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# Eosinophils, but Not Eosinophil Peroxidase or Major Basic Protein, Are Important for Host Protection in Experimental *Brugia pahangi* Infection

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**The attenuation of eosinophilia by the administration of monoclonal antibodies to CCR3 consistently correlates with impairment in worm elimination following primary intraperitoneal *Brugia pahangi* infections in mice. Host protection was unimpaired in mice deficient in eosinophil peroxidase (EPO) or major basic protein 1 (MBP-1), suggesting that eosinophils are essential in host protection but that neither EPO nor MBP-1 alone is.**

Despite the consistent association of eosinophilia with parasitic infections, evidence supporting a host-protective role in vivo remains elusive (1–4, 16, 22). The function of eosinophils has been studied by modulating their numbers by manipulating interleukin-5 levels (5) (13) or depleting them selectively with a monoclonal antibody against the eotaxin receptor CCR3 (8). Eosinophilia correlates with improved host protection in *Angiostrongylus cantonensis* (19), *Strongyloides ratti* (17), *Strongyloides stercoralis* (12), *Onchocerca volvulus* (14), and *Litomosoides sigmodontis* (15) infections. In contrast, the depletion of eosinophils did not alter the course of *Schistosoma mansoni* (20), *Mesocestoides corti* (13), and *Trichuris muris* (3) infections. In the case of filarial parasites, eotaxin and eosinophils have been shown to play a role in host protection (21).

The role of eosinophil granule constituents in host protection is also unclear. Purified eosinophil granule proteins have been shown to effectively kill *Brugia* microfilariae (11), schistosomulae (2), and *Trichinella spiralis* newborn larvae (10) in vitro. Gutierrez-Pena et al. (9) have shown that the death of onchocercal microfilariae following amocarzine treatment was associated with eosinophil degranulation. Electron microscopy analyses showed apposition of eosinophil granule material on the microfilarial surface. However, the requirement of eosinophil granule protein for in vivo host protection has not been studied extensively. In a recent study, $EPO^{-/-}$ and wild-type (WT) mice challenged with *O. volvulus* manifested similar parasite recoveries, suggesting that eosinophil peroxidase (EPO) is not required for host protection in this model (1). In order to investigate the role of the eosinophil granule proteins in host protection, we have now examined the course of *Brugia pahangi* infection in mice that have undergone targeted mutations in the genes encoding two of the major proteins in the eosinophil granules.

C57BL/6 (hereafter WT) mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). $EPO^{-/-}$ and MBP-$1^{-/-}$ mice (6, 7) were transferred from the Mayo Clinic, Scottsdale, Arizona, where they were generated and backcrossed with C57BL/6 mice for six generations at the UCHC AAALAC-accredited facility. *Brugia pahangi* third-stage larvae (L3) were provided by one of the following sources: TRS Inc. (Athens, GA), John McCall (University of Georgia, Athens, GA), or Thomas Klei (State University of Louisiana, Baton Rouge, LA). Mice were injected with 50 *B. pahangi* L3 intraperitoneally and sacrificed at various time points postinfection. Live worm recoveries were enumerated in the peritoneal lavage fluid and carcass soak fluid. The total numbers of peritoneal cells (PECs) and various cell types were enumerated as described previously (18). Monoclonal antibodies against CCR3 (6S2-19-4) were obtained from DNAX (Palo Alto, CA) (8). Monoclonal antibodies against rat RT 6.1 (DS4.23) and 6.2 (6A5) were obtained from Dale Greiner, UMass Medical Center, Worcester, MA. Antibodies were enriched from hybridoma ascites fluid by 50% ammonium sulfate precipitation. The precipitate was dialyzed against phosphate-buffered saline, and the protein content was measured by the bicinchoninic acid protein assay reagent (Pierce, Rockford, IL). The purity of the antibody preparations was ascertained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue. Student's *t* test was used to deduce statistical significance using Microsoft Excel or Graphpad Prism. *P* values of less than 0.05 were considered statistically significant.

We administered 1 mg of eosinophil-depleting anti-CCR3 or an isotype-matched control intraperitoneally to two groups of mice (five mice per group) at the same time as the worm infection. Mice were necropsied 2 weeks postinfection. PECs analyzed as described earlier (18) to enumerate lymphocytes and macrophages revealed no significant differences between the two groups. Mice that received anti-CCR3 had significantly fewer eosinophils [$(0.57 \pm 0.2) \times 10^6$ cells per mouse] than mice treated with the isotype control antibody [$(4.3 \pm 1.2) \times 10^6$ eosinophils per mouse; $P < 0.01$]. Anti-CCR3-treated mice also retained higher parasite numbers ($21\% \pm 4.73\%$) than the isotype control-treated group ($7.5\% \pm 1.9\%$; $P < 0.01$). These data are representative of two similar experiments.

We next sought to determine whether eosinophil granule contents are essential for eliminating parasite infection. On

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two independent occasions, we injected groups of EPO$^{-/-}$ ($n = 7$) and WT ($n = 8$) mice with *B. pahangi* L3 and necropsied them on day 14. The absence of EPO does not impair the ability of the mice to eliminate the parasites, since both groups of mice have nearly identical worm recoveries (11.14% ± 6.4% in EPO$^{-/-}$ mice versus 11.75% ± 8.1% in WT mice; $P = 0.86$). Eosinophil numbers were significantly lower in EPO$^{-/-}$ than in WT mice [$(3.2 \pm 2.1) \times 10^6$ eosinophils/mouse in EPO$^{-/-}$ mice versus $(5.9 \pm 2.0) \times 10^6$ eosinophils/mouse in WT mice; $P < 0.01$], though total PEC numbers were comparable. Data from the duplicate experiment were similar.

MBP-1$^{-/-}$ mice also did not manifest a defect in their ability to eliminate infection. We injected 50 *B. pahangi* L3 organisms into each of five mice. The larval recoveries from these mice were comparable to those from WT mice at 2 weeks (28.3% ± 18.8% in MBP-1$^{-/-}$ mice compared to 36.0% ± 12.9% in WT mice; $P = 0.72$). In another experiment, the recoveries were lower in both strains of mice but equivalent (0.5% ± 0.5% in MBP-1$^{-/-}$ mice compared to 3.75% ± 1.9% in WT mice; $P = 0.1$). PEC numbers were comparable between the two groups. Unlike EPO$^{-/-}$ mice, MBP-1$^{-/-}$ mice did not have a defect in eosinophil numbers. However, the side-scatter profile (representing granularity or complexity) of eosinophils measured by flow cytometry was significantly lower in the MBP-1$^{-/-}$ eosinophils, reflecting a substantial contribution by this one protein for this parameter (data not shown).

In summary, treatment with the anti-CCR3 antibody was consistently associated with higher worm recoveries than was isotype control treatment, and this increase in worm recovery was associated with a significant and selective depletion of eosinophils. The results from the two approaches seem to suggest that eosinophils do play an important and nonredundant role in the elimination of the parasite. Our results also suggest that neither EPO nor MBP-1, the two most abundant eosinophil secondary granule proteins, is crucial in worm elimination. It is likely that the repertoire of eosinophil granule contents is redundant and that the absence of one particular protein is compensated for by the other(s). Likewise, in a murine model for airway hyperreactivity, in which these mice were originally studied, the absence of either one of these granule proteins failed to affect the airway response (6, 7).

An unexpected finding for the EPO$^{-/-}$ mice was that their eosinophil numbers were about half of those in WT mice. It is not yet clear how EPO might regulate eosinophil survival or recruitment. It is still possible that the defect in eosinophil numbers is due to an artifactual defect unrelated to EPO function.

Our data argue that eosinophils are required for optimal clearance of *B. pahangi* larvae from the peritoneal cavity following a primary infection and that this eosinophil-mediated protection is not abrogated by the absence of EPO or MBP-1.

## REFERENCES


1. **Abraham, D., O. Leon, S. Schnyder-Candrian, C. C. Wang, A. M. Galioto, L. A. Kerepesi, J. J. Lee, and S. Lustigman.** 2004. Immunoglobulin E and eosinophil-dependent protective immunity to larval *Onchocerca volvulus* in mice immunized with irradiated larvae. Infect. Immun. **72:**810–817.
2. **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.
3. **Betts, C. J., and K. J. Else.** 1999. Mast cells, eosinophils and antibody-mediated cellular cytotoxicity are not critical in resistance to Trichuris muris. Parasite Immunol. **21:**45–52.
4. **Chandrashekar, R., U. R. Rao, P. B. Parab, and D. Subrahmanyam.** 1986. Brugia malayi: rat cell interactions with infective larvae mediated by complement. Exp. Parasitol. **62:**362–369.
5. **Coffman, R. L., B. W. Seymour, S. Hudak, J. Jackson, and D. Rennick.** 1989. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. Science **245:**308–310.
6. **Denzler, K. L., M. T. Borchers, J. R. Crosby, G. Cieslewicz, E. M. Hines, J. P. Justice, S. A. Cormier, K. A. Lindenberger, W. Song, W. Wu, S. L. Hazen, G. J. Gleich, J. J. Lee, and N. A. Lee.** 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.
7. **Denzler, K. L., S. C. Farmer, J. R. Crosby, M. Borchers, G. Cieslewicz, K. A. Larson, S. Cormier-Regard, N. A. Lee, and J. J. Lee.** 2000. Eosinophil major basic protein-1 does not contribute to allergen-induced airway pathologies in mouse models of asthma. J. Immunol. **165:**5509–5517.
8. **Grimaldi, J. C., N. X. Yu, G. Grunig, B. W. Seymour, F. Cottrez, D. S. Robinson, N. Hosken, W. G. Ferlin, X. Wu, H. Soto, A. O'Garra, M. C. Howard, and R. L. Coffman.** 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.
9. **Gutierrez-Pena, E. J., J. Knab, and D. W. Buttner.** 1998. Immunoelectron microscopic evidence for release of eosinophil granule matrix protein onto microfilariae of Onchocerca volvulus in the skin after exposure to amocarzine. Parasitol. Res. **84:**607–615.
10. **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.
11. **Hamann, K. J., G. J. Gleich, J. L. Checkel, D. A. Loegering, J. W. McCall, and R. L. Barker.** 1990. In vitro killing of microfilariae of Brugia pahangi and Brugia malayi by eosinophil granule proteins. J. Immunol. **144:**3166–3173.
12. **Herbert, D. R., J. J. Lee, N. A. Lee, T. J. Nolan, G. A. Schad, and D. Abraham.** 2000. Role of IL-5 in innate and adaptive immunity to larval Strongyloides stercoralis in mice. J. Immunol. **165:**4544–4551.
13. **Kopf, M., F. Brombacher, P. D. Hodgkin, A. J. Ramsay, E. A. Milbourne, W. J. Dai, K. S. Ovington, C. A. Behm, G. Kohler, I. G. Young, and K. I. Matthaei.** 1996. IL-5-deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. Immunity **4:**15–24.
14. **Lange, A. M., W. Yutanawiboonchai, P. Scott, and D. Abraham.** 1994. IL-4- and IL-5-dependent protective immunity to Onchocerca volvulus infective larvae in BALB/cBYJ mice. J. Immunol. **153:**205–211.
15. **Le Goff, L., P. Loke, H. F. Ali, D. W. Taylor, and J. E. Allen.** 2000. Interleukin-5 is essential for vaccine-mediated immunity but not innate resistance to a filarial parasite. Infect. Immun. **68:**2513–2517.
16. **McLaren, D. J., F. J. Ramalho-Pinto, and S. R. Smithers.** 1978. Ultrastructural evidence for complement and antibody-dependent damage to schistosomula of Schistosoma mansoni by rat eosinophils in vitro. Parasitology **77:**313–324.
17. **Ovington, K. S., K. McKie, K. I. Matthaei, I. G. Young, and C. A. Behm.** 1998. Regulation of primary Strongyloides ratti infections in mice: a role for interleukin-5. Immunology **95:**488–493.
18. **Ramalingam, T., L. Ganley-Leal, P. Porte, and T. V. Rajan.** 2003. Impaired clearance of primary but not secondary Brugia infections in IL-5 deficient mice. Exp. Parasitol. **105:**131–139.
19. **Sasaki, O., H. Sugaya, K. Ishida, and K. Yoshimura.** 1993. Ablation of eosinophils with anti-IL-5 antibody enhances the survival of intracranial worms of Angiostrongylus cantonensis in the mouse. Parasite Immunol. **15:**349–354.
20. **Sher, A., R. L. Coffman, S. Hieny, and A. W. Cheever.** 1990. Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against Schistosoma mansoni in the mouse. J. Immunol. **145:**3911–3916.
21. **Simons, J. E., M. E. Rothenberg, and R. A. Lawrence.** 2005. Eotaxin-1-regulated eosinophils have a critical role in innate immunity against experimental Brugia malayi infection. Eur. J. Immunol. **35:**189–197.
22. **Yoshimura, K., K. Uchida, K. Sato, and H. Oya.** 1984. Ultrastructural evidence for eosinophil-mediated destruction of Angiostrongylus cantonensis transferred into the pulmonary artery of non-permissive hosts. Parasite Immunol. **6:**105–118.


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