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Major Basic Protein from Eosinophils and Myeloperoxidase from Neutrophils Are Required for Protective Immunity to *Strongyloides stercoralis* in Mice[▽]

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Eosinophils and neutrophils contribute to larval killing during the primary immune response, and neutrophils are effector cells in the secondary response to *Strongyloides stercoralis* in mice. The objective of this study was to determine the molecular mechanisms used by eosinophils and neutrophils to control infections with *S. stercoralis*. Using mice deficient in the eosinophil granule products major basic protein (MBP) and eosinophil peroxidase (EPO), it was determined that eosinophils kill the larvae through an MBP-dependent mechanism in the primary immune response if other effector cells are absent. Infecting *PHIL* mice, which are eosinophil deficient, with *S. stercoralis* resulted in development of primary and secondary immune responses that were similar to those of wild-type mice, suggesting that eosinophils are not an absolute requirement for larval killing or development of secondary immunity. Treating *PHIL* mice with a neutrophil-depleting antibody resulted in a significant impairment in larval killing. Naïve and immunized mice with neutrophils deficient in myeloperoxidase (MPO) infected with *S. stercoralis* had significantly decreased larval killing. It was concluded that there is redundancy in the primary immune response, with eosinophils killing the larvae through an MBP-dependent mechanism and neutrophils killing the worms through an MPO-dependent mechanism. Eosinophils are not required for the development or function of secondary immunity, but MPO from neutrophils is required for protective secondary immunity.

Control of helminth infections represents a unique challenge to the immune response, in part because of the large size of the worms relative to the cells of the immune system. Primary or secondary protective immune responses to helminths comprise many specific interactions of host cells and molecules (5). In particular, both eosinophils and neutrophils have been identified to be effector and immunomodulatory cells during helminth infections (14).

Eosinophilia is a hallmark of helminth infections, and in some host-parasite relationships, eosinophils have been observed to kill worms (40, 47). *In vitro* studies have shown that eosinophils, in association with specific antibody, can kill the nematodes *Trichinella spiralis* (38, 44, 64, 65), *Haemonchus contortus* (53), and *Onchocerca volvulus* (23). Specific ablation of eosinophils with a monoclonal antibody (MAb) to chemokine receptor type 3 (CCR3) (24) in mice immunized against the nematode *O. volvulus* not only eliminated protective immunity but also significantly increased the survival of the parasites in immunized mice to levels exceeding those seen in the controls (1). Elimination of eosinophils with the anti-CCR3 MAb also blocked resistance to primary infections with *Brugia pahangi* (54). Similar observations were made studying resistance to *T. spiralis* using CCR3-knockout mice, where there was an absence of eosinophil recruitment with a concomitant elevation in larval parasite survival (25). Experiments have been performed in *PHIL* (42) and ΔdblGATA (33) mice, both of which constitutively lack eosinophils, to determine whether eosinophils are required for protective immunity against specific nematodes. Resistance to *Nippostrongylus brasiliensis* was impaired in ΔdblGATA mice for relatively short periods of time within primary and secondary infections (41). Immunity to the intestinal phase of *T. spiralis* was not affected by the absence of eosinophils in *PHIL* and ΔdblGATA mice, whereas the absence of eosinophils resulted in a decrease in the survival of larvae in muscle, suggesting that the presence of eosinophils enhances the survival of the larvae (19). Studies were performed in mice deficient in major basic protein (MBP) and (17) and eosinophil peroxidase (EPO) (16) to determine if these eosinophil granule products were required for resistance to nematode infections. Protective immunity in immunized mice to the larvae of *O. volvulus* is dependent on eosinophils but was unaffected in $EPO^{-/-}$ mice (1). Studies performed on *B. pahangi* in mice deficient in MBP or EPO also concluded that eosinophils were essential for protective immunity, yet neither MBP nor EPO was required (54). In contrast, both MBP and EPO are required for protective immunity to *Litomosoides sigmodontis* in mice (61). Therefore, eosinophils and their granule products are associated with both resistance and susceptibility to nematode infections in a species-specific manner.

Neutrophils have been associated with killing of nematode

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parasites in the secondary immune response. Larvae recovered from mice immunized against *Ancylostoma caninum* were surrounded by neutrophils and had extensive ultrastructural damage (60). *In vitro* assays have shown that neutrophils in conjunction with specific antibody kill *O. volvulus* (34), *T. spiralis* (65), *Brugia malayi* (15), *Acanthocheilonema viteae* (4), and *Acanthocheilonema cantonensis* (59). Interestingly, synergy has been described between gamma interferon and interleukin-5 (IL-5) in the control of *Litomosoides sigmodontis* in mice. In the absence of both cytokines, mice are nearly devoid of eosinophils and display reduced neutrophil-mediated activities that allow an increase in parasite survival, suggesting a potential link between neutrophil and eosinophil function (58). Efforts to identify the molecular mechanisms used by neutrophils to kill nematodes have demonstrated that myeloperoxidase (MPO) purified from human neutrophils is toxic to *T. spiralis* in the presence of hydrogen peroxide and halide (13).

Mechanisms of primary and secondary protective immunity to the nematode *Strongyloides stercoralis* have been studied in mice (2). Infective third-stage larvae of *S. stercoralis* are killed in naïve mice within 5 to 7 days postinfection through an immune response dependent on complement activation (39), neutrophils, and eosinophils (21, 29). Eosinophils (62) and neutrophils (49) are recruited directly by the parasite, and eosinophils can serve as the bridge between the primary and the secondary immune responses, acting in their role as antigen-presenting cells for the induction of the primary and expansion of the secondary Th2 immune responses (50, 51). Secondary immunity to *S. stercoralis*, induced in mice by immunization with live larvae, kills more than 90% of larvae within 24 h and requires $CD4^+$ Th2 cells for IL-4 and IL-5 (29, 56), B-1a B cells for IgM antibody (11, 30, 45), complement component C3 (11, 39), and neutrophils but not eosinophils (21, 45). If neutrophil recruitment in mice was blocked, because of a defect either in Gαi2 signaling (52) or in the expression of CXCR2 (21), the capacity of naïve and immunized mice to kill *S. stercoralis* larvae is significantly decreased. Adding neutrophils isolated from $CXCR2^{-/-}$ mice directly into the larval microenvironment in recipient $CXCR2^{-/-}$ mice restores larval killing (21). Therefore, neutrophil recruitment to the parasite requires CXCR2 for protective immunity in both naïve and immunized mice, while larvicidal function is independent of this receptor.

The objective of this study was to determine the molecular mechanisms used by eosinophils and neutrophils to control primary and secondary infections of *S. stercoralis* in mice. It was determined that there was redundancy in the primary immune response, with eosinophils killing the larvae through an MBP-dependent mechanism and neutrophils killing the worms through an MPO-dependent mechanism. Eosinophils were not required for secondary immunity, but MPO from neutrophils was required for protective secondary immunity.

MATERIALS AND METHODS

Experimental animals. The C57BL/6 and C3 $^{-/-}$ mice used in experiments were purchased from Jackson Laboratories (Bar Harbor, ME). IL-5 TG (43), $EPO^{-/-}$ (16), $MBP^{-/-}$ (17), *PHIL* (42), $EPO^{-/-}$ × IL-5 TG, and $MBP^{-/-}$ × IL-5 TG mice were bred either in the Thomas Jefferson University Laboratory Animal Sciences facility (Philadelphia, PA) or in the Mayo Clinic—Scottsdale Laboratory Animal Sciences facility (Scottsdale, AZ). $EPO^{-/-}$ × IL-5 TG and $MBP^{-/-}$ × IL-5 TG mice were bred to provide a sufficient source of eosinophils deficient in either EPO or MBP. $MPO^{-/-}$ mice on a C57BL/6 background were a generous gift from Aldons J. Lusis (University of California at Los Angeles) and were bred in the Thomas Jefferson University Laboratory Animal Sciences facility (Philadelphia, PA). All mice were used at the age of 7 to 14 weeks, with the exception of the IL-5 TG and the IL-5 TG crosses, being up to 16 weeks of age. Experimental animals were housed in filter-top microisolator boxes under pathogen-free and light- and temperature-controlled conditions in the Thomas Jefferson University animal facility.

Parasites. *S. stercoralis* larvae were obtained from the feces of laboratory infected dogs by previously described methods (2). Larvae were washed five times in a 1:1 mixture of NCTC-135 and Iscove's modified Dulbecco's media supplemented with 100 U/ml penicillin plus 100 μg/ml streptomycin (Cellgro Inc., Herndon, VA), 0.1 mg/ml gentamicin (Invitrogen, Carlsbad, CA), and 0.25 mg/ml levofloxacin (Levaquin; Ortho-McNeil, Raritan, NJ).

Diffusion chambers. Cell-permeable diffusion chambers were constructed from 2.0-μm-pore-size Isopore membranes glued to one side of 14-mm Lucite rings (Millipore, Bedford, MA) using cyanoacrylate adhesive (Superglue Corp., Hollis, NY). The sides of the chambers were then assembled using a 1:1 adhesive mixture of 1,2-dichloroethane (Fisher Scientific, Pittsburgh, PA) and acryloid resin (Rohm and Haas, Philadelphia, PA). Cell-impermeable diffusion chambers were constructed with 0.1-μm-pore-size Durapore membranes attached to 14-mm Lucite rings using a 1:1 mixture of 1,2-dichloroethane to acryloid resin adhesive, and the sides were assembled with an extra, membrane-free Lucite ring between them. The diffusion chambers were sterilized with 100% ethylene oxide exposure, followed by 12 h of aeration.

Experimental infections. Mice were immunized with 5,000 larvae injected subcutaneously between the scapulae; on day 14 they were vaccinated with an equivalent booster immunization, and on day 21 they received challenge infections. Challenge infections consisted of a diffusion chamber containing 50 larvae implanted subcutaneously in the dorsal flank of naïve and immunized mice. All surgical procedures were performed on mice anesthetized with isoflurane (Webster Veterinary, Sterling, MA). Diffusion chambers were recovered from the mice between 1 and 5 days later, as indicated, and larval survival was assessed on the basis of their motility and morphology. Host cells recovered within the diffusion chambers were counted using a hemocytometer and then centrifuged onto slides using a Cytospin 3 apparatus (Shandon, Pittsburgh, PA), followed by staining with DiffQuik (Baxter Healthcare, Miami, FL).

Granulocyte depletion. MAb 6S2-19-4 (anti-CCR3) (cell lines were a gift from D. L. Coffman, Dynax Corp.) was used to deplete eosinophils from mice (24). Naïve mice received intraperitoneal injections of 350 μg of the anti-CCR3 MAb on the day prior to implantation of the challenge infection, 100 μg was injected into the subcutaneous pocket surrounding the diffusion chamber at the time of the challenge infections, and 350 μg was injected intraperitoneally 2 days after the challenge. Eosinophils and neutrophils were eliminated from mice by injecting them intraperitoneally with 0.5 mg of MAb RB6-8C5 (32) 3 days prior to challenge and on the day of challenge.

Eosinophil purification. Eosinophils were purified from the spleens of IL-5 TG mice as previously described (29). Briefly, spleens were collected from IL-5 TG mice, homogenized, and then resuspended in 2% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) in phosphate-buffered saline (PBS). The cells were then separated on a Percoll (Sigma) density column by centrifugation. The eosinophil-lymphocyte layer was collected and washed with 2% BSA–PBS to remove the excess Percoll. Contaminating red blood cells were removed by hypotonic lysis. Cells were then incubated with anti-mouse CD45R and CD90 microbeads (MACS; Miltenyi Biotech, Auburn, CA) to remove B cells and T cells, respectively. Eosinophils then were collected via negative selection after being passed over magnetic cell sorter LS columns (Miltenyi Biotech). The purity of the eosinophils was between 90 and 95% in all experiments.

Neutrophil isolation. Bone marrow was flushed from the femurs, tibias, and humeri from C57BL/6 and $MPO^{-/-}$ mice using 2.0% fetal bovine serum (FBS) in PBS (Mediatech, Herndon, VA) and 100 U/ml penicillin plus 100 μg/ml streptomycin. The cells were passed through a cell strainer (BD Falcon, San Diego, CA), centrifuged, and resuspended in a 45% Percoll solution. The cell solution was layered onto a Percoll gradient column of 80%, 62%, 55%, and 50% Percoll as previously described (28). The Percoll columns were spun at 660 × *g* for 30 min. Following the centrifugation step, the cell layer between the 62% and 80% Percoll layers was removed and the total cell number and percent neutrophils were determined. If the purity of the neutrophils was less than 90% following the Percoll column step, 1 μl each of anti-B220 and anti-Thy1.2 MACS beads (Miltenyi Biotec, Auburn, CA) was added per 1 million total cells to remove contaminating lymphocytes. The cells were incubated with the beads for 15 min at 4°C and then purified using MACS LS columns (Miltenyi) according

to the manufacturer's instructions. The cells were then reanalyzed microscopically for the total number and percentage of neutrophils. The purity of the neutrophil preparations was, on average, 95%.

Eosinophil *in vitro* killing assays. Purified eosinophils (5×10^6) from IL-5 TG, EPO$^{-/-}$ × IL-5 TG, and MBP$^{-/-}$ × IL-5 TG mice were incubated with 50 larvae in 96-well flat bottom plates (Corning Inc., Corning, NY) for 48 h at 37°C. The culture medium consisted of RPMI with 10% heat-inactivated FBS (Mediatech Inc.) or 50% naïve mouse serum, with the total volume of the well being 200 μl. Mouse serum was collected from naïve C57BL/6 and C3$^{-/-}$ mice and was used untreated or heat inactivated at 56°C for 30 min. After 48 h, parasite survival and cell viability were assessed in each well.

***In vivo* cell transfer studies.** Cell-impermeable diffusion chambers constructed with 0.1-μm-pore-size membranes to prevent cell ingress or egress were loaded with 50 larvae and 5×10^4 purified eosinophils from IL-5 TG, EPO$^{-/-}$ × IL-5 TG, or MBP$^{-/-}$ × IL-5 TG mice or 2×10^6 purified neutrophils from C57BL/6 or MPO$^{-/-}$ mice. The diffusion chambers were surgically implanted into mice, after which they were recovered to evaluate parasite survival and cell viability.

***S. stercoralis* antigen preparation.** Antigen used for enzyme-linked immunosorbent assays (ELISAs) was prepared using previously described methods (31). Briefly, frozen larvae were ground using a homogenizer with protease inhibitor cocktail (Sigma), followed by sonication. The homogenate was incubated in PBS overnight at 4°C, after which the PBS fraction was removed and sterilized with a 0.22-μm-pore-size filter. The remaining insoluble proteins were combined with 20 mM Tris-HCl–0.5% deoxycholate (DOC; Sigma) with protease inhibitor cocktail (Sigma). DOC-soluble proteins were dialyzed overnight against PBS and then filter sterilized. The concentration of extract/antigen soluble in PBS or DOC was quantified using a Micro BCA protein assay kit (Pierce Biotechnology, Rockford, IL).

Serum IgM determination. Maxisorp plates (Nunc, Naperville, IL) were coated with DOC-soluble *S. stercoralis* antigen at 10 μg/ml. Borate blocking buffer solution (BBS; 0.17 M boric acid, 0.12 M NaCl, 0.05% Tween 20, 0.25% BSA, 1 mM EDTA, pH 8.5) was added for 1 h to block the plates. Wells were washed with distilled water, and serial dilutions of serum samples in BBS were added to duplicate wells and incubated for 2 h. Biotinylated goat anti-mouse IgM (Vector Laboratories, Burlingame, CA) was added, and plates were incubated for an additional 2 h. After the plates were washed, Extravidin peroxidase solution (Sigma) was added for 30 min, followed by peroxidase substrate 2,2′-azinodi(3-ethylbenzthiazoline-6-sulfonate) (ABTS; Kirkegaard & Perry Laboratories, Gaithersburg, MD). The color reaction was measured at 405 nm on a microplate reader.

Spleen cell stimulation and cytokine analysis. Spleen cells cultured for 3 days at 2×10^6/well in a 96-well plate were stimulated with *S. stercoralis* antigens in the presence of anti-IL-4Rα MAb (BD Pharmingen) in Dulbecco modified Eagle medium (Sigma) supplemented with 10% heat-inactivated and filtered fetal calf serum (HyClone), 2 mM L-glutamine (Life Technologies), 100 U/ml penicillin plus 100 μg/ml streptomycin, and 50 μM 2-mercaptoethanol (Sigma). Culture supernatants were analyzed for IL-5 and IL-4 production by ELISAs using appropriately matched MAbs (TRFK.5 and TRFK.4 for measuring IL-5 and BVD6-24G2 and BVD4-1D11 for measuring IL-4 [BD Pharmingen]) for capturing and detection. Incubation with Extravidin peroxidase (Sigma) followed by the ABTS peroxidase substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) resulted in a color reaction that was measured at 405 nm.

Statistics. All experiments consisted of at least 5 mice per group, and independent experiments were performed at least twice, with each experiment having similar outcomes. Data from all experiments performed are presented in the table and figures. Statistical analysis was performed using multivariate general linear hypothesis, multifactorial analysis of variance using the SYSTAT (version 11) software (SYSTAT Inc., Evansville, IL). Fisher's least significant difference test was performed for *post hoc* analysis, and probability values less than 0.05 were considered significant.

RESULTS

Role of eosinophil-derived MBP and EPO in primary and secondary immunity. Treatment of naïve mice with the anti-CCR3 MAb to eliminate eosinophils resulted in an increase in larval survival, indicating that eosinophils are active in the protective primary immune response (Fig. 1A). To determine if either MBP or EPO from eosinophils was required as an effector molecule in the primary immune response, larvae were implanted in naïve C57BL/6, MBP$^{-/-}$, and EPO$^{-/-}$ mice for 5 days in cell-permeable diffusion chambers. All three strains of mice killed the larvae at equal rates (Fig. 1A), and cellular infiltration into the parasite microenvironment was also comparable between the strains of mice (Fig. 1B). MBP$^{-/-}$ and EPO$^{-/-}$ mice had a mean number of 6,700 eosinophils per diffusion chamber which was reduced to essentially 0 by the treatment with the anti-CCR3 MAb (Fig. 1B). The elimination of eosinophils resulted in an increase in larval survival (Fig. 1A), suggesting that eosinophils were required for optimal killing of larvae in the primary immune response but that MBP and EPO were not essential in the killing process. C57BL/6, MBP$^{-/-}$, and EPO$^{-/-}$ mice were immunized to determine whether the absence of a single granule product from eosinophils would affect the development of secondary immunity. Both the MBP$^{-/-}$ and EPO$^{-/-}$ mice developed protective immunity equal to that of the C57BL/6 mice, with greater than 90% of the larvae eliminated in the immunized animals (Fig. 1C).

In vitro assays were performed to determine whether MBP or EPO from eosinophils functions in killing *S. stercoralis* larvae, if other cell types are absent. Larvae incubated with eosinophils purified from IL-5 TG mice and naïve serum killed the larvae, whereas worms incubated with eosinophils in heat-inactivated serum or serum from C3$^{-/-}$ mice were not killed (Fig. 2A). Similar *in vitro* assays were performed using MBP$^{-/-}$- and EPO$^{-/-}$-derived eosinophils produced by crossing MBP$^{-/-}$ or EPO$^{-/-}$ mice with IL-5 TG mice. In the presence of naïve serum, MBP$^{-/-}$ eosinophils killed fewer larvae than control eosinophils or EPO$^{-/-}$ eosinophils (Fig. 2B). Experiments were performed *in vivo* using eosinophils from control IL-5 TG, MBP$^{-/-}$, and EPO$^{-/-}$ mice to corroborate the *in vitro* observation that MBP was required for killing the larvae, whereas EPO was not. Eosinophils were placed with larvae into cell-impermeable diffusion chambers and implanted into naïve C57BL/6 mice. MBP$^{-/-}$ eosinophils killed fewer larvae than control or EPO$^{-/-}$ eosinophils (Fig. 2C). These studies indicate that eosinophils act as effector cells in primary immunity and demonstrate that eosinophils are able to kill worms through the release of MBP.

Primary and secondary immunity in mice lacking eosinophils. Naïve and immunized *PHIL* mice, which constitutively lack eosinophils, were challenged with *S. stercoralis* larvae within diffusion chambers to further examine the role of eosinophils in primary and secondary immunity to larval *S. stercoralis*. Parasite survival was assessed after 1 day in immunized mice and after 1, 3, or 5 days in naïve mice. *PHIL* and control mice had similar levels of parasite survival at all time points for naïve mice and equal rates of parasite killing for mice that had been immunized (Fig. 3A). *PHIL* and control mice had similar numbers of neutrophils and macrophages in the larval microenvironment, whereas no eosinophils were detected in *PHIL* mice (Fig. 3B). Parasite-specific IgM antibody titers were not different in the *PHIL* mice (Fig. 3C), and production of the Th2 cytokines IL-4 and IL-5 by spleen cells also was not diminished in *PHIL* mice compared to C57BL/6 mice (Fig. 3D).

It has been previously reported that in addition to eosinophils, neutrophils participate in the protective primary immune response to *S. stercoralis* (21). Naïve *PHIL* mice were treated with the MAb RB6-8C5 to eliminate neutrophils and deter-

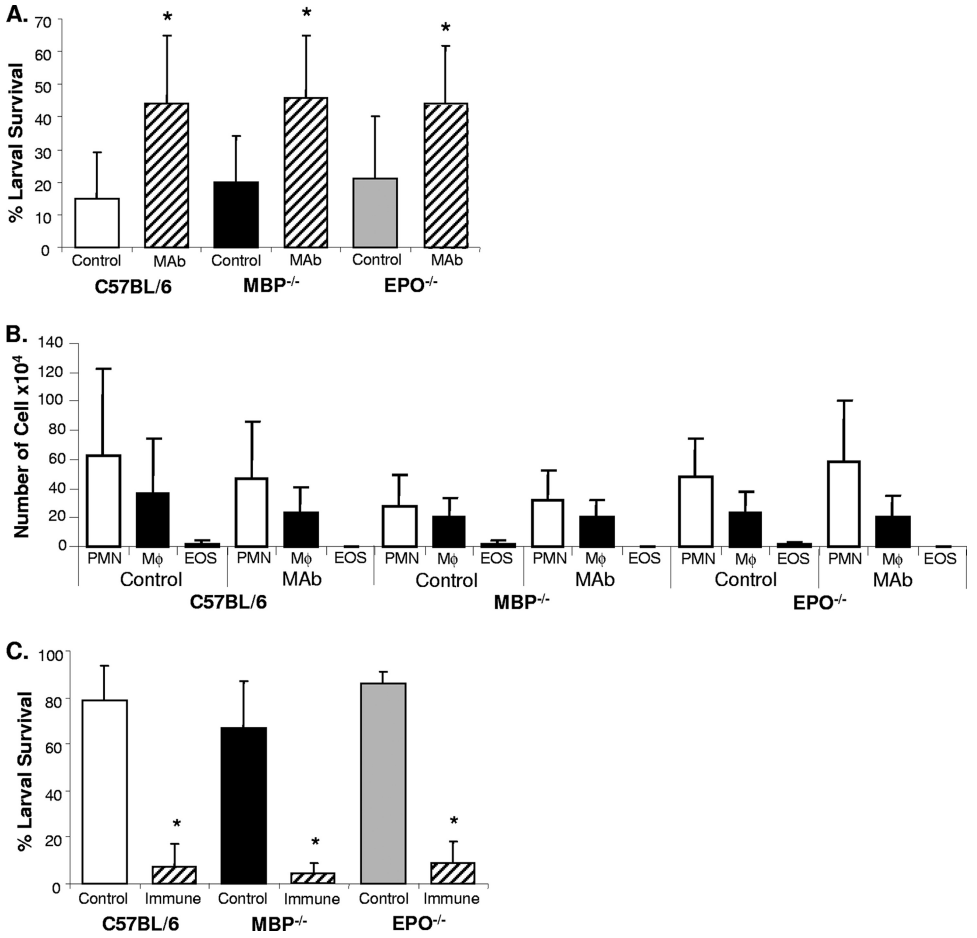

FIG. 1. Primary and secondary immunity to *S. stercoralis* in mice deficient in either MBP or EPO. (A) *S. stercoralis* larvae were implanted in cell-permeable diffusion chambers in naïve C57BL/6, MBP $^{-/-}$, or EPO$^{-/-}$ mice for 5 days, and parasite survival was determined. In addition, mice were treated with an anti-CCR3 MAb to determine the effect of eosinophil depletion on parasite survival in C57BL/6, MBP $^{-/-}$, or EPO$^{-/-}$ mice. *, statistically significant difference between larval recoveries from treated and untreated mice. (B) Number of neutrophils (polymorphonuclear leukocytes [PMN]), macrophages (Mϕ), and eosinophils (EOS) found within cell-permeable diffusion chambers implanted in untreated naïve C57BL/6, MBP $^{-/-}$, or EPO$^{-/-}$ mice or mice treated with an anti-CCR3 MAb. (C) *S. stercoralis* larvae were implanted in cell-permeable diffusion chambers in naïve and immunized C57BL/6, MBP $^{-/-}$, or EPO$^{-/-}$ mice for 1 day, and parasite survival was determined. *, statistically significant difference between larval recoveries from control and immunized mice. Data shown represent the means and standard deviations from 8 to 22 mice per group.

mine whether neutrophils function as effector cells in *PHIL* mice. Reduction of neutrophils by the MAb treatment (54% reduction in C57BL/6 mice and 82% reduction in *PHIL* mice) (Fig. 4A) resulted in increased survival of parasites in naïve control and *PHIL* mice (Fig. 4B). These studies show that eosinophils are not required for the development of a protective Th2 secondary immune response to *S. stercoralis*, nor are eosinophils essential for control of the infection in the primary immune response. In the absence of eosinophils, neutrophils function as effector cells in the primary response against the parasite.

Role of neutrophil-derived MPO in primary and secondary immunity. The ability of MPO$^{-/-}$ mice to kill *S. stercoralis* larvae during the primary immune response was evaluated. Diffusion chambers containing larvae were implanted into naïve C57BL/6 and MPO$^{-/-}$ mice for 1, 3, or 5 days. MPO$^{-/-}$ mice exhibited a significant decrease in larval killing at day 3 postchallenge, while at days 1 and 5 postchallenge, MPO$^{-/-}$ and wild-type mice had similar levels of parasite survival (Fig. 5). MPO$^{-/-}$ mice also were tested to determine their ability to kill larvae during the secondary immune response. It was determined that immunized MPO$^{-/-}$ mice had a defect in their ability to kill larvae compared to immunized C57BL/6 controls, thereby demonstrating that MPO was required for protective secondary immunity (Fig. 5).

Neutrophils were isolated from the bone marrow of C57BL/6 and MPO$^{-/-}$ mice and transferred with larvae into C57BL/6 mice inside cell-impermeable diffusion chambers to assess whether MPO was specifically required by neutrophils to kill the larvae. Implanting larvae with neutrophils derived from C57BL/6 mice resulted in significant larval killing in naïve (P = 0.001) and immunized (P = 0.001) mice compared to larval survival in diffusion chambers without neutrophils. However, neutrophils from MPO$^{-/-}$ mice had a significantly reduced capacity to kill the larvae in both naïve (P = 0.001) and immunized (P = 0.001) C57BL/6 mice compared to neutrophils from C57BL/6 mice. Furthermore, it was determined that the

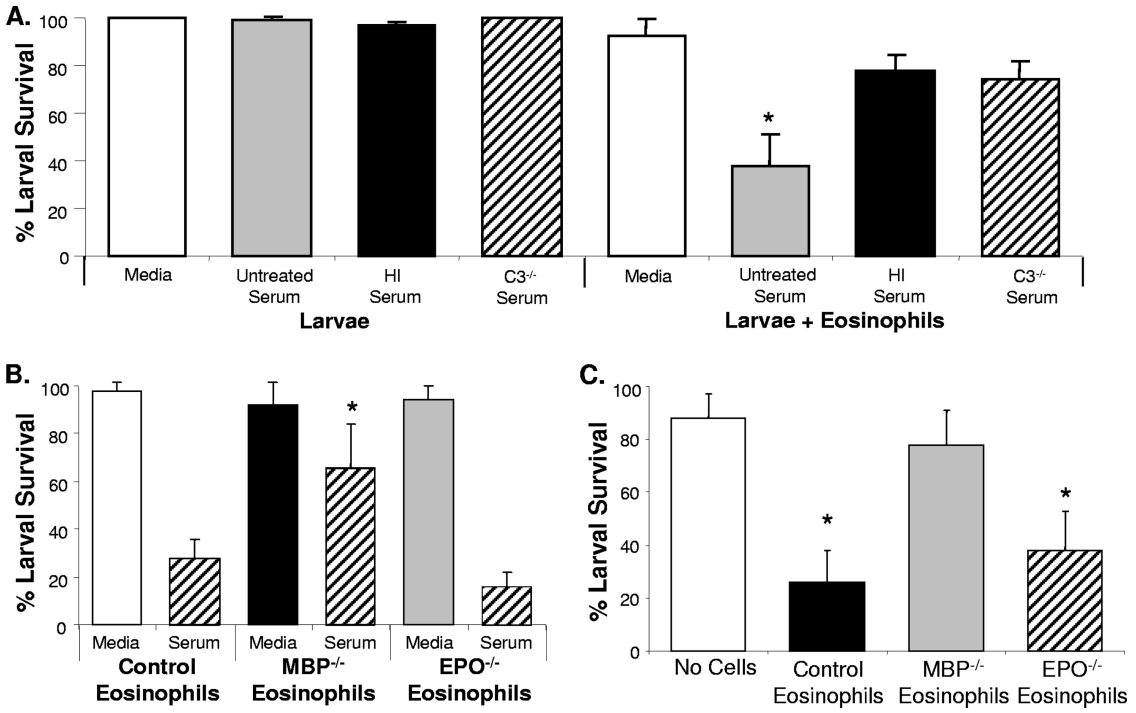

FIG. 2. *In vitro* and *in vivo* studies on the role of MBP and EPO on killing of larvae by eosinophils. (A) Larvae *in vitro* with eosinophils derived from IL-5 TG mice were killed if untreated serum was added but not if serum that was heat inactivated (HI) or that was derived from $C3^{-/-}$ mice was added to the cultures. *, statistically significant difference between larval survival from culture wells in which untreated serum was added and wells in which no serum was added or heat-inactivated serum or serum that was derived from $C3^{-/-}$ mice was added. (B) Larvae were placed *in vitro* in wells containing eosinophils derived from IL-5 TG, $MBP^{-/-}$ × IL-5 TG, and $EPO^{-/-}$ × IL-5 TG mice in the presence of untreated naïve serum. *, statistically significant difference between larval survival in culture wells in which $MBP^{-/-}$ × IL-5 TG eosinophils were inserted and survival in wells containing eosinophils from either IL-5 TG or $EPO^{-/-}$ × IL-5 TG mice. (C) Transfer of eosinophils derived from IL-5 TG, $MBP^{-/-}$ × IL-5 TG, and $EPO^{-/-}$ × IL-5 TG mice into cell-impermeable diffusion chambers with larvae and implantation for 1 day in naïve C57BL/6 mice. *, statistically significant difference between parasite survival in the presence of IL-5 TG and $EPO^{-/-}$ × IL-5 eosinophils and the absence of cells or the presence of eosinophils from $MBP^{-/-}$ × IL-5 TG mice. Data shown represent the means and standard deviations from 11 to 13 mice per group.

defect in primary and secondary immunity in $MPO^{-/-}$ mice was limited to neutrophil function, since naïve and immunized $MPO^{-/-}$ mice that received C57BL/6 neutrophils killed larvae at a level equal to that for C57BL/6 mice (Table 1). Therefore, *in vitro*- and *in vivo*-derived data confirm that MPO from neutrophils is required for killing of *S. stercoralis* larvae in primary and secondary immunity.

DISCUSSION

The objective of this study was to determine the molecular mechanisms used by eosinophils and neutrophils to kill the larvae of *S. stercoralis*. It was concluded that there is redundancy within the protective immune response, with both eosinophils and neutrophils being capable of acting as effector cells. Eosinophils required MBP to kill the worms in the primary response, and neutrophils required MPO in the primary and secondary responses to kill the worms.

Initial studies on primary and secondary immunity to *S. stercoralis* in $MBP^{-/-}$ and $EPO^{-/-}$ mice indicated that these molecules were not required in either immune response for eosinophils to function as killing cells when other host cells were accessible. Yet, it is was clear that eosinophils were essential components of the primary immune response, based on the observation that elimination of eosinophils in wild-type, $MBP^{-/-}$, and $EPO^{-/-}$ mice blocked protective immunity in naïve mice. When experiments were performed *in vitro* using isolated eosinophils to test their ability to kill the worms, it was determined that eosinophils deficient in MBP did not kill the worms, whereas eosinophils deficient in EPO killed the worms at levels equivalent to those by wild-type cells. Interestingly, killing of larvae by eosinophils *in vitro* required complement as a cofactor, as previously reported *in vivo* (39). Eosinophils deficient in MBP also did not kill the worms *in vivo* when placed in diffusion chambers with the larvae, whereas eosinophils deficient in EPO killed the worms at rates equal to those of wild-type cells if no other host cells were present.

Granule products purified from human eosinophils have been tested for their ability to directly kill the larvae of *S. stercoralis*. Human eosinophil-derived MBP and eosinophil cationic protein killed the mammal-adapted larvae of *S. stercoralis* but not the infective larvae, whereas EPO- and eosinophil-derived neurotoxin did not kill either form of the larvae (57). The findings of the current study using $MBP^{-/-}$ and $EPO^{-/-}$ mice provide further support for the findings of these earlier studies, confirming similar activities of MBP as well as the inactivity of EPO. Other studies using granule products derived from human eosinophils have demonstrated that MBP

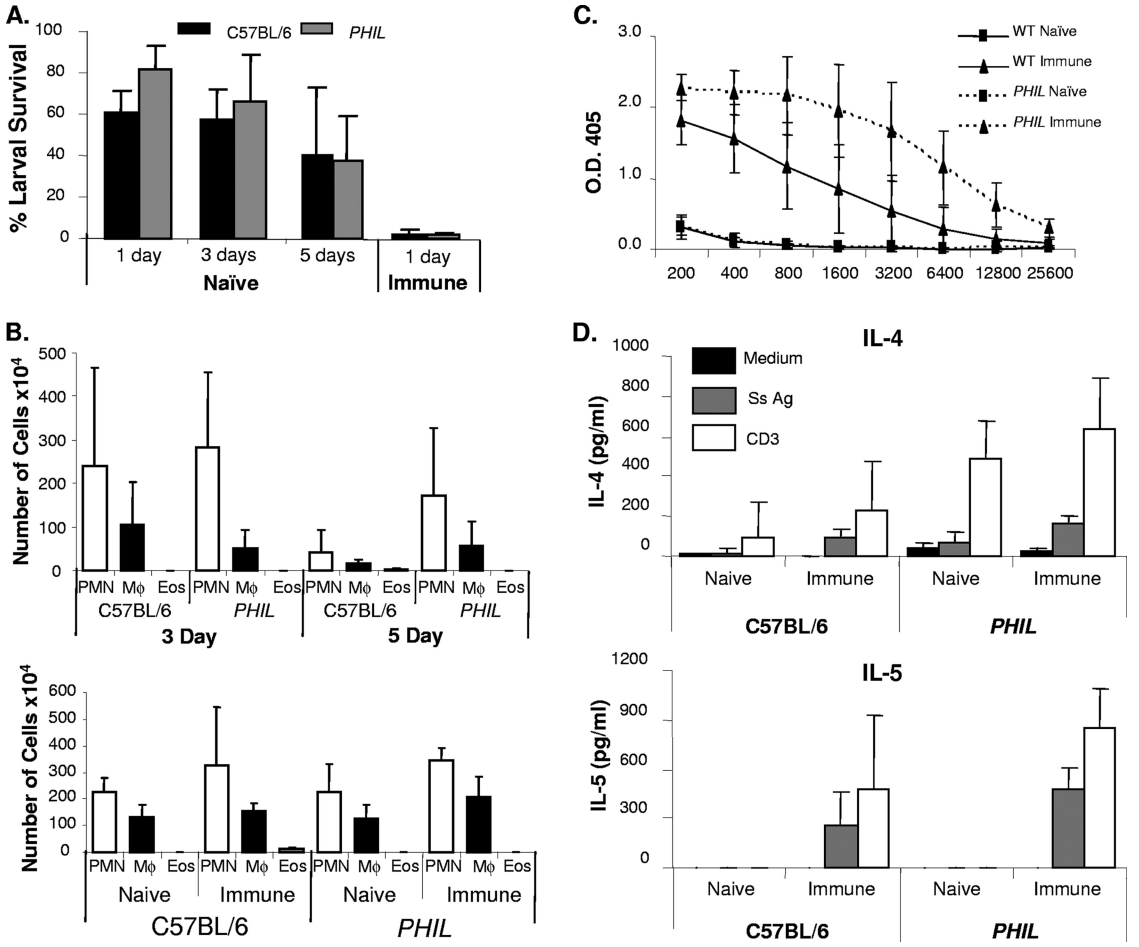

FIG. 3. Primary and secondary immunity to *S. stercoralis* in *PHIL* mice. (A) Parasite survival in naïve or immunized C57BL/6 or *PHIL* mice after 1, 3, or 5 days. (B) Number of neutrophils (polymorphonuclear leukocytes [PMN]), macrophages (Mϕ), and eosinophils (EOS) found within diffusion chambers implanted in naïve or immunized C57BL/6 and *PHIL* mice. (C) Serial dilutions of parasite-specific IgM response in naïve or immunized C57BL/6 or *PHIL* mice. OD, optical density; WT, wild type. (D) Production of IL-4 and IL-5 by spleen cells derived from naïve or immunized C57BL/6 or *PHIL* mice after stimulation with medium, *S. stercoralis* antigen (Ss Ag), or CD3. Data shown represent the means and standard deviations from 9 to 10 mice per group.

and EPO are both toxic to *Schistosoma mansoni* (3, 12, 37), *T. spiralis* (13, 26, 66), and the microfilariae of *Brugia pahangi* and *Brugia malayi* (27). In contrast, studies performed on *B. pahangi* in mice deficient in MBP or EPO concluded that eosinophils were required for protective immunity, yet neither MBP nor EPO was required (54). It is possible that MBP and EPO are effective at killing the microfilarial stage of *Brugia* but not other stages of these filarial worms. Alternatively, the observations on immunity to *B. pahangi* in mice deficient in MBP and EPO may be comparable to those seen in $MBP^{-/-}$ and $EPO^{-/-}$ mice infected with *S. stercoralis* in the present study, in that while eosinophil granule products are toxic to worms, this is not the only mechanism by which eosinophils control the infections. Finally, immunity to the larvae of *O. volvulus* in mice depends on eosinophils, but not on EPO (1). In contrast, it was shown in $MBP^{-/-}$ and $EPO^{-/-}$ mice that protective immunity to *Litomosoides sigmodontis* required both eosinophil granule products (61), demonstrating that even within filarial worms the mechanisms used by eosinophils to control the infections can differ dramatically.

Experiments from the current study using mice deficient in MBP indicated that eosinophils are required for killing of worms in the primary response, although MBP was not essential. However, *in vitro* and *in vivo* experiments using eosinophils isolated from $MBP^{-/-}$ mice indicated that MBP was required for eosinophils to kill the worms. An explanation for these disparate findings is that eosinophils participate in killing the larvae through two distinct mechanisms. The direct killing mechanism used by eosinophils requires complement and is MBP dependent. The indirect killing mechanism requires the interaction between eosinophils and other cells in the host and is MBP independent. Optimal killing of larvae in naïve mice depends on the presence of both eosinophils and neutrophils (21). It is possible that the mechanism of optimal killing seen in wild-type mice is not the additive result of independent killing mechanisms by eosinophils and neutrophils but, rather, a synergistic mechanism requiring the cells to collaborate in an MBP-independent mechanism.

Experiments were performed in eosinophil-deficient *PHIL* mice to further investigate the role of eosinophils in primary

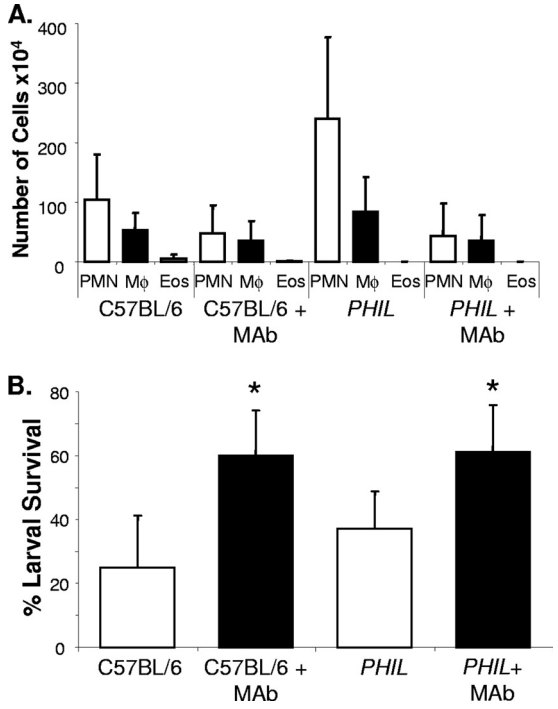

FIG. 4. Treatment of *PHIL* mice with MAb to eliminate neutrophils. (A) Number of neutrophils (polymorphonuclear leukocytes [PMN]), macrophages (Mϕ), and eosinophils (EOS) found within cell-permeable diffusion chambers implanted for 3 days in untreated naïve C57BL/6 or *PHIL* mice and mice treated with MAb RB6-8C5 to eliminate neutrophils. (B) Parasite survival in naïve C57BL/6 and *PHIL* mice treated with a MAb to eliminate neutrophils. *, statistically significant difference between larval survival in mice receiving treatment to eliminate neutrophils and untreated controls. Data shown represent the means and standard deviations from 8 to 10 mice per group.

and secondary immunity to the larvae of *S. stercoralis. PHIL* mice developed primary and secondary immunity to infection with *S. stercoralis* in a manner indistinguishable from that for wild-type mice. Previous studies have shown that eosinophils are not required as effector cells in the secondary immune response (21). However, eosinophils have the capacity to act as antigen-presenting cells for antigens from *S. stercoralis* to induce Th2 responses and specific antibody production (50, 51). Results from immunized *PHIL* mice indicate that although eosinophils can function as antigen-presenting cells, they are not required for this role in *PHIL* mice, as immunized *PHIL* mice had intact T and B cell responses to the infection. Clearly, other cells act as antigen-presenting cells when eosinophils are absent.

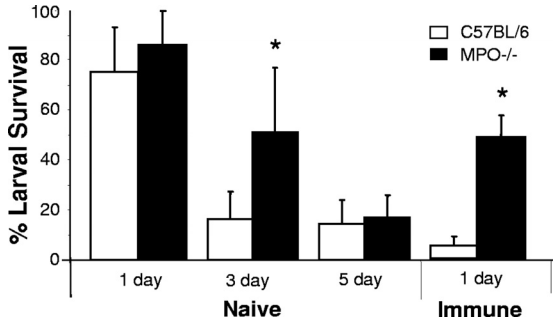

FIG. 5. Primary and secondary immunity to *S. stercoralis* in $MPO^{-/-}$ mice. Parasite survival in naïve or immunized C57BL/6 or $MPO^{-/-}$ mice for 1, 3 or 5 days. *, statistically significant difference between larval survival in $MPO^{-/-}$ mice and C57BL/6 mice. Data shown represent the means and standard deviations from 8 to 10 mice per group.

TABLE 1. Effect of neutrophils derived from C57BL/6 or $MPO^{-/-}$ mice on survival of larvae of *S. stercoralis* larvae implanted in cell-impermeable diffusion chambers in naïve or immunized C57BL/6 and $MPO^{-/-}$ mice

Recipient mouse	Mean % survival ± SD by neutrophil source[a]		
	No cells	C57BL/6	$MPO^{-/-}$
C57BL/6 naïve	97 ± 5 (10)	70 ± 16 (19)	96 ± 06 (9)
C57BL/6 immune	96 ± 5 (9)	44 ± 26 (19)	76 ± 20 (10)
$MPO^{-/-}$ naïve	ND	74 ± 14 (8)	ND
$MPO^{-/-}$ immune	ND	34 ± 22 (9)	ND

[a] Data shown represent the mean percent survival and standard deviation from 8 to 10 mice per group. The specific number of mice in each experiment is indicated in parentheses. ND, not determined.

A spectrum of results has been obtained from studies of the role of eosinophils in protective immunity to various pathogens utilizing *PHIL* and ΔdblGATA mice (33), both of which lack eosinophils. Infection and disease caused by *S. mansoni* in *PHIL* mice and ΔdblGATA mice did not differ from those seen in wild-type mice (63). *PHIL* mice had impaired ability to control infection with the bacterium *Pseudomonas aeruginosa* (46), and resistance to *Nippostrongylus brasiliensis* was transiently impaired in ΔdblGATA mice within primary and secondary infections (41). Finally, infection of *PHIL* and ΔdblGATA mice with *T. spiralis* demonstrated that the intestinal phase of the infection was not affected by the absence of eosinophils, whereas the absence of eosinophils resulted in a decrease in the survival of larvae in muscle, thus showing that the eosinophils can enhance parasite survival (19). Results obtained with *S. stercoralis* in *PHIL* mice are consistent with those obtained with *S. mansoni* (63), in that immunity was not impaired in the absence of eosinophils.

The role that eosinophils play in the primary immune response to *S. stercoralis* appears to be dependent on the host in which they are found. Treatment of control C57BL/6 mice to eliminate eosinophils decreased primary immunity, whereas *PHIL* mice, which constitutively lack eosinophils, did not have a diminished protective primary immune response to the infection. *PHIL* mice may have developed enhanced alternative killing mechanisms to compensate for the absence of eosinophils. Treatment of *PHIL* mice with MAb to eliminate neutrophils blocked the primary immune response, indicating that in the absence of eosinophils, neutrophils assume sole responsibility for eliminating the larvae of *S. stercoralis*. Purified MPO from neutrophils is toxic to *S. mansoni* (37) and *T. spiralis* (13). Human neutrophils deficient in MPO have decreased ability to kill the amoeba *Naegleria fowleri* (20) and the bacterium *Escherichia coli* (55). Mice deficient in MPO have increased susceptibility to a variety of bacteria and fungi (6–10, 22). In the present study, $MPO^{-/-}$ mice had a time-limited reduction in protective primary immunity to *S. stercoralis* and a decrease in protective secondary immunity. It was concluded that MPO from neutrophils functions in killing *S. stercoralis* larvae. This

finding confirms the observation that the toxicity of MPO is not limited to intracellular pathogens but also will target extracellular organisms, as previously reported (13, 20, 37).

Both eosinophils and neutrophils are effector cells against parasites, although their functions differ on the basis of the species and stage of the organism. Killing microfilariae of *O. volvulus* (23, 35) and *Dirofilaria immitis* (18) is mediated by either neutrophils or eosinophils. Alternatively, killing the adult stage of *O. volvulus* appears to depend on eosinophils and is blocked by the presence of neutrophils (48). A third mechanism, demonstrating synergy between neutrophils and eosinophils, has been observed in the human neutrophil killing of *S. mansoni* schistosomula. Maximum killing by neutrophils occurred when EPO from eosinophils was bound to the surface of the worm (36). The present study demonstrates that both eosinophils and neutrophils can independently kill the larvae of *S. stercoralis* in the primary immune response. Results from the *PHIL* mice show that in the absence of eosinophils, neutrophils compensate and kill the larvae. Furthermore, killing larvae in the secondary immune response depends on MPO from neutrophils. The observation that MPO from neutrophils was required on day 3 postinfection and MBP was required from eosinophils on day 5 postinfection suggests that there is sequential killing of the larvae, with neutrophils preceding eosinophils. Alternatively, the observation that eosinophils did not require either MBP or EPO to kill the worms in the primary immune response if other cell types were available suggests that in addition to being able to kill independently, eosinophils can also kill the worms with neutrophils through a synergistic mechanism. Human infections with *S. stercoralis* differ from other nematode infections in terms of longevity, with infections that can persist for decades, and the development of hyperinfection related to immunosuppression. It is possible that the primary immune response in humans mirrors that seen in mice and was developed with redundancies to ensure a carefully controlled yet effective protective immune response.

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REFERENCES

1. **Abraham, D., et al.** 2004. Immunoglobulin E and eosinophil-dependent protective immunity to larval *Onchocerca volvulus* in mice immunized with irradiated larvae. Infect. Immun. **72:**810–817.
2. **Abraham, D., et al.** 1995. *Strongyloides stercoralis*: protective immunity to third-stage larvae in BALB/cByJ mice. Exp. Parasitol. **80:**297–307.
3. **Ackerman, S. J., G. J. Gleich, D. A. Loegering, B. A. Richardson, and A. E. Butterworth.** 1985. Comparative toxicity of purified human eosinophil granule cationic proteins for schistosomula of *Schistosoma mansoni*. Am. J. Trop. Med. Hyg. **34:**735–745.
4. **Aime, N., A. Haque, B. Bonnel, G. Torpier, and A. Capron.** 1984. Neutrophil-mediated killing of *Dipetalonema viteae* microfilariae: simultaneous presence of IgE, IgG antibodies and complement is required. Immunology **51:**585–594.
5. **Anthony, R. M., L. I. Rutitzky, J. F. Urban, Jr., M. J. Stadecker, and W. C. Gause.** 2007. Protective immune mechanisms in helminth infection. Nat. Rev. Immunol. **7:**975–987.
6. **Aratani, Y., et al.** 1999. Severe impairment in early host defense against *Candida albicans* in mice deficient in myeloperoxidase. Infect. Immun. **67:** 1828–1836.
7. **Aratani, Y., et al.** 2006. Contribution of the myeloperoxidase-dependent oxidative system to host defence against *Cryptococcus neoformans*. J. Med. Microbiol. **55:**1291–1299.
8. **Aratani, Y., et al.** 2002. Critical role of myeloperoxidase and nicotinamide adenine dinucleotide phosphate-oxidase in high-burden systemic infection of mice with *Candida albicans.* J. Infect. Dis. **185:**1833–1837.
9. **Aratani, Y., et al.** 2002. Relative contributions of myeloperoxidase and NADPH-oxidase to the early host defense against pulmonary infections with *Candida albicans* and *Aspergillus fumigatus*. Med. Mycol. **40:**557–563.
10. **Aratani, Y., et al.** 2000. Differential host susceptibility to pulmonary infections with bacteria and fungi in mice deficient in myeloperoxidase. J. Infect. Dis. **182:**1276–1279.
11. **Brigandi, R. A., et al.** 1996. *Strongyloides stercoralis*: role of antibody and complement in immunity to the third stage of larvae in BALB/cByJ mice. Exp. Parasitol. **82:**279–289.
12. **Butterworth, A. E., D. L. Wassom, G. J. Gleich, D. A. Loegering, and J. R. David.** 1979. Damage to schistosomula of *Schistosoma mansoni* induced directly by eosinophil major basic protein. J. Immunol. **122:**221–229.
13. **Buys, J., R. Wever, and E. J. Ruitenberg.** 1984. Myeloperoxidase is more efficient than eosinophil peroxidase in the in vitro killing of newborn larvae of *Trichinella spiralis*. Immunology **51:**601–607.
14. **Cadman, E. T., and R. A. Lawrence.** 2010. Granulocytes: effector cells or immunomodulators in the immune response to helminth infection? Parasite Immunol. **32:**1–19.
15. **Chandrashekar, R., U. R. Rao, and D. Subrahmanyam.** 1990. Antibody-mediated cytotoxic effects in vitro and in vivo of rat cells on infective larvae of *Brugia malayi*. Int. J. Parasitol. **20:**725–730.
16. **Denzler, K. L., et al.** 2001. Extensive eosinophil degranulation and peroxidase-mediated oxidation of airway proteins do not occur in a mouse ovalbumin-challenge model of pulmonary inflammation. J. Immunol. **167:**1672–1682.
17. **Denzler, K. L., et al.** 2000. Eosinophil major basic protein-1 does not contribute to allergen-induced airway pathologies in mouse models of asthma. J. Immunol. **165:**5509–5517.
18. **El-Sadr, W. M., M. Aikawa, and B. M. Greene.** 1983. In vitro immune mechanisms associated with clearance of microfilariae of *Dirofilaria immitis*. J. Immunol. **130:**428–434.
19. **Fabre, V., et al.** 2009. Eosinophil deficiency compromises parasite survival in chronic nematode infection. J. Immunol. **182:**1577–1583.
20. **Ferrante, A., N. L. Hill, T. J. Abell, and H. Pruul.** 1987. Role of myeloperoxidase in the killing of *Naegleria fowleri* by lymphokine-altered human neutrophils. Infect. Immun. **55:**1047–1050.
21. **Galioto, A. M., et al.** 2006. Role of eosinophils and neutrophils in innate and adaptive protective immunity to larval *Strongyloides stercoralis* in mice. Infect. Immun. **74:**5730–5738.
22. **Gaut, J. P., et al.** 2001. Neutrophils employ the myeloperoxidase system to generate antimicrobial brominating and chlorinating oxidants during sepsis. Proc. Natl. Acad. Sci. U. S. A. **98:**11961–11966.
23. **Greene, B. M., H. R. Taylor, and M. Aikawa.** 1981. Cellular killing of microfilariae of *Onchocerca volvulus*: eosinophil and neutrophil-mediated immune serum-dependent destruction. J. Immunol. **127:**1611–1618.
24. **Grimaldi, J. C., et al.** 1999. Depletion of eosinophils in mice through the use of antibodies specific for C-C chemokine receptor 3 (CCR3). J. Leukoc. Biol. **65:**846–853.
25. **Gurish, M. F., et al.** 2002. CCR3 is required for tissue eosinophilia and larval cytotoxicity after infection with *Trichinella spiralis*. J. Immunol. **168:**5730–5736.
26. **Hamann, K. J., R. L. Barker, D. A. Loegering, and G. J. Gleich.** 1987. Comparative toxicity of purified human eosinophil granule proteins for newborn larvae of *Trichinella spiralis*. J. Parasitol. **73:**523–529.
27. **Hamann, K. J., et al.** 1990. In vitro killing of microfilariae of *Brugia pahangi* and *Brugia malayi* by eosinophil granule proteins. J. Immunol. **144:**3166–3173.
28. **Hart, P. H., L. K. Spencer, M. F. Nulsen, P. J. McDonald, and J. J. Finlay-Jones.** 1986. Neutrophil activity in abscess-bearing mice: comparative studies with neutrophils isolated from peripheral blood, elicited peritoneal exudates, and abscesses. Infect. Immun. **51:**936–941.
29. **Herbert, D. R., et al.** 2000. Role of IL-5 in innate and adaptive immunity to larval *Strongyloides stercoralis* in mice. J. Immunol. **165:**4544–4551.
30. **Herbert, D. R., T. J. Nolan, G. A. Schad, and D. Abraham.** 2002. The role of B cells in immunity against larval *Strongyloides stercoralis* in mice. Parasite Immunol. **24:**95–101.
31. **Herbert, D. R., T. J. Nolan, G. A. Schad, S. Lustigman, and D. Abraham.** 2002. Immunoaffinity-isolated antigens induce protective immunity against larval *Strongyloides stercoralis* in mice. Exp. Parasitol. **100:**112–120.
32. **Hestdal, K., et al.** 1991. Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cells. J. Immunol. **147:**22–28.
33. **Humbles, A. A., et al.** 2004. A critical role for eosinophils in allergic airways remodeling. Science **305:**1776–1779.
34. **Johnson, E. H., et al.** 1994. *Onchocerca volvulus*: in vitro cytotoxic effects of human neutrophils and serum on third-stage larvae. Trop. Med. Parasitol. **45:**331–335.
35. **Johnson, E. H., S. Lustigman, B. Brotman, J. Browne, and A. M. Prince.** 1991. *Onchocerca volvulus*: in vitro killing of microfilaria by neutrophils and

eosinophils from experimentally infected chimpanzees. Trop. Med. Parasitol. **42:**351–355.
36. **Jong, E. C., E. Y. Chi, and S. J. Klebanoff.** 1984. Human neutrophil-mediated killing of schistosomula of *Schistosoma mansoni*: augmentation by schistosomal binding of eosinophil peroxidase. Am. J. Trop. Med. Hyg. **33:**104–115.
37. **Jong, E. C., A. A. Mahmoud, and S. J. Klebanoff.** 1981. Peroxidase-mediated toxicity to schistosomula of *Schistosoma mansoni*. J. Immunol. **126:**468–471.
38. **Kazura, J. W., and M. Aikawa.** 1980. Host defense mechanisms against *Trichinella spiralis* infection in the mouse: eosinophil-mediated destruction of newborn larvae in vitro. J. Immunol. **124:**355–361.
39. **Kerepesi, L. A., J. A. Hess, T. J. Nolan, G. A. Schad, and D. Abraham.** 2006. Complement component C3 is required for protective innate and adaptive immunity to larval *Strongyloides stercoralis* in mice. J. Immunol. **176:**4315–4322.
40. **Klion, A. D., and T. B. Nutman.** 2004. The role of eosinophils in host defense against helminth parasites. J. Allergy Clin. Immunol. **113:**30–37.
41. **Knott, M. L., et al.** 2007. Impaired resistance in early secondary *Nippostrongylus brasiliensis* infections in mice with defective eosinophilopoeisis. Int. J. Parasitol. **37:**1367–1378.
42. **Lee, J. J., et al.** 2004. Defining a link with asthma in mice congenitally deficient in eosinophils. Science **305:**1773–1776.
43. **Lee, N. A., et al.** 1997. Expression of IL-5 in thymocytes/T cells leads to the development of a massive eosinophilia, extramedullary eosinophilopoiesis, and unique histopathologies. J. Immunol. **158:**1332–1344.
44. **Lee, T. D.** 1991. Helminthotoxic responses of intestinal eosinophils to *Trichinella spiralis* newborn larvae. Infect. Immun. **59:**4405–4411.
45. **Ligas, J. A., et al.** 2003. Specificity and mechanism of immunoglobulin M (IgM)- and IgG-dependent protective immunity to larval *Strongyloides stercoralis* in mice. Infect. Immun. **71:**6835–6843.
46. **Linch, S. N., et al.** 2009. Mouse eosinophils possess potent antibacterial properties in vivo. Infect. Immun. **77:**4976–4982.
47. **Meeusen, E. N., and A. Balic.** 2000. Do eosinophils have a role in the killing of helminth parasites? Parasitol. Today **16:**95–101.
48. **Nfon, C. K., et al.** 2006. Eosinophils contribute to killing of adult *Onchocerca ochengi* within onchocercomata following elimination of *Wolbachia*. Microbes Infect. **8:**2698–2705.
49. **O'Connell, A. E., et al.** 2011. Soluble extract from the nematode *Strongyloides stercoralis* induces CXCR2 dependent/IL-17 independent neutrophil chemotaxis. Microbes Infect. **13:**536–544.
50. **Padigel, U. M., et al.** 2007. Eosinophils act as antigen-presenting cells to induce immunity to *Strongyloides stercoralis* in mice. J. Infect. Dis. **196:**1844–1851.
51. **Padigel, U. M., J. J. Lee, T. J. Nolan, G. A. Schad, and D. Abraham.** 2006. Eosinophils can function as antigen-presenting cells to induce primary and secondary immune responses to *Strongyloides stercoralis*. Infect. Immun. **74:**3232–3238.
52. **Padigel, U. M., et al.** 2007. Signaling through Galphai2 protein is required for recruitment of neutrophils for antibody-mediated elimination of larval *Strongyloides stercoralis* in mice. J. Leukoc. Biol. **81:**1120–1126.
53. **Rainbird, M. A., D. Macmillan, and E. N. Meeusen.** 1998. Eosinophil-mediated killing of *Haemonchus contortus* larvae: effect of eosinophil activation and role of antibody, complement and interleukin-5. Parasite Immunol. **20:**93–103.
54. **Ramalingam, T., P. Porte, J. Lee, and T. V. Rajan.** 2005. Eosinophils, but not eosinophil peroxidase or major basic protein, are important for host protection in experimental *Brugia pahangi* infection. Infect. Immun. **73:**8442–8443.
55. **Rosen, H., et al.** 2009. Methionine oxidation contributes to bacterial killing by the myeloperoxidase system of neutrophils. Proc. Natl. Acad. Sci. U. S. A. **106:**18686–18691.
56. **Rotman, H. L., et al.** 1997. IL-12 eliminates the Th-2 dependent protective immune response of mice to larval *Strongyloides stercoralis*. Parasite Immunol. **19:**29–39.
57. **Rotman, H. L., et al.** 1996. *Strongyloides stercoralis*: eosinophil-dependent immune-mediated killing of third stage larvae in BALB/cByJ mice. Exp. Parasitol. **82:**267–278.
58. **Saeftel, M., M. Arndt, S. Specht, L. Volkmann, and A. Hoerauf.** 2003. Synergism of gamma interferon and interleukin-5 in the control of murine filariasis. Infect. Immun. **71:**6978–6985.
59. **Shaio, M. F., et al.** 1990. Immunoglobulin G-dependent classical complement pathway activation in neutrophil-mediated cytotoxicity to infective larvae of *Angiostrongylus cantonensis*. Ann. Trop. Med. Parasitol. **84:**185–191.
60. **Shuhua, X., et al.** 2001. Electron and light microscopy of neutrophil responses in mice vaccinated and challenged with third-stage infective hookworm (*Ancylostoma caninum*) larvae. Parasitol. Int. **50:**241–248.
61. **Specht, S., et al.** 2006. Lack of eosinophil peroxidase or major basic protein impairs defense against murine filarial infection. Infect. Immun. **74:**5236–5243.
62. **Stein, L. H., et al.** 2009. Eosinophils utilize multiple chemokine receptors for chemotaxis to the parasitic nematode *Strongyloides stercoralis.* J. Innate Immun. **1:**618–630.
63. **Swartz, J. M., et al.** 2006. *Schistosoma mansoni* infection in eosinophil lineage-ablated mice. Blood **108:**2420–2427.
64. **Venturiello, S. M., G. H. Giambartolomei, and S. N. Costantino.** 1995. Immune cytotoxic activity of human eosinophils against *Trichinella spiralis* newborn larvae. Parasite Immunol. **17:**555–559.
65. **Venturiello, S. M., G. H. Giambartolomei, and S. N. Costantino.** 1993. Immune killing of newborn *Trichinella larvae* by human leucocytes. Parasite Immunol. **15:**559–564.
66. **Wassom, D. L., and G. J. Gleich.** 1979. Damage to *Trichinella spiralis* newborn larvae by eosinophil major basic protein. Am. J. Trop. Med. Hyg. **28:**860–863.

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