

Mouse Eosinophils Possess Potent Antibacterial Properties In Vivo[†]

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Eosinophils are best known as the predominant cellular infiltrate associated with asthma and parasitic infections. Recently, numerous studies have documented the presence of Toll-like receptors (TLRs) on the surfaces of eosinophils, suggesting that these leukocytes may participate in the recognition and killing of viruses and bacteria. However, the significance of this role in the innate immune response to bacterial infection is largely unknown. Here we report a novel role for eosinophils as antibacterial defenders in the host response. Isolated mouse eosinophils possessed antipseudomonal properties in vitro. In vivo, interleukin-5 transgenic mice, which have profound eosinophilia, demonstrated improved clearance of *Pseudomonas aeruginosa* introduced into the peritoneal cavity. The findings of improved bacterial clearance following adoptive transfer of eosinophils, and impaired bacterial clearance in mice with a congenital eosinophil deficiency, established that this effect was eosinophil specific. The data presented also demonstrate that eosinophils mediate this antibacterial effect in part through the release of cationic secondary granule proteins. Specifically, isolated eosinophil granules had antibacterial properties in vitro, and administration of eosinophil granule extracts significantly improved bacterial clearance in vivo. These data suggest a potent yet underappreciated antibacterial role for eosinophils in vivo, specifically for eosinophil granules. Moreover, the data suggest that the administration of eosinophil-derived products may represent a viable adjuvant therapy for septic or bacteremic patients in the intensive care unit.

Sepsis, defined as the systemic inflammatory response to infection, is currently the leading cause of death in the intensive care unit (ICU). It affects more than 700,000 people each year in the United States and costs more than \$17 billion annually (4, 18). Ninety percent of cases are caused by bacterial infections (29). Despite maximal supportive care and antimicrobial therapy, mortality remains in excess of 25%, and the use of inappropriate antibiotics further increases mortality (4, 18, 21). These observations highlight the need for novel antimicrobial adjuvant therapies for patients, especially with the increasing prevalence of antibiotic resistance (4, 18, 39).

Innate immune responses are central to the containment of bacterial pathogens through nonspecific pattern recognition receptors present on innate effector cells, such as neutrophils and macrophages. Activation of innate immunity upregulates inflammatory cytokine production and recruitment of additional effector cells to sites of infection. However, in spite of an overwhelming cytokine response in sepsis, inhibition of either pathogen recognition, through blockade of Toll-like receptors (TLRs), or proinflammatory cytokines, such as tumor necrosis factor alpha (TNF- α) or interleukin-1 β (IL-1 β), has failed to improve survival (2, 12, 33, 41, 42). In addition, patients with neutropenia or congenital defects in the innate response have increased susceptibility to, and mortality from, severe bacterial infections (14, 30, 35). Finally, recent studies suggest that sepsis is associated with acquired impairment of innate immune

responses, including evidence of reduced bacterial killing by neutrophils isolated from septic patients (13, 17, 46, 54). Collectively, these data highlight the importance of the pathogen control function of the innate immune response in sepsis.

Recent studies have focused on augmenting innate immune responses in order to improve host defense and clinical outcomes in sepsis. For example, administration of granulocyte-macrophage colony stimulating factor to septic patients increased neutrophil recruitment and enhanced monocyte activation, resulting in increased pathogen control and bacterial clearance (34, 40, 44). Investigators have also focused on neutrophil-derived products such as bactericidal/permeability-increasing protein (BPI), a dominant component of neutrophil granules, which possesses both endotoxin-neutralizing capabilities and a potent nonspecific bactericidal activity against multiple bacterial species (15, 32, 51). A randomized, controlled trial of BPI treatment for meningococcal sepsis demonstrated a significant improvement in morbidity and a trend toward decreased mortality in the treatment group (28). Thus, these studies suggest that either granulocyte activation or administration of granulocyte-derived products may enhance the innate immune response and provide a viable adjuvant therapy for sepsis or bacterial infections.

Eosinophils are another granulocyte subset recently implicated in the innate response to bacterial infections. Recent data documented the expression of multiple TLRs on human eosinophils (31, 52). As a result, human eosinophils recognize and are activated by multiple bacterial species in vitro (8, 37, 47, 52). Once activated, these cells secrete cytotoxic granule proteins, including major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO), all of which have antibacterial properties in vitro (8, 24, 27). How-

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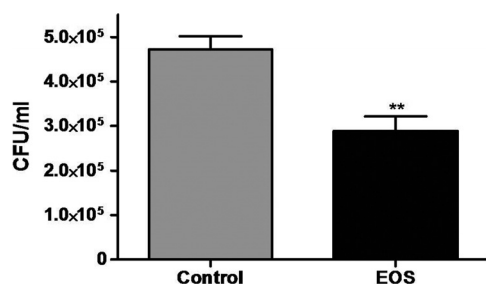


FIG. 1. Eosinophils kill *P. aeruginosa* in vitro. Eosinophils isolated from NJ.1638 mice or PBS was incubated at a multiplicity of infection of 10 with 10^6 CFU of *P. aeruginosa* for 1 h, and quantitative colony counts were made by serial dilution. Shaded bar, vehicle controls; filled bar, eosinophil-treated samples (EOS). Data are means from three independent experiments. Error bars, SEM. **, $P = 0.01$.

ever, little is known about the role of eosinophils in vivo during bacterial infection. Multiple observational studies showed that the number of circulating eosinophils correlates inversely with disease severity in patients with sepsis or severe bacterial infections (1, 5, 6, 45, 50). Conversely, we have shown recently that hypereosinophilic mice have improved survival in a mouse model of polymicrobial sepsis (53). The mechanism of this protection remains enigmatic; therefore, the goal of this study was to fully define the antibacterial properties of mouse eosinophils in vitro, but more importantly in vivo, and to specifically address the role of mouse eosinophil granules in host responses to bacterial infection.

MATERIALS AND METHODS

Mice. Female 8- to 12-week-old C57BL/6 mice were purchased from Jackson Labs, and mice were allowed to acclimatize 1 week prior to use. NJ.1638 and PHIL mice were generated by James J Lee, Mayo Clinic, and genotyping was performed as previously described (25, 26). Mice were bred and housed in a maximal-barrier specific-pathogen-free facility at Oregon Health and Science University, and all experiments with mice were approved by the IACUC. All experiments were performed with age- and sex-matched mice.

Eosinophil isolation. Peripheral blood was collected from female 8- to 12-week-old NJ.1638 mice, diluted with phosphate-buffered saline (PBS) plus 2% fetal bovine serum (FBS), and subjected to density gradient centrifugation using Percoll (GE Healthcare), with a density of 1.084 g/ml (45 min, 2,000 rpm, 4°C). Interface cells were removed and washed with PBS plus 2% FBS. Red blood cells were lysed using sodium chloride lysis buffer. Eosinophils were further isolated on the basis of size and granularity using a Vantage cell sorter; they were then resuspended in PBS plus 6% FBS.

Eosinophil granule isolation. Eosinophils were isolated and purified from tail vasculature-derived blood of IL-5 transgenic mice (26). Heparinized blood was layered onto a Percoll E gradient (60% Percoll E [$\rho = 1.084$], 1× Hanks balanced salt solution, 15 mM HEPES [pH 7.4], and 0.003 N HCl) and centrifuged (45 min, 3,000 rpm, 4°C). The buffy coat was recovered and washed in PBS plus 2% fetal calf serum. Eosinophils were isolated using a magnetic cell separation system (MACS; Miltenyi Biotec). B cells and T cells were removed by positive selection with antibody-conjugated magnetic beads specific for CD45-R (B220) and CD90 (Thy 1.2), respectively. Granules were extracted by washing eosinophils with ice-cold 0.25 M sucrose and lysed with 0.25 M sucrose, 300 U/ml heparin, and 200 U/ml DNase. The granules were recovered by centrifuging the lysate (20 min, 10,000 × g, 4°C), and the pellet was frozen at -80°C.

Ex vivo eosinophil killing assay. Purified eosinophils were resuspended to a concentration of 10^6 /ml in RPMI plus 2% FBS and were added to a 96-well plate. A clinical strain of *Pseudomonas aeruginosa* (Boston 41501; ATCC) was grown with shaking in LB broth at 37°C until an optical density of 1.0, or a concentration of 10^9 CFU/ml, was reached. *P. aeruginosa* was resuspended to approximately 10^7 CFU/ml in RPMI plus 2% FBS and was then added to a 96-well plate with or without eosinophils. Plates were incubated at 37°C for 1 h; suspensions were removed and centrifuged (10,000 rpm). Cell pellets were resuspended in

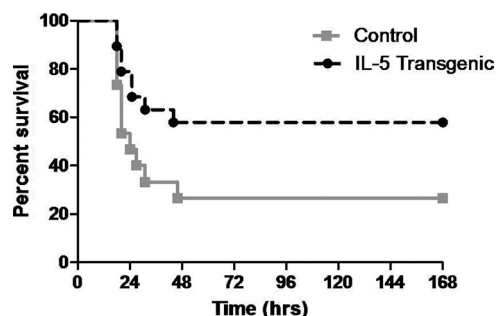


FIG. 2. IL-5 transgenic mice have higher survival rates than their littermate controls in a *Pseudomonas* peritonitis model. Kaplan-Meier survival curves of IL-5 transgenic (NJ.1638) mice ($n = 15$) and littermate controls ($n = 19$) following i.p. infection with 10^7 CFU of *Pseudomonas* are shown. Four independent experiments were performed ($P = 0.05$).

100 μ l PBS, diluted in water, plated on LB agar (Sigma), and incubated at 37°C overnight. Viable-cell counts were performed on the cultured plates.

In vivo assessment of bacterial killing in NJ.1638 and PHIL mice. Eight- to 12-week-old male NJ.1638 mice, PHIL mice, and their respective littermate controls were infected with approximately 10^7 CFU of *P. aeruginosa* intraperitoneally (i.p.). Eighteen hours postinfection, peritoneal lavage and blood samples were taken. Bacterial viability was determined as described above. Samples were also analyzed for the cytokines IL-6, IL-10, IL-12, TNF- α , and IL-1 β by an enzyme-linked immunosorbent assay (ELISA) (R&D Systems) using a VersaMax tunable microplate reader and SoftMax Pro 5 software (Molecular Devices). ELISAs were performed according to the manufacturer's instructions.

Ex vivo eosinophil granule killing assay. Eosinophil granule protein was resuspended in distilled water, sonicated to lyse the granules, and centrifuged at 300 × g. The supernatant was removed, and granule protein was resuspended in 0.01 M HCl to a concentration of protein equivalent to 2×10^7 cells/ml. *P. aeruginosa* was grown with shaking in LB broth as described above. *P. aeruginosa* was resuspended to a concentration of 10^6 CFU/ml in RPMI plus 2% FBS and was added to a 96-well plate. Eosinophil granule protein concentrations were determined using a bicinchoninic acid assay (Pierce Biosciences). Eosinophil granule protein or a vehicle control was added to wells with *P. aeruginosa* at varying doses (8.5 μ g and 350 μ g). Plates were incubated for 1 h at 37°C; suspensions were removed and centrifuged. Bacterial viability was determined as described above.

Adoptive transfer of eosinophils or eosinophil granules and in vivo infection. Purified eosinophils were resuspended to a concentration of 10^6 /ml in PBS. Eosinophils or a saline control was injected i.p. into female C57BL/6 mice 1 h prior to i.p. infection with approximately 10^7 CFU *P. aeruginosa*. Eosinophil granule protein concentrations were determined as described above. Eosinophil granules (130 to 175 μ g) were administered i.p. 1 h following *P. aeruginosa* infection of female C57BL/6 mice. Peritoneal lavage and blood samples were taken from all four groups at 18 h postinfection. Bacterial viability was determined as described above. Samples were also analyzed for the cytokines IL-6, IL-10, IL-12, TNF- α , and IL-1 β by ELISA.

Eosinophil granule treatment in the CLP model. Cecal ligation and puncture (CLP) were performed as previously described (16). Briefly, a small incision was made in the abdominal cavity of each female C57BL/6 mouse. The cecum was isolated and punctured once with a 19-gauge needle, and feces was extruded. The abdominal cavity was sutured, and mice received 1 ml of saline for resuscitation. Eosinophil granules were administered i.p. 1 h following CLP, and animals were monitored for survival.

Statistical analysis. Data were compared by Student's *t* test or analysis of variance where appropriate using GraphPad Prism software. Graphs represent the means $\pm$ standard errors of the means (SEM) for three or more separate experiments. Chi-square analysis was used on blood cultures of PHIL mice.

RESULTS

Eosinophils were isolated from IL-5 transgenic mice (NJ.1638) to determine if mouse eosinophils possess antibacterial properties. These mice constitutively express elevated

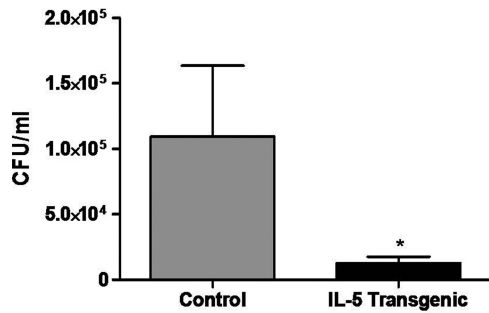


FIG. 3. IL-5 transgenic mice have lower bacterial burdens than their littermate controls. IL-5 transgenic (NJ.1638) mice ($n = 13$) or littermate controls ($n = 16$) were infected i.p. with 10^7 CFU of *P. aeruginosa* and were sacrificed at 18 h. Quantitative colony counts of the peritoneal lavage fluid were made by serial dilution. Bars represent means $\pm$ SEM. Four independent experiments were performed. *, $P = 0.04$.

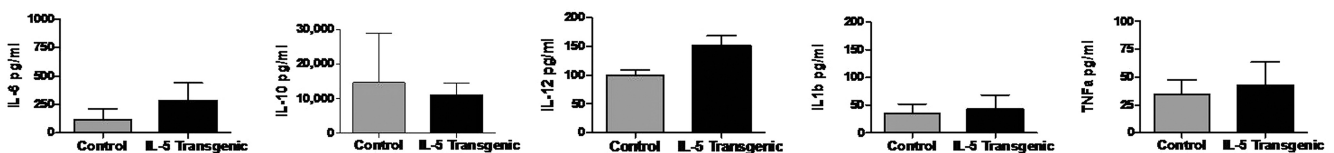
levels of IL-5—the primary hematopoietic growth factor controlling eosinophil maturation and survival—under the control of the CD3 δ promoter, resulting in profound eosinophilia (26). Circulating levels of IL-5 in NJ.1638 mice are 425 pg/ml; those in littermate controls are <10 pg/ml. Eosinophils isolated from NJ.1638 mice were incubated for 1 h with *Pseudomonas aeruginosa* in vitro at a multiplicity of infection of 10. Eosinophils killed approximately 40% of total viable bacteria (Fig. 1). In order to determine if eosinophils are capable of participating in bacterial clearance in vivo, *P. aeruginosa* was administered i.p. to NJ.1638 mice or littermate controls, and the animals were monitored for survival. NJ.1638 mice had significantly higher survival rates following *Pseudomonas* infection than their littermate controls (Fig. 2), which was associated with a 77% decrease in the bacterial burden in peritoneal lavage specimens (Fig. 3) and a 74% decrease in the bacterial burden in peripheral blood cultures (data not shown). Previous studies have shown elevation of both pro- and anti-inflammatory cytokines, including IL-6, IL-10, IL-12, TNF- α , and IL-1 β , in septic humans and animal models of sepsis (3, 9, 11, 19, 36, 48, 49). Interestingly, the decrease in the bacterial burden in

NJ.1638 mice was not associated with alterations in the levels of these cytokines in the blood or peritoneal lavage fluid relative to those for littermate controls (Fig. 4A and B). Baseline levels of IL-6 are approximately 18 pg/ml in the plasma of NJ.1638 mice and below the limit of detection in littermate controls. IL-10 and IL-12 in the peritoneal lavage fluid and plasma, and IL-6 in the peritoneal lavage fluid, are below the limits of detection for both NJ.1638 mice and their littermate controls.

In order to identify the specific role of eosinophils in the absence of elevated levels of IL-5, eosinophils isolated from NJ.1638 mice were adoptively transferred into the peritoneal cavities of wild-type C57BL/6 mice. We have previously shown that adoptive transfer of eosinophils significantly improves the survival of mice following CLP, a polymicrobial model of sepsis (53). Through the use of a *Pseudomonas* peritonitis model, mice that received adoptively transferred eosinophils had a 95% reduction in the bacterial burden in peritoneal lavage fluid compared to that for animals receiving the vehicle control (Fig. 5). To further assess the role of eosinophils in vivo, *P. aeruginosa* was injected i.p. into eosinophil-deficient PHIL transgenic mice, which have a diphtheria toxin transgene under the control of the EPO promoter (25). PHIL mice had higher bacterial burdens in the peritoneal lavage fluid than their littermate controls 18 h following infection (Fig. 6). In addition, 60% of PHIL mice had bacteria present in the blood, while none of their littermate controls did ($P = 0.04$). Furthermore, levels of IL-6, IL-10, IL-12, TNF- α , and IL-1 β in the blood and peritoneal lavage fluid did not differ between PHIL mice and their littermate controls (data not shown).

Previous studies have indicated that human eosinophils degranulate in response to bacteria in vitro (37, 47). In order to determine if the mechanism of antibacterial activity could be attributed in part to eosinophil granule proteins, eosinophil granules were purified and incubated with *P. aeruginosa* in vitro. Eosinophil granule proteins directly killed *P. aeruginosa* in a dose-dependent manner (Fig. 7). The dose of granule proteins equivalent to 10^5 eosinophils, the number used in our in vitro studies, showed an equivalent level of killing (approximately 40%).

A. Plasma



B. Peritoneal Lavage

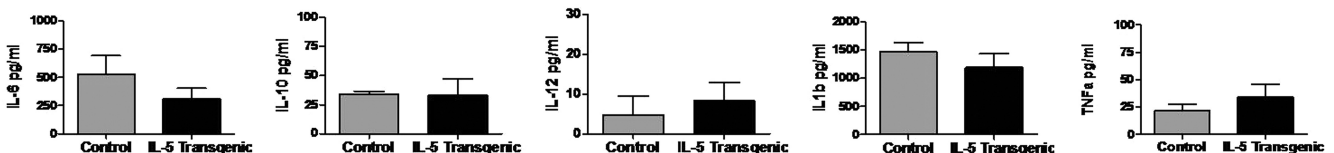


FIG. 4. IL-5 transgenic mice show no alteration in the levels of inflammatory cytokines following *Pseudomonas* infection. IL-5 transgenic (NJ.1638) mice or their littermate controls were infected i.p. with 10^7 CFU of *P. aeruginosa* and were sacrificed at 18 h. Levels of the cytokines IL-6, IL-10, IL-12, TNF- α , and IL-1 β in plasma (A) and peritoneal lavage fluid (B) were measured by ELISA. Shaded bars, littermate controls ($n = 10$); solid bars, IL-5 transgenic mice ($n = 11$). Each sample was run in duplicate. Data are means $\pm$ SEM.

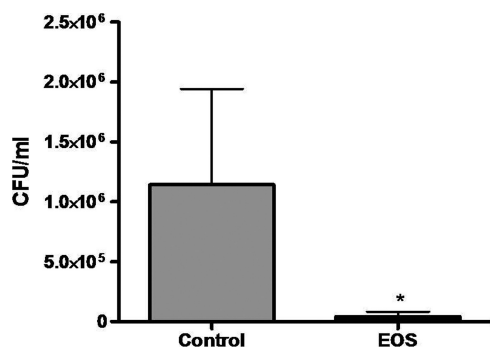


FIG. 5. Adoptive transfer of eosinophils (EOS) reduces the bacterial burden in a *Pseudomonas* peritonitis model. C57BL/6 mice were injected either with PBS ($n = 14$) or with 2×10^5 eosinophils ($n = 9$) isolated from NJ.1638 mice. The C57BL/6 mice were infected i.p. with 10^7 CFU of *P. aeruginosa* 1 h later and were sacrificed at 18 h. Quantitative colony counts of the peritoneal lavage fluid were made by serial dilution. Bars represent means $\pm$ SEM. Three independent experiments were performed. *, $P = 0.03$.

To address the ability of eosinophil granules to enhance bacterial clearance in vivo, granule proteins were injected into the peritoneal cavities of wild-type mice 1 h following i.p. infection with *P. aeruginosa*. Mice treated with eosinophil granules had an 82% reduction in the bacterial burden in the peritoneal cavity from those for mice treated with the vehicle control (Fig. 8). Surprisingly, increased bacterial clearance was again not associated with any change in the levels of inflammatory cytokines in the blood or peritoneal cavity (Fig. 9). Finally, to assess the benefits of eosinophil granule proteins in the context of sepsis, CLP was performed on wild-type mice, followed by i.p. administration of granule proteins 1 h later. Eosinophil granule proteins prolonged the survival of treated mice compared to that of mice receiving the vehicle control ($P = 0.03$) (data not shown).

DISCUSSION

Understanding the host innate immune response to sepsis is essential for creating new and effective immunomodulatory

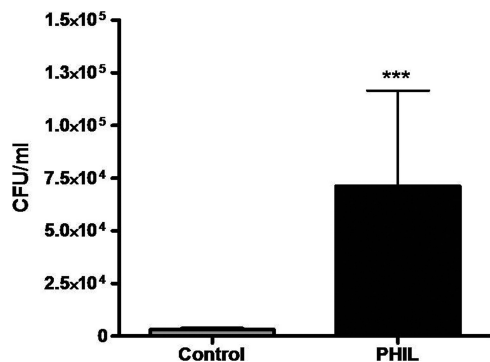


FIG. 6. PHIL mice have increased bacterial burdens in a *Pseudomonas* peritonitis model. PHIL mice ($n = 5$) or littermate controls ($n = 5$) were infected i.p. with 10^7 CFU of *P. aeruginosa* and were sacrificed at 18 h. Quantitative colony counts of the peritoneal lavage fluid were made by serial dilution. Bars represent means $\pm$ SEM. ***, $P = 0.007$.

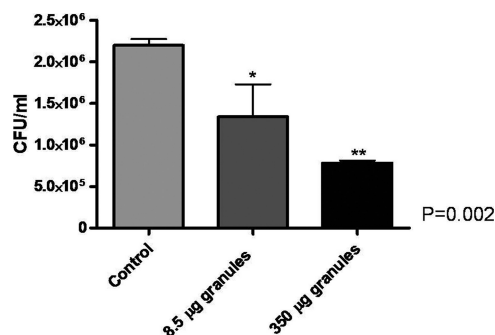


FIG. 7. Eosinophil granules kill bacteria in vitro. An eosinophil granule extract or a vehicle control was incubated with 10^6 CFU of *P. aeruginosa* for 1 h, and quantitative colony counts were made by serial dilution. Bars represent means $\pm$ SEM from three independent experiments. *, $P < 0.05$ for comparison of 8.5 µg of granules with the control; **, $P < 0.01$ for comparison of 350 µg of granules with the control ($P = 0.002$ by analysis of variance).

therapies for patients. Our findings suggest not only that eosinophils are beneficial in host defense against bacterial infection but also that administration of eosinophil granule proteins may be a viable adjuvant therapy to improve the control of bacterial infections.

Eosinophils are traditionally thought to be important to host responses following parasitic infections. In addition, eosinophils appear to be a critical component of Th2-mediated allergic responses, including those associated with asthma (22, 25). Mounting in vitro evidence suggests that human eosinophils can have a potentially important role in innate immune responses to viral and bacterial infections (37, 38, 43, 47, 52). This role likely stems from an ability to recognize and kill bacteria through the expression of multiple TLRs, specifically TLR-2, -4, -5, and -9 (52). Our results indicate that mouse eosinophils also have potent antibacterial properties in vitro. Specifically, we demonstrated this antibacterial activity against *P. aeruginosa*, a highly antibiotic resistant species important in the clinical setting. These data confirm the work of Persson et al. and Svensson and Wenneras, which demonstrated that human eosinophils are capable of killing bacteria, specifically *Escherichia coli* and *Staphylococcus aureus*, in vitro (37, 47).

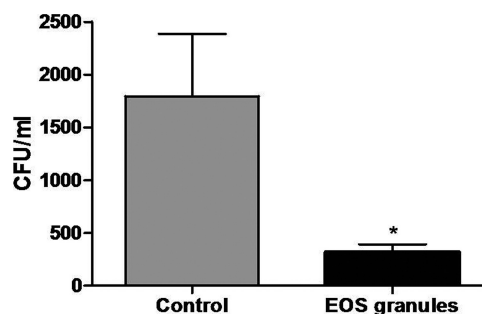
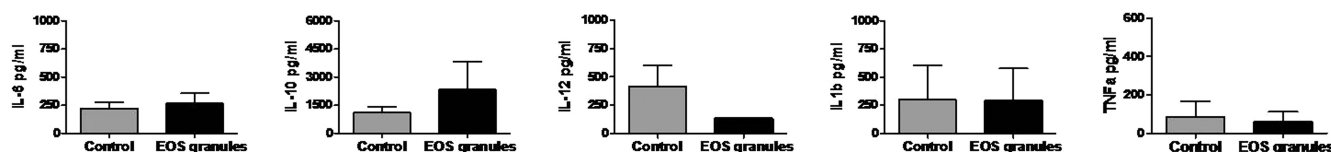


FIG. 8. Eosinophil (EOS) granules reduced the bacterial burden in vivo in a *Pseudomonas* peritonitis model. C57BL/6 mice were administered either an eosinophil granule extract ($n = 9$) or a vehicle control ($n = 12$) 1 h following i.p. infection with 10^7 CFU of *P. aeruginosa*. Mice were sacrificed at 18 h. Quantitative colony counts of the peritoneal lavage fluid were made by serial dilution. Bars represent means $\pm$ SEM. Three independent experiments were performed. *, $P = 0.03$.

A. Plasma



B. Peritoneal Lavage

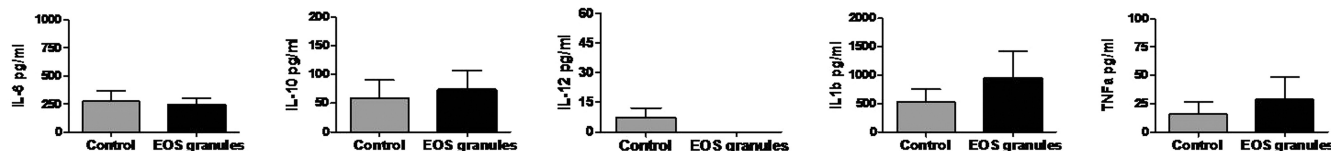


FIG. 9. Granule-treated mice show no change in the levels of inflammatory cytokines following *Pseudomonas* peritonitis. C57BL/6 mice were administered either an eosinophil (EOS) granule extract or a vehicle control 1 h following i.p. infection with 10^7 CFU of *P. aeruginosa*. Mice were sacrificed at 18 h, and levels of the cytokines IL-6, IL-10, IL-12, TNF- α , and IL-1 β in plasma (A) and peritoneal lavage fluid (B) were measured by ELISA. Shaded bars, vehicle controls ($n = 7$); solid bars, granule-treated mice ($n = 9$). Samples were run in duplicate. Data are means $\pm$ SEM.

Importantly, after establishing that mouse eosinophils also possess antibacterial properties in vitro, we used a mouse model of *Pseudomonas* peritonitis and showed the biological significance of this activity in vivo. Furthermore, IL-5 transgenic mice (NJ.1638), in which 40% of the circulating leukocytes are eosinophils by the age of 4 weeks, had improved bacterial clearance, a finding that is biologically significant because it was associated with a significant improvement in survival. Moreover, adoptive transfer of eosinophils to wild-type mice following *Pseudomonas* infection recapitulated these effects, further establishing the antibacterial potential of eosinophils in vivo. The specificity of the antibacterial role for eosinophils is further established by the impairment of bacterial clearance in mice (PHIL) with a congenital eosinophil deficiency. Our recent study showed that IL-5 overexpression in NJ.1638 mice, or adoptive transfer of eosinophils to wild-type mice, rescues animals from the lethality of polymicrobial sepsis (53). While this was associated with a reduction in the peritoneal bacterial burden, the finding of degranulated eosinophils in the cecum and the contribution of necrotic tissue to the inflammatory response make it difficult to determine whether this represented a true antibacterial role or whether other inflammatory mediators and/or cecal wound healing was also modulated by eosinophils. In addition, the polymicrobial nature of the infection and the lack of anaerobic cultures in that study make it impossible to accurately assess the direct role of eosinophils in bacterial clearance. Our use of a defined bacterial challenge model now establishes that mouse eosinophils demonstrate potent antibacterial properties in vivo. Further, the improved survival in the absence of inflammatory cytokine modulation suggests that the increase in bacterial clearance is highly specific, though the possibility remains that other inflammatory pathways are affected by eosinophils in vivo. It should be noted that other studies have demonstrated that the hypereosinophilic state induced by ovalbumin sensitization impairs *Pseudomonas* clearance in the lung (7). This apparent discrepancy is likely due to the induction of multiple Th2 cytokines in the ovalbumin model, including IL-4, which has recently been shown to promote bacterial growth in vivo (20, 23).

Given that adoptive transfer of eosinophils is a difficult therapeutic strategy that may have limited use for patients in the ICU, we investigated whether eosinophil-derived products could mediate a similar antibacterial effect in vivo, as demonstrated with the use of neutrophil-derived BPI in meningococcal sepsis (28). Our data suggest that the bacterial killing afforded by eosinophils is due in part to the degranulation and release of cytotoxic granule proteins. These results support previous data showing that MBP possesses potent antibacterial properties against *E. coli* and *S. aureus*, ECP possesses antibacterial activity against *E. coli*, and purified EPO, another granule protein, can kill *Mycobacterium* spp. in vitro (8, 24, 27). We advanced these findings to establish a similar role for mouse eosinophil granules in vitro and the ability of eosinophil granule proteins to enhance bacterial clearance in vivo. This now establishes the potential for eosinophil granule proteins to be used as a unique adjuvant therapy, which can be exploited by physicians to augment bacterial clearance without altering inflammatory cytokine production. The importance of this is underscored by the growing prevalence of antimicrobial resistance, including resistance among other *Pseudomonas* species. While we cannot exclude the possibility that contaminating components, such as mitochondrial DNA, also mediate bacterial killing, as shown recently by Yousefi and colleagues, our method of granule isolation makes this possibility unlikely (53). Furthermore, our data provide evidence of a specific antibacterial activity afforded by granule proteins alone.

While we have documented an important role for eosinophils in bacterial clearance in vivo, further studies are necessary to fully define the role of specific granule proteins (MBP, EPO, and eosinophil-associated RNases, etc.) in order to determine the active component in eosinophil granules. It may be that one of these proteins, or a combination, impairs bacterial clearance or has other detrimental effects on the host response that we have not observed. Based on numerous studies, it is likely that MBP and EPO play a prominent role in bacterial clearance (8, 24, 27, 47). However, the description of BPI as a component of human eosinophil granules implicates this protein as another potential candidate due to its known antibacterial activity in vivo (10).

In conclusion, we provide evidence that mouse eosinophils and eosinophil granules play a beneficial but poorly defined role in innate immune responses to bacterial infections. These data suggest that the use of eosinophil-derived granule proteins, or administration of a specific granule protein, may be a useful adjuvant therapy for patients with sepsis or other bacterial infections. In addition, these data suggest that patients receiving eosinophil depletion therapies may experience potentially adverse effects on innate immune responses and the resolution of bacterial/viral infections.

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