-}$ genotype were sense 5′-CGG GAA GAC AAT AAC TG-3′ and antisense 5′-CAT TTC CGA TAA GGC TTG G-3′. The Δdbl-GATA$^{-/-}$ mice on a BALB/c background (18) were purchased from The Jackson Laboratory. BALB/c^{NHsd}, C57BL/6^{NHsd}, and C57BL/10^{ScNHsd} mice were purchased from Harlan Sprague-Dawley. All knockout strains had been backcrossed with the indicated inbred strain for at least eight generations. Animal care was in accordance with the guidelines of the American Association for Accreditation of Laboratory Animal Care and experiments were performed with the approval of the Institutional Animal Care and Use Committee of Cornell University.

Parasite and Ags

T. spiralis infectious larvae (L_1) and NBL were recovered from rats as previously described (16, 21). Mice were injected in the lateral tail vein with 20,000 NBL suspended in serum-free DMEM (Mediatech). Alternatively, L_1 were suspended in 2% nutrient broth (Difco), 0.6% gelatin (Fisher Scientific), and doses of 300 L_1 were administered by gavage. For studies of immune responses elicited by the two routes of infection, oral infections were started four days before the i.v. infections, as worms do not reproduce until 4 days after establishment in the intestine (12). Mice were euthanized by CO_2 inhalation at the times indicated in each experiment. Muscle larvae burdens were assessed in whole carcasses as previously described (16). Somatic Ags from L_1 were prepared as previously described (22).

Histology

Microscopy was performed using a BX51 microscope (Olympus). Cellular infiltration was quantified by measuring the distance from the nurse cell capsule to the margin of the infiltrate at eight different positions using Microsuite Basic edition software (Olympus). Values are expressed as length in microns. Means were calculated from 60 nurse cells from each group. Developing nurse cells per low power field (LPF), measured at a magnification of ×20, were counted in sections of tongue. The entire tongue section (10–15 LPF) was evaluated and the number per mouse used to calculate the mean of the group. Histochemical staining and immunohistochemistry were conducted as previously described (23). Immunohistochemistry experiments used rat mAbs specific for tyvelose (clone 18H (24)) and rabbit polyclonal anti-inducible NO synthase (iNOS) (23). Masson's trichrome staining was performed following the method described by Luna (25).

Culture of leukocytes and cytokine ELISA

Cells from cervical lymph nodes (CLN) or mesenteric lymph nodes (MLN) were obtained as previously described (23). ELISA tests to measure IL-4, IL-5, IL-10, IL-13, and IFN-γ in culture supernatants were performed as previously described (23) except that clone R4A2L (eBioscience) was used in the IFN-γ assay.

NO determination

Total NO end products (nitrates and nitrites) were measured in CLN culture supernatants using the method described by Misko et al. (26). The 96-well black plates (Nunc) were used for this assay. The sensitivity of the assay was 0.2 μM.

Muscle and blood counts

Leukocytes were recovered from diaphragms as previously described (16). Cells for cytologic staining were prepared using a cytocentrifuge (Cytospin 3; Shandon Scientific). Blood was collected by cardiac puncture into EDTA-containing tubes at indicated times. Blood smears were made from each sample. Slides were stained with Hema3 (Protocol; Fisher Scientific) and differential counts were performed. Three hundred cells were counted under magnification at ×40.

iNOS inhibition

L-NIL (N^6(2-iminoethyl) L-lysine dihydrochloride; Cayman Chemicals) was dissolved in sterile water and administered daily by i.p. injection (75 mg/kg). Aminoguanidine hemisulfate (AMG) salt (Sigma-Aldrich) was dissolved in sterile PBS and administered daily by i.p. injection (800 mg/kg). Drug treatment began 12 days after oral infection and continued until the day of euthanasia. Control mice received sterile water or PBS.

Statistical analysis

All experiments were performed two to four times with similar results. Mean ± SD was calculated from data collected from individual mice. Significant differences were determined using Student's *t* test. Statistical analysis was performed with GraphPad Prism 4 software.

Results

Intestinal infection promotes myositis

Muscle infection by *T. spiralis* is initiated at a time coincident with a potent intestinal Th2 immune response that causes expulsion of adult worms from the intestine (27). To investigate the influence of intestinal immunity induced by *T. spiralis* on muscle infection and myositis, we infected mice by the natural oral route and compared their responses with those of mice i.v. injected with NBL. The latter method causes muscle infection in the absence of the intestinal colonization (16). To accommodate the 4-day period between initiation of intestinal infection and production of NBL, oral infections were initiated 4 days before i.v. infections (12). Parasite doses delivered by the two routes were calibrated to produce similar muscle burdens (I.V.: 6080 ± 3141, oral: 6864 ± 2293; $p > 0.05$) and therefore, differences in immune responses in muscle would not be attributable to differences in Ag dose. Furthermore, the tongue and diaphragm are preferred sites of colonization in natural infection and we have shown previously that both sites are colonized when infection is initiated by injection of NBL (16). Thus, the primary differences between the two routes of infection that may influence immunity are the concurrent intestinal infection during the early stages of muscle colonization and the rate at which NBL are delivered to muscle. NBL arrive in muscle over a period of several days in natural infection, whereas i.v. infections are performed with a single injection of NBL. The relative contribution of each influence has not been tested.

Examination of histologic sections from both groups of mice revealed that myositis was more severe in orally infected mice (Fig. 1*A*). Leukocytic infiltrates around nurse cells were significantly larger in naturally infected mice at 24 and 28 days post oral infection (dpoi) compared with i.v. infected mice at 20 and 28 days

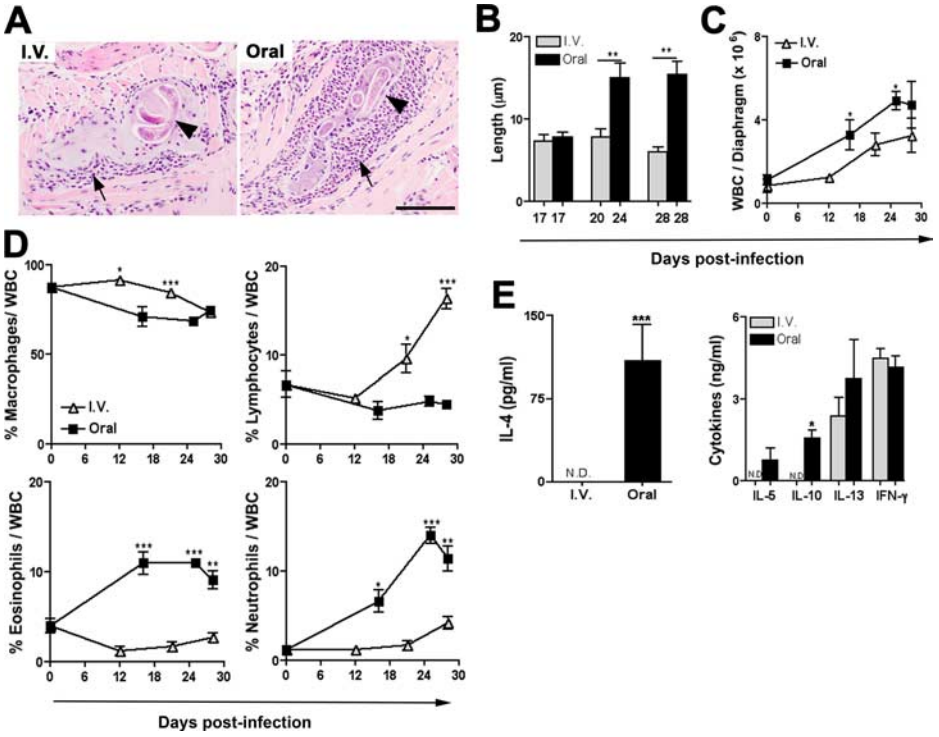

FIGURE 1. Intestinal infection promotes myositis, neutrophilia, eosinophilia, and a peripheral Th2 response. *A*, H&E stained sections of tongue collected from C57BL/10 mice at 20 dpi i.v. (I.V.) or 24 dpoi. Arrow indicates infiltrating cells and arrowhead indicates larva within the nurse cell. Scale bar = 100 μm. *B*, The cellular response around nurse cells was quantified by measuring the distance from the capsule to the margin of the infiltrate. (Note: Cellular infiltrates do not attain sufficient size for measurement before 17 dpoi/dpi) *C*, The number of white blood cells (WBC) recovered from diaphragms of mice after i.v. or oral infection. *D*, Differential counts of leukocytes recovered from diaphragms of mice after i.v. or oral infection. *C* and *D*, values at 12 and 20 dpi should be compared with values at 16 and 24 dpoi. *E*, Cytokines produced in cultures of Ag-stimulated CLN cells collected at 12 dpi or 16 dpoi. N.D., not detected. Results represent mean ± SD, $n = 4–8$ mice. *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$.

postinfection (dpi) (Fig. 1*B*). This difference correlated with the number of leukocytes recovered from diaphragms (Fig. 1*C*).

To better understand the nature of the inflammatory response, we enumerated specific cell types. Macrophages comprised 90% of the infiltrating cell population, regardless of the route of infection. Eosinophils and neutrophils were more numerous among cells recovered from orally infected mice (Fig. 1*D*) and the number of these cells was significantly increased over those in NBL recipients at all times assayed. The more severe myositis and enhanced granulocyte infiltration in naturally infected mice was associated with a robust IL-4 response in the CLN that drain the tongue (Fig. 1*E*).

Eosinophil deficiency compromises parasite survival in skeletal muscle

We tested the significance of eosinophils at sites of infection in two mouse models of eosinophil ablation. First, PHIL or C57BL/6 mice were infected orally with 300 L_1, and larval burdens were assessed after 28 days. Eosinophils were not detected in blood smears or cytologic preparations of diaphragm cells from infected PHIL mice (see Supplemental Fig. 1).[5] When compared with C57BL/6 controls, PHIL mice harbored significantly fewer larvae in muscle (67–77% reduction in three experiments; Fig. 2*A*), implicating eosinophils in parasite protection rather than destruction. Furthermore, when larvae recovered from PHIL (PHIL L_1) or from C57BL/6 (C57BL/6 L_1) mice at 22 dpoi were fed to C57BL/6 mice, larval burdens measured 24 days later revealed that PHIL L_1 were much less efficient in propagating infection (Fig. 2*B*). Thus, although PHIL mice did not completely clear the infection by 22 dpoi, the surviving larvae were severely compromised in their ability to transmit infection to another host. These observations suggest that sustained eosinophil responses to nematode infection may not reflect the effort of the host to clear the parasite, but rather the effort of the worm to maintain its position in the host.

To confirm that the effect of eosinophil deficiency on parasite survival was independent of the gene-targeting strategy, we infected Δdbl-GATA$^{-/-}$ mice. Similar to results obtained in PHIL mice, larval burdens were significantly reduced (48%) in orally infected Δdbl-GATA$^{-/-}$ mice compared with BALB/c controls (Fig. 2*C*).

The intensity of muscle infection by *T. spiralis* is influenced by retention time and fecundity of female worms in the intestine, the success of NBL in migrating to skeletal muscle, and larval survival in muscle. When we investigated each of these factors, we found that PHIL mice accepted and expelled intestinal worms at the same rate as C57BL/6 mice (Fig. 2*D*). Further evidence that intestinal infection was unaffected by eosinophil deficiency was provided by analysis of the cytokine response in MLN. In response to Ag restimulation in vitro, MLN cells from PHIL and C57BL/6 mice produced similar quantities of IL-4 and IL-13, cytokines that are crucial for clearance of intestinal worms (see Supplemental Fig. 2).[5] To determine whether a similar number of NBL initiated infection in muscle, we counted the number of infected myotubes at 12 dpoi in sections of tongues. Rates of muscle infection in PHIL and C57BL/6 mice were comparable (Fig. 2*E*). To better understand the developmental stage at which larvae were susceptible to killing, we evaluated histologic sections of tongues collected at intervals between 12 and 28 dpoi. In muscle collected 15 dpoi, nurse cells in both strains had produced collagen capsules (see Supplemental Fig. 3)[5] and larvae were producing tyvelose, a sugar synthesized and stored by mature L_1 (Fig. 2*F*). At 22 dpoi, many nurse cells in PHIL mice were infiltrated and counts of larvae digested from carcasses showed that a large number had been destroyed by day 28 (Fig. 2*G*). These observations indicate that larvae are attacked at a time when they have established in muscle and are maturing, between 15 and 22 dpoi. Further confirmation that developing muscle larvae are targets of attack was obtained in experiments in which mice were i.v. infected with NBL. In two experiments, muscle burdens in PHIL mice were reduced 31 or 62% compared with C57BL/6 mice. Thus, concurrent intestinal infection is not necessary for inducing eosinophil-dependent

[5] The online version of this article contains supplemental material.

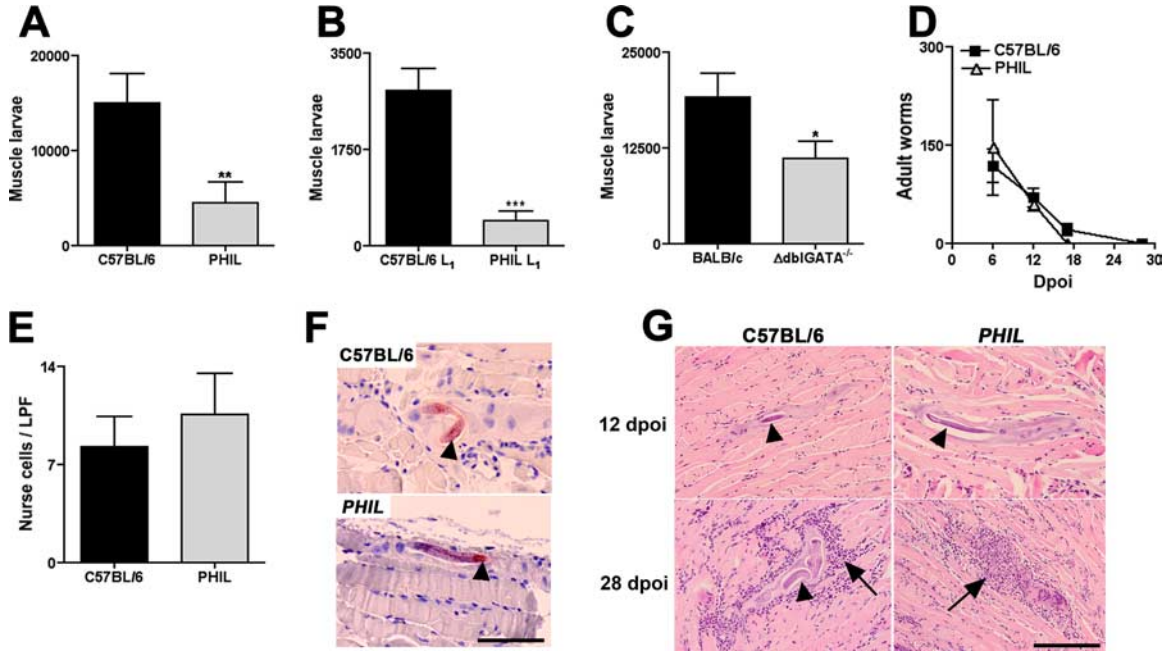

FIGURE 2. Eosinophils promote survival of *T. spiralis* larvae in muscle. *A*, Larval burdens in muscles of C57BL/6 or PHIL mice, 28 dpoi. *B*, Infectivity of L_1 recovered from C57BL/6 or PHIL muscles 24 dpoi. The viability of L_1 is reflected in their ability to transmit infection to C57BL/6 mice. *C*, Muscle burdens in BALB/c or Δdbl-GATA$^{-/-}$ mice, 28 dpoi. *D*, Kinetics of adult worm expulsion from small intestines of C57BL/6 or PHIL mice. *E*, Number of developing nurse cells per LPF at 12 dpoi. *F*, Detection of tyvelose (red color) in muscle larvae, 15 dpoi. *G*, H&E stained sections of tongue collected from mice at 12 and 28 dpoi. Arrowheads indicate *T. spiralis* larva and arrows indicate infiltrating cells. Scale bar = 100 μm. Results are mean ± SD, n = 3–4 mice. *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$.

protection of muscle stage larvae and the comparatively low eosinophil numbers present in i.v. infected mice are both necessary and sufficient for larval survival.

Overall, the intestinal phase of infection by *T. spiralis* was not discernibly affected by eosinophils, whereas the chronic phase was dependent upon them.

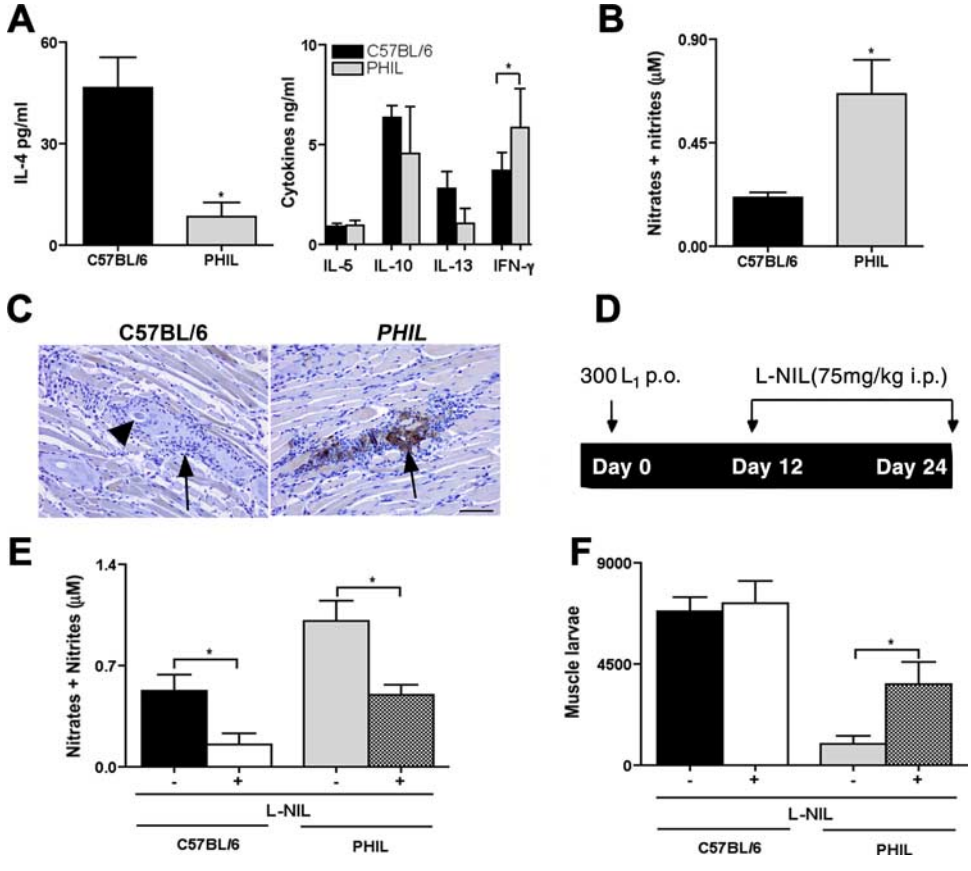

FIGURE 3. Parasite killing is associated with a Th1 immune response. Cytokines (*A*) and NO end products (*B*) measured in Ag-stimulated cultures of CLN cells collected from PHIL and C57BL/6 mice at 17 dpoi. *C*, Detection of iNOS in skeletal muscle (brown color), 17 dpoi. Arrowhead indicates larva and arrow indicates cellular infiltrates. Scale bar = 100 μm. *D*, Design of L-NIL treatment study. *E*, Effect of L-NIL treatment on NO end products in cultures of CLN, 24 dpoi. *F*, Effect of L-NIL treatment on larval burdens, 24 dpoi. Results represent mean ± SD (n = 3–4 mice). *, $p < 0.05$.

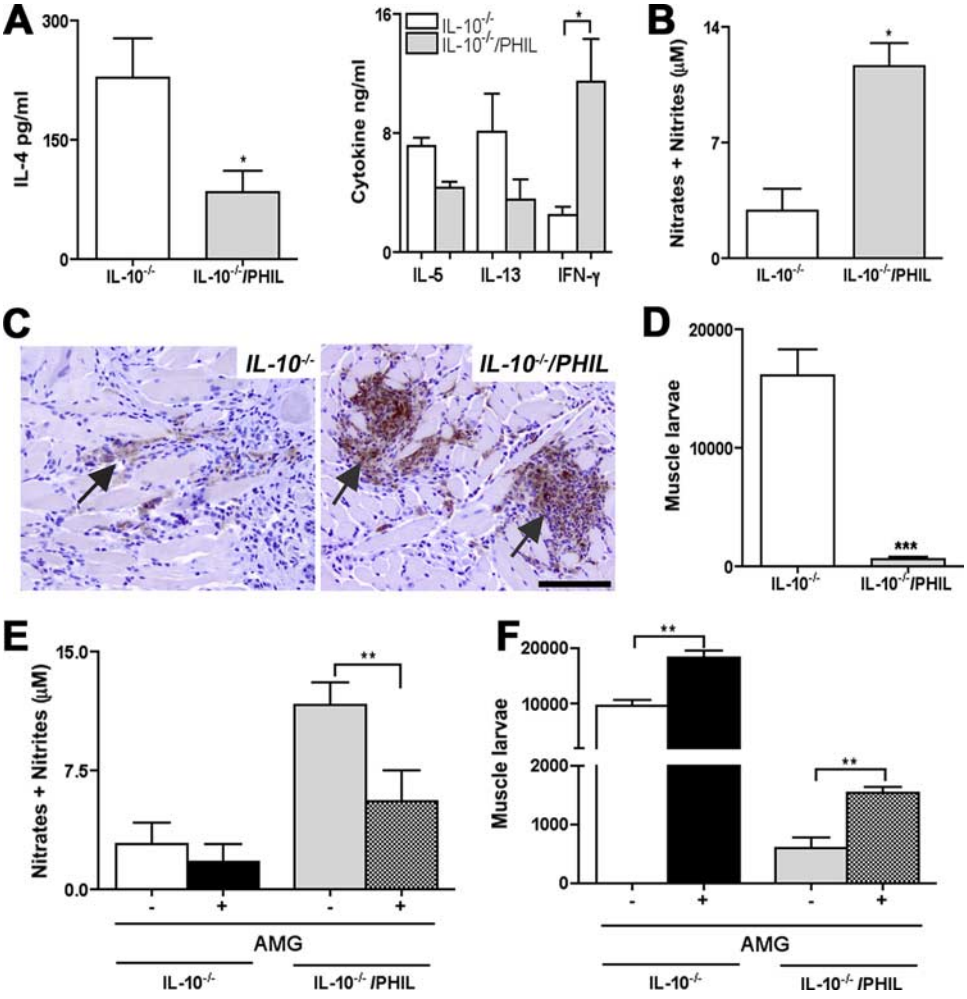

FIGURE 4. Enhanced Th1 response in eosinophil-deficient mice enhances parasite killing. Cytokines (*A*) and NO end products (*B*) measured in cultures of Ag-stimulated CLN cells collected 24 dpoi. *C*, Detection of iNOS in muscle. Arrow indicates positive areas, 24 dpoi. Scale bar = 100 μm. *D*, Larval burdens in muscles of IL-$10^{-/-}$ and IL-$10^{-/-}$/PHIL mice, 28 dpoi. *E*, Effect of AMG treatment on NO end products in CLN cultures. *F*, Larval burdens in IL-$10^{-/-}$ and IL-$10^{-/-}$/PHIL mice, 24 dpoi. Treatment design replicated that in Fig. 3. Results represent mean ± SD (n = 3–4 mice). *, $p < 0.05$; **, $p < 0.001$.

Parasite killing is dependent upon iNOS

To characterize the immune mechanism responsible for parasite killing, we analyzed the cytokines secreted by cells in lymph nodes draining infected muscles. Significantly more IFN-γ and less IL-4 were detected in CLN cell cultures of PHIL vs C57BL/6 mice (Fig. 3*A*). IFN-γ activates macrophages to express iNOS (28). Consistent with increased IFN-γ, NO end products (nitrates and nitrites) were increased in CLN cultures (Fig. 3*B*) and iNOS-positive cells were more numerous in muscles of PHIL mice. The latter cells colocalized with areas of severe nurse cell infiltration and parasite death (Fig. 3*C*).

Based on these findings, together with the observation that macrophages dominated local infiltrates, we hypothesized that classically activated macrophages, through the production of NO and other reactive nitrogen species, were responsible for parasite damage and death. To test the role of NO in parasite death, PHIL mice were treated between 12 and 24 dpoi (Fig. 3*D*) with a specific iNOS inhibitor, L-NIL (29). Treatment reduced NO production in both strains by 50% (Fig. 3*E*), but only PHIL mice showed significant increases in larval burdens that were attributable to drug treatment (Fig. 3*F*). In summary, our results show that in the absence of eosinophils, IFN-γ and iNOS were increased, and iNOS contributed to parasite clearance in *T. spiralis*-infected muscle.

In the absence of eosinophils, poorly regulated Th1 responses correlate with enhanced parasite killing

We have previously reported that IL-10 inhibits iNOS and IFN-γ production during muscle infection by *T. spiralis* (23). Both factors are dramatically up-regulated in IL-10-deficient mice. We hypothesized that if IFN-γ-dependent responses contributed to parasite killing in PHIL mice, then mice that were deficient in both IL-10 and eosinophils should demonstrate an enhanced ability to kill muscle larvae. Indeed, doubly deficient mice mounted stronger Th1 responses compared with IL-10-deficient mice as evidenced by higher concentrations of IFN-γ, reduced production of IL-4 (Fig. 4*A*), enhanced production of NO end products (Fig. 4*B*) in CLN cultures, and enhanced iNOS detection in muscle (Fig. 4*C*). Consistent with these differences, there was a dramatic reduction in larval burdens (93%) in IL-$10^{-/-}$/PHIL mice compared with IL-$10^{-/-}$ controls (Fig. 4*D*). Furthermore, when IL-$10^{-/-}$ or IL-$10^{-/-}$/PHIL mice were treated with the iNOS inhibitor AMG, NO end products in CLN cultures were reduced, and larval survival improved (Fig. 4, *E* and *F*). These results further implicate Th1-driven immunity in destruction of muscle larvae and support a mechanism by which eosinophils limit the Th1 response and NO production.

Discussion

We evaluated *T. spiralis* infection using two models of eosinophil ablation in which the mechanisms of ablation and the genetic backgrounds of the mice were distinctly different. Parasite clearance occurred in both strains, demonstrating that the effect on parasite survival is unlikely to be an artifact or secondary effect of the construct. Both models have been tested previously in models of allergy. The reduction in allergic disease reported was associated

with reduced Th2 cytokines and decreased recruitment of T lymphocytes to the lung (20, 30). Based on the outcome of adoptive transfer experiments, it was concluded that eosinophils regulate the immune response in this model of allergy by promoting production of chemokines that recruit Th2 lymphocytes to the lung. A similar mechanism may be at work in *T. spiralis* infection whereby protective Th2 cells would be recruited to muscle by eosinophil-dependent chemokines.

In addition to this sort of indirect inhibition of Th1 responses, eosinophils have been shown to directly inhibit Th1 cells. Human eosinophils produce IDO in response to IFN-γ and thereby induce apoptosis in IFN-γ-producing T cells (31). Eosinophils further inhibit Th1 responses via release of Th2 cytokines, lipid mediators, and chemokines (1). In addition, eosinophil-derived neurotoxin has been demonstrated to function as an alarmin, promoting Th2 responses by signaling dendritic cells via TLR2 (32). In aggregate, such properties are compatible with a regulatory function for eosinophils, an influence that is supported further by the demonstration that eosinophils can present Ag to T cells (reviewed in Ref. 1). This activity has been confirmed in vitro with Ags from the nematode, *Strongyloides stercoralis* (33).

We found that the intestinal phase of infection by *T. spiralis* was not discernibly affected by eosinophil deficiency. In contrast, the muscle phase was dependent upon eosinophils. Intestinal infection induced a marked eosinophilia, whereas i.v. infection elicited a comparatively weak response. Muscle larvae were killed in PHIL mice infected by either route, suggesting that even weak eosinophil responses were associated with parasite protection. Previously, we have shown that infiltrating cells in i.v. infected wild-type mice did not produce detectable quantities of iNOS (23). Thus, in the presence of low level of eosinophilia induced by i.v. infection, a destructive Th1 response fails to develop.

In eosinophil-deficient mice, larvae were not prevented from invading muscle cells but rather they were attacked between 15 and 22 dpoi. This interval coincides with the emergence of a dominant Th2 response in normal mice and also with a period of larval growth and maturation (13). Our data do not reveal whether the target of immune attack is the nurse cell or the larva within it. The parasite may be killed directly by toxic mediators that infiltrate the cell, or indirectly by an assault of leukocytes on the integrity of the nurse cell. Proteolytic disruption of the collagen capsule or, alternatively, damage to endothelial cells in the circulatory rete surrounding the infected cell may compromise larval viability. This issue is important to resolve, as understanding the mechanism of killing will provide insight into the interdependence of the larva and its host cell, a relationship that is undefined at present.

Although Th2 responses mediate immunity to nematodes in the gastrointestinal tract (27), Th1 responses and NO have been implicated in resistance to *Taenia crassiceps*, a helminth that colonizes extraintestinal sites (34). In our experiments, orally infected IL-10-deficient mice produced a great deal more NO than did PHIL mice and treatment with AMG significantly improved survival of muscle larvae in these mice. This finding was surprising, as previously we have shown that intestinal and muscle burdens are not different in wild-type vs IL-10-deficient mice infected with *T. spiralis*, despite greater IFN-γ and iNOS production in the latter strain. Our results contradicted those of Helmby and Grencis (35) that showed similar effects on cytokine production but reduced muscle burdens and delayed adult worm expulsion in IL-10-deficient mice. At the time, the difference in results from our two laboratories was unexplained. If, in our experiments, production or survival of NBL is greater in IL-10-deficient mice, but developing muscle larvae are killed in numbers that reduce the eventual burden to approximate wild-type numbers at 28 dpi, then the effect of AMG would be to increase larval burdens only in IL-10-deficient mice, as reported in this study. This model is compatible with the findings of Helmby and Grencis (35). The effect of AMG treatment indicates that iNOS contributes to parasite killing, even in the presence of eosinophils. Because inhibition of iNOS did not completely reverse the dramatic effect of eosinophil deficiency, other mechanisms are likely to contribute to parasite killing.

Our data support a role for eosinophils in limiting parasite destruction in muscle, while intestinal infection with *T. spiralis* is not altered by eosinophil deficiency. Similarly, eosinophil deficiency had no effect on *Schistosoma mansoni* infection, as PHIL and Δdbl-GATA$^{-/-}$ mice demonstrated normal courses of infection and typical liver lesions (8). In contrast, a weak protective role for eosinophils was demonstrated in Δdbl-GATA$^{-/-}$ mice infected with *Nippostrongylus brasiliensis* (36). Alternatively activated macrophages have been implicated as sources of chemotactic factors for eosinophil recruitment to the lung and peritoneal cavity during *N. brasiliensis* infection (37). Our data are compatible with a central role for macrophages in protecting *T. spiralis* larvae; however, direct interaction between eosinophils and macrophages remains to be proven, as well as any direct mechanism of action for eosinophils as parasite-protective cells. Overall, research to date indicates that the influence of the eosinophil varies by organ system and also among helminth infections.

Immune regulation appears to be the dominant function of eosinophils in allergy (38). Although eosinophils contributed to the inflammatory disease that accompanies *T. spiralis* infection in muscle, they were required also for the immune response that is crucial to transmission of the parasite to the next host and to its survival in nature. The profound impact of eosinophil ablation on the outcome of a nematode infection invites consideration of a new paradigm for the function of eosinophils in infectious diseases.

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Disclosures

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