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The Role of Eosinophils in Non-Parasitic Infections

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Abstract

Eosinophils are a subset of leukocytes the traditionally associated with Th2-related diseases and helminth infections. However, accumulating evidence suggests that eosinophils play a more prominent role in the immune response to bacterial and viral pathogens than previously realized. Specifically, eosinophils possess antimicrobial properties against a broad range of pathogens, and release specific and secondary granules as a result of pathogen recognition. Pathogen recognition is accomplished through expression of Toll-like receptors, as well as other surface and intracellular receptors expressed by the eosinophil. Interestingly, specific killing mechanisms employed by each granule protein differ based on pathogen recognition, but ultimately release of eosinophil granules leads to direct killing of many different pathogens. The precise mechanisms of killing by granule proteins and the circumstances in which specific proteins are secreted are only now being determined. Future efforts to understand these mechanisms may lead toward clinical use of granule proteins as antimicrobial agents in humans, in addition to revealing implications regarding the use of eosinophil-depleting therapies for allergic disorders. This review will summarize the literature to date regarding the role of eosinophils in non-parasitic infections.

Keywords

Bacteria; eosinophils; fungi; granules; toll-like receptors; viruses

INTRODUCTION TO EOSINOPHIL BIOLOGY

Eosinophils are a granulocyte subset comprising 1–3% of all circulating leukocytes in humans. They are multifunctional effector leukocytes traditionally associated with parasitic infections and their role in the pathology of asthma and other allergic diseases. Eosinophils were initially discovered by Paul Ehrlich in 1879 due to their characteristic staining with eosin, a negatively charged fluorescein dye. They are highly granular cells comprised of a crystalline core of Major Basic Protein (MBP), surrounded by a matrix of Eosinophil Peroxidase (EPO), and two ribonucleases--Eosinophil Cationic Protein (ECP) and Eosinophil-Derived Neurotoxin (EDN). In rodents, the latter two proteins are replaced by a family of proteins known as Eosinophil Associated Ribonucleases (EARs), with over eleven different family members described to date [1]. Though these are the best characterized proteins to date, some labs have described the presence of additional proteins within these

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granules [2, 3]. While these granule proteins are capable of causing tissue damage and cytotoxicity, it is important to understand their function in and contribution to the eosinophilic response during infection, will be discussed in detail in this review.

Eosinophils are generated in the bone marrow from pluripotent CD34⁺ stem cells and are terminally differentiated leukocytes. IL-5 in combination with IL-3 and GM-CSF are responsible for regulating eosinophil development [4–6], though IL-5 is the most specific to eosinophils and is known to trigger activation, prolong survival, and stimulate recruitment of eosinophils from the bone marrow [7–9]. Eosinophil activation is associated with changes in adhesion molecule expression, including upregulation of CD11b and CD69, and loss of CD62L. Activation is also associated with intracellular calcium fluctuations, which are required for adhesion, chemotaxis, and degranulation in eosinophils [10]. Following eosinophil activation, ultrastructural changes occur including loss of secondary granules and the MBP crystalline core [10]. There are several mechanisms for degranulation in eosinophils and all are tightly regulated; this is discussed in detail elsewhere [1, 10, 11].

Eosinophils have been traditionally associated with parasitic infections. However, the generation of both transgenic and knockout mice has allowed investigators to determine that while many parasitic infections are associated with eosinophilia, these cells are not required to kill most parasites or clear an infection, with the exception of *Strongyloides* sp. and filarial diseases [1, 11, 12]. In fact, their role in parasitic diseases is still being debated. Since eosinophils are believed to be unnecessary to control the vast majority of parasitic infections, several groups have postulated that eosinophils migrate to sites of infection and degranulate, and that this contributes to tissue remodeling and debris clearance for many parasitic diseases [12]. Moreover, eosinophil accumulation in the lung during viral-associated asthma exacerbations suggests that aside from their classical anti-parasitic function, there are additional accessory roles for these cells. Indeed, it is known that eosinophils and their granule proteins cause tissue destruction and bronchoconstriction, but as was stated by Rosenberg and Domachowske, “even the most rudimentary understanding of evolution and biology leads to the incontrovertible conclusion that the ability to induce severe pathology cannot possibly be a ‘raison d’être’ for any existing cell type or function” [13]. This review will cover the evidence to support this statement, and discuss in detail the literature available as it pertains to eosinophil-mediated destruction of bacteria, viruses, and fungi.

PATHOGEN RECOGNITION BY EOSINOPHILS

Toll-Like Receptor Expression

One of the most studied mechanisms for pathogen recognition is the family of Toll-like receptors (TLR), capable of recognizing a wide range of pathogens based on specific, conserved pathogen associated molecular patterns (PAMP) found in cellular membranes, secreted proteins, and nucleic acid sequences. There are numerous reports documenting the presence of TLR in and on eosinophils; however, there are conflicting data regarding the presence of and circumstances required for expression of specific TLR.

One early report demonstrated that human eosinophils express CD14 and TLR4, which both recognize different portions of the lipopolysaccharide (LPS) molecule found in the cell wall of gram negative bacteria [14]. In accordance with this, other labs showed that isolated human eosinophils expressed surface TLR2 (peptidoglycan) and TLR4, indicating that eosinophils recognize and distinguish a wide range of bacterial species [3, 15]. Furthermore, this is an inducible phenomenon, as stimulation of eosinophils with BCG or lipomannan, a component of the Mycobacterial cell wall, induced cell surface expression of TLR2 and TLR4 [3]. Conversely, another lab showed that peripheral blood eosinophils fail to exhibit cell surface expression of CD14, TLR4 or TLR2, but do express mRNA for TLR4 [16]. Additionally, eosinophils are not activated in response to LPS, as evidenced by a lack of CD62L shedding or upregulation of CD11b [16]. Supporting these data, Wong and colleagues also found that human eosinophils do not respond to LPS stimulation through cytokine secretion or degranulation [15]. These results collectively suggest that while human eosinophils seem to express TLR4, they do not appear responsive to the ligand.

One potential reason for these discrepant findings is the source of eosinophils. Interestingly, it appears that there is an inherent difference between eosinophils from patients who are predisposed to allergic disease versus those who are not. Specifically, eosinophils from healthy volunteers failed to express TLR2 and TLR4, whereas eosinophils from hypereosinophilic donors spontaneously expressed these receptors [3]. Furthermore, Mansson and colleagues stated that expression of TLR7 and TLR9 was the same between healthy and atopic patients. However, upon *in vitro* TLR stimulation with R-837 (TLR7) or CpG (TLR9), eosinophils from atopic patients had increased cytokine production and granule protein secretion compared to those from healthy patients [17]. These data suggest that important differences exist between these two conditions (atopic vs. healthy status) and immunological environments [17]. It is important to consider these observations, as they may explain some of the discrepancies regarding specific TLR expression and subsequent activation of eosinophils.

Another important difference between many studies is that the choice of ligand for TLR stimulation will affect the measured outcome of eosinophil activation. TLR2 stimulation of human eosinophils using peptidoglycan induced a potent cytokine response including release of IL-1 β , IL-6, IL-8, and GRO- α , which could be blocked using a TLR2-specific antibody [15]. Moreover, peptidoglycan stimulation also induced upregulation of ICAM-1 and CD18 on the surface of eosinophils [15]. However, Nagase *et al.* concluded that no significant effects were observed in eosinophils following TLR2 stimulation with Pam3CSK4. One important consideration for this discrepancy is that TLR2 forms a heterodimer with either TLR1 or TLR6, which have both been detected in human eosinophils [15, 18], and the recognition of either triacylated lipopeptides of gram-negative bacteria (TLR1) or diacylated lipopeptides of gram-positive bacteria (TLR6) depends on this. Therefore, using Pam3CSK4 or lipomannan stimulation will trigger a TLR2-TLR1 complex, whereas peptidoglycan will trigger a TLR2-TLR6 complex [19, 20]. The levels of surface expression of TLR1 and TLR6 will affect recognition of these ligands by TLR2, further complicating the comparison and interpretation of these studies.

More conclusive data exists regarding the expression of TLR7, TLR8 and TLR9 in human eosinophils. Several studies found that human eosinophils constitutively express mRNA for TLR7, TLR8, and TLR9 and cell surface receptors for TLR7 and TLR8. These receptors are detectable by flow cytometry, though surface expression levels were lower in eosinophils than in neutrophils [15, 17, 18]. Additionally, these receptors are functional as TLR9 stimulation using CpG induced secretion of EDN from eosinophils, and enhanced chemotaxis [17]. These receptors are inducible as stimulation with IFN- γ increased expression of TLR7, and TLR8 mRNA [18]. Nagase and colleagues showed that stimulation of TLR7 and TLR8 using the agonist R-848 induced superoxide generation, CD11b expression, and L-selectin shedding, indicating functional activation of TLR signaling and eosinophil activation [18]. These data provide strong evidence for the presence and importance of TLR in human eosinophils.

There are little data regarding TLR expression in mouse eosinophils. One study showed that peripheral eosinophils from IL-5 overexpressing transgenic mice, which have a profound eosinophilia, express TLR3, TLR4, TLR5, and TLR7, with higher levels of TLR3 and TLR7 observed intracellularly [21]. This study also demonstrated that following ssRNA stimulation of TLR7, eosinophils released significant amounts of EPO and exhibited rearrangement of the MBP granular core, further indicating activation and degranulation [21]. Collectively, though there is still debate regarding specific TLR expression in and on eosinophils, it is clear that they recognize PAMP belonging to a broad spectrum of pathogens and are activated as a result of this recognition.

Alternative Receptors Involved in Eosinophil Activation

Eosinophils can also respond to microbes through the stimulation of additional receptors. It is well established that eosinophils can be activated through complement receptors by the anaphylatoxins C3a and C5a [10], which are cleaved from their parent molecules following bacterial recognition by the complement system. Additional evidence indicates that they express the formyl peptide receptor, as stimulation with fMLF induced a rapid increase in intracellular calcium [22]. Furthermore, blockade of this receptor using two different antagonists blocked the respiratory burst and release of EPO following recognition of various bacterial species or fMLF, indicating that this receptor is important for bacterial recognition and activation in eosinophils [23].

Eosinophils are also known to express CD11b, which is important in cellular migration and adhesion, as well as phagocytosis. One group demonstrated that eosinophil degranulation required direct contact with the fungus *Alternaria alternata*, specifically through CD11b recognition of β -glucan [24], which is found in various bacteria and fungi. Interestingly, it seems that recognition of *A. alternata* induced upregulation of CD11b, as well as CD63 [24], indicating that similar to what was observed for TLR2 and TLR4 in eosinophils, a positive feedback mechanism exists for β -glucan recognition. These data collectively imply that in addition to TLR, eosinophils are capable of recognizing bacterial products through multiple receptors resulting in cellular activation and degranulation.

ANTIBACTERIAL EFFECTS OF EOSINOPHILS

Lessons from Parasites: the Role of Granule Proteins and Degranulation in Pathogen Killing

In one of the earliest studies on antibacterial properties of eosinophils, Yazdanbakhsh *et al.* demonstrated that human eosinophils phagocytose roughly 50% less *Staphylococcus aureus* than neutrophils, but at a higher multiplicity of infection (MOI) there is no significant difference between these two cell types in bacterial killing [25]. Moreover, purified mouse eosinophils killed *Pseudomonas aeruginosa in vitro* providing further evidence to support the role of eosinophils in bacterial infections [26]. While eosinophils are capable of phagocytosis, it is well known that in order for them to kill large parasites such as *Strongyloides* sp., they must use alternative mechanisms. Eosinophils rely on secreted mediators—cytotoxic granules—to attack and kill parasites [27–30]. Emerging data suggest that parasites are not the only organisms susceptible to the toxic effects of these highly cationic proteins. Increasing evidence indicates that eosinophil granule proteins are bind to and kill several different bacterial species, as well as fungi. A recent article showed that human eosinophil crude protein extracts alone have the ability to induce *Escherichia coli* killing in a dose-dependent manner [31]. Moreover, another report indicated that isolated mouse eosinophil granules also possessed dose-dependent antibacterial activity against *P. aeruginosa in vitro*, further confirming the killing potential of these highly cationic proteins [26].

To determine the specific contribution of individual granule proteins, several groups used purified granule proteins, MBP and ECP [32] or EPO [33], and showed that they all have significant bactericidal activity against *E. coli* and *S. aureus* (Table 1) [32–34]. Interestingly, these studies also showed that the degree of bacterial killing is dependent upon pH, temperature, and metabolic activity of the bacteria [32, 33]. By measuring the conversion of a beta-lactamase substrate, Lehrer and colleagues were able to show that the mechanisms of both MBP- and ECP-mediated bacterial killing is due to outer membrane permeabilization of *E. coli*, but ECP had limited activity compared to MBP, as ECP was not effective at killing mid-logarithmic phase *E. coli* [32]. These data suggest that each granule protein may have similar but non-redundant modes of action and that environmental circumstances affect these activities.

Within the last few years, the mechanism of ECP-mediated bacterial killing was further examined. Torrent and colleagues found that ECP caused membrane depolarization of both gram positive and gram negative species, and that it bound with high affinity to LPS, lipid A and peptidoglycan on the surface of bacteria [35]. The N-terminal portion of ECP, specifically amino acid residues 1–45, is necessary and sufficient for its membrane-altering and antibacterial ability [36].

Other studies have investigated the killing mechanism of another granule protein, EPO. EPO constitutes 40% of the total granule protein in eosinophils, and oxidizes several specific substrates in the presence of hydrogen peroxide to create hypohalous acids, which are lethal to pathogens [33, 37, 38]. EPO possessed significant antibacterial activity, which was inhibited with the use of azide, establishing the generation of hypohalous acids as a

mechanism of EPO-mediated killing [31]. Blockade of superoxide production through NADPH oxidase also inhibited *E. coli* killing. However, there was no combined impairment in bacterial killing caused by blockade of EPO (via azide) and superoxide generation, suggesting that NADPH oxidase works in conjunction with EPO in eosinophils, similar to myeloperoxidase in neutrophils [31]. Additional studies are needed to further understand the unique contribution of individual granule proteins, and to determine their usefulness as adjuvants during infection.

While several granule proteins have antibacterial properties, the relative contribution of each to bacterial killing may depend upon the bacterial species. A recent study examined the ability of eosinophils to recognize various species of bacteria and found that the release of specific granule proteins is dependent upon the organism [23]. Specifically, *Haemophilus influenzae* or *E. coli* (both gram negative) triggered the release of ECP, EPO, and MBP from human eosinophils. However, *S. aureus* (gram positive) only induced the release of EPO [23]. While most bacterial species that induced secretion of ECP were gram negative, *Clostridium perfringens* (a gram positive organism that often stains gram negative due to a thinner peptidoglycan layer) was also found to induce ECP, making it difficult to discern a specific pattern for granule release [23]. It could be speculated that granule secretion is related to the recognition of other specific PAMP in addition to TLR2/4 (e.g. fMLF). Another possibility for the differences observed in granule protein release is that the secretion of a given protein is determined by the concentration of a given bacterium. For example, greater amounts of EPO were released at a MOI of 1 compared to a MOI of 100. In contrast, ECP required a higher MOI to induce secretion [23]. Additional studies will be necessary to determine the precise mechanism by which eosinophils release specific granule proteins in response to a particular bacterial species, and exactly how this is detected by the eosinophil.

In addition to bacterial recognition and killing, eosinophils also kill fungi through degranulation. Yoon and colleagues showed that human eosinophils released EDN and MBP in response to a common environmental fungus, *A. alternata*, which resulted in significant membrane damage [24]. These results were confirmed by another group, who in addition showed release of EDN following recognition of *Penicillium notatum* [39]. Interestingly, *Aspergillus versicolor* and *Candida albicans* failed to induce eosinophil degranulation [39]. These data indicate that degranulation in response to fungal recognition by eosinophils is also dependent on the pathogen detected, and may be related to specific PAMP.

While it is known that MBP, ECP, and EPO have antibacterial activity, eosinophils contain additional antibacterial proteins. It was reported that human eosinophil granules possess Bactericidal/Permeability Increasing protein (BPI), which is capable of LPS recognition and pore formation in gram negative bacteria [2]. Moreover, they also produce α -defensins following stimulation with microbial products [3]; α -defensins are potent antimicrobial peptides active against many bacterial species, fungi, and enveloped viruses [40]. Additionally, other granule proteins may exist in eosinophils that have not been fully described and these proteins may possess antimicrobial activity. More studies are necessary in order to determine the contribution of these alternative proteins to pathogen killing *in vitro* and *in vivo*.

Eosinophil Catapults and Alternative Mechanisms of Pathogen Control

Recently, another mechanism of antibacterial activity was described by Yousefi and colleagues. They detected the release of DNA from IL-5 or IFN- γ primed eosinophils following stimulation with LPS, and this DNA was bound to ECP and MBP as well as bacteria [41]. Interestingly, this DNA was mitochondrial in origin unlike neutrophil extracellular traps (NETs), which are comprised of nuclear DNA [41, 42]. This method of mitochondrial DNA extrusion provides the eosinophil the distinct ability to survive following pathogen recognition and delivery of DNA, whereas neutrophil DNA extrusion is lethal to the cell [41, 42]. Moreover, they determined that human eosinophils kill *E. coli* *in vitro* and killing was dependent upon release of mitochondrial DNA as much of this effect was abrogated by DNase [41]. *In vivo*, using the cecal ligation and puncture (CLP) model of polymicrobial sepsis, release of DNA and MBP was detected in the ceca of IL-5 overexpressing, hypereosinophilic mice, which was absent in wild-type controls. Additionally, IL-5 overexpressing mice had decreased circulating bacterial burden and improved survival over wild-type mice with CLP sepsis, further suggesting a protective role for eosinophils in bacterial disease [41]. These results suggest an additional mechanism for bacterial killing involving both mitochondrial DNA and cationic proteins.

Indirect Mechanisms of Pathogen Killing: Antigen Presentation and Cytokine Secretion

Eosinophils secrete various cytokines and chemokines, which activate and recruit other inflammatory cells to sites of infection. Specifically, stimulation with TLR ligands including peptidoglycan (TLR2), flagellin (TLR5), and R837 (TLR7) induced release of IL-1 β , IL-6 and IL-8 from eosinophils [15]; TLR3 stimulation with polycytidylic acid induced IL-8 secretion [17]. Additionally, recognition of β -glucans by eosinophils induced secretion of MCP-1, IL-8 and MIP-1 α [24, 43], and direct recognition of both bacteria and fungi induced IL-8 release from eosinophils [39, 43]. These chemokines are important in recruiting both neutrophils and macrophages to sites of infection, though it is not known to what extent chemokine secretion by eosinophils affects the clearance of an infection *in vivo*.

Eosinophils also produce various cytokines, including IL-2, IL-4, IL-6, IL-10, and IL-12, which are important in promoting T cell polarization (Th1/Th2), activation, and proliferation [1]. This suggests eosinophils may provide an important link between the innate and the adaptive immune system. Further, growing evidence suggests eosinophils directly participate in the adaptive immune response. Several groups showed that eosinophils process and present antigen, and express costimulatory molecules [44, 45]. Specifically, eosinophils presented soluble antigen to CD4 T cells [46], and in culture they induced T cell proliferation and IFN- γ secretion following rhinovirus infection [47]. Moreover, blockade of various costimulatory molecules using neutralizing antibodies inhibited eosinophil-mediated T cell proliferation and cytokine secretion [45]. These data collectively suggest eosinophils provide a connection between innate and adaptive immunity, which may be an important but poorly recognized ability *in vivo*.

In Vivo Evidence Supporting the Protective Role of Eosinophils During Bacterial Infection

Despite extensive *in vitro* data, studies have just begun examining the antimicrobial effects of eosinophils *in vivo*. Recently, two studies demonstrated that adoptive transfer of isolated

mouse eosinophils into wild-type C57BL/6 mice increased bacterial clearance in the peritoneal cavity using a *Pseudomonas peritonitis* model, as well as in the peripheral blood, using the CLP model of polymicrobial sepsis [26, 41]. In both models, the improvement in survival was linked to the antibacterial capabilities of eosinophils. Hypereosinophilic mice had decreased circulating bacterial burden, and improved survival compared to littermate controls [41], whereas congenitally eosinophil-deficient PHIL mice had significantly impaired bacterial clearance in the peritoneal cavity [26]. Antibacterial activity is attributed in part to eosinophil granules as administration of mouse eosinophil granule extracts increased bacterial clearance in the peritoneal cavity of wild-type mice following *Pseudomonas peritonitis* [26]. This effect is specific to bacterial killing, as evidenced by no change in IL-6, IL-10, IL-12, IL-1 β , or TNF- α in plasma or peritoneal lavage fluid, though there may be other changes not assessed [26]. Collectively, these studies suggest that *in vivo* eosinophils and eosinophil granule proteins participate in bacterial killing.

ANTIVIRAL EFFECTS OF EOSINOPHILS

In Vitro Antiviral Activity of Eosinophil Granules

Human eosinophil granules contain two proteins ECP and EDN, which are both members of the RNase A superfamily, though EDN has 100-fold greater ribonuclease activity than ECP [48]. Since other RNases have been shown to have antiviral activity, investigators began to look at the role of secreted eosinophil ribonucleases in viral infection [49–51]. It was known that in respiratory syncytial virus (RSV) infection, eosinophils are recruited to and degranulated in the lung, and that wheezing during RSV infection was associated with increased eosinophils in the airways and ECP in respiratory secretions [52, 53]. Domachowske *et al.* investigated the role of EDN in RSV infection. They found that exposing RSV to human eosinophils reduced the infectivity of the virus, which was restored following addition of a ribonuclease inhibitor [54]. Moreover, the mechanism by which RSV induced eosinophil degranulation was CD18-dependent [55]. Interestingly, recombinant EDN alone exhibited similar antiviral activity against both RSV and parainfluenza virus *in vitro*, though it should be stated that the structure of the recombinant protein is different from native EDN, which is heavily glycosylated [54]. Additionally, EPO has antiviral activity against human immunodeficiency virus-1 (HIV-1) *in vitro* [56]. Klebanoff and colleagues determined that PMA-stimulated human eosinophils are virucidal against HIV-1 and this activity was reversed by peroxidase inhibition and catalase, which destroys hydrogen peroxide [56]. These data demonstrated that generation of hypohalous acids by EPO and hydrogen peroxide is vital to viral killing. Collectively, these data document the important role of eosinophil granule proteins in viral killing *in vitro*.

Eosinophil Recognition of RSV *In Vivo*: Coming Full Circle

The role of eosinophils in viral infection *in vivo* has only recently been studied. Adamko *et al.* demonstrated using a parainfluenza virus model that ovalbumin-sensitized guinea pigs had dramatically decreased viral titers and increased pulmonary eosinophilia, which was reversed by eosinophil depletion suggesting eosinophils have antiviral effects *in vivo* [57]. The strongest evidence to date for the role of eosinophils *in vivo* during viral infection comes from an RSV model in hypereosinophilic mice. *In vivo*, IL-5 overexpressing mice

have accelerated viral clearance over wild-type control mice, but more importantly, adoptive transfer of eosinophils into wild-type or MyD88-deficient mice accelerated viral clearance in the lungs of RSV-infected animals [21]. Interestingly, this may be through a MyD88-dependent pathway in eosinophils as adoptive transfer of MyD88-deficient eosinophils into wild-type mice did not accelerate viral clearance, implicating the role of TLR *in vivo* in eosinophils [21]. Recent data makes these results more interesting though difficult to reconcile; eosinophils may be specifically targeted by viruses during infection [58]. A recent study observed that eosinophils are infected by mouse pneumovirus, where the virus replicated and released infectious virions. Moreover, pneumovirus replication was accelerated in MyD88-deficient eosinophils, further underscoring the importance of TLR in this phenomenon [58]. More studies are needed to definitively assess the role of eosinophils in the innate immune response during viral infection, and specifically the role of MyD88 and TLR signaling.

CONCLUSION

It is clear that eosinophils kill various pathogens, using both direct and indirect mechanisms (Fig. 1). However, this role is incompletely understood, as is the frequency at which this occurs *in vivo*. Continuing efforts must be made to evaluate the precise mechanisms required for pathogen distinction by eosinophils. The need for greater understanding of eosinophil anti-bacterial and antiviral effects is highlighted by the increased use of eosinophil depletion strategies for the treatment of allergic disorders, since these therapies may have potentially unknown impacts on the host immune response. Moreover, the ability to distinguish various pathogens and secrete granule proteins according to the organism detected may provide researchers new insights in pathogen or pattern recognition and may provide clinicians with new ways in which to treat patients with infection. Harnessing the killing activity of granule proteins may provide an adjuvant therapy to antibiotics, especially since antibiotic resistant species are increasing rapidly, further underscoring the importance of research on eosinophil- and eosinophil granule-mediated pathogen killing.

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Fig. 1.
Eosinophils possess various mechanisms to attack and kill various pathogens.

Table 1

Summary of Research Involving Specific Granule Proteins Discussed in This Review

Granule Protein	Microbial Species	Key Findings [References]
MBP	<i>E. coli</i>	Released at low and high MOI [23]; Kills <i>E. coli</i> and killing is temperature, pH, and time dependent [32]
	<i>S. aureus</i>	Kills <i>S. aureus</i> and killing is temperature dependent [32]
	<i>H. influenzae</i>	Released at low and high MOI [23]
	<i>C. perfringens</i>	Released at low and high MOI [23]
	<i>A. alternata</i>	Released by <i>A. alternata</i> stimulation [39]; Kills in a CD11b-mediated contact-dependent manner [24]
ECP	<i>E. coli</i>	MOI of 100 required for secretion of ECP [23]; Kills <i>E. coli</i> and killing is temperature and time dependent [32]
	<i>S. aureus</i>	Kills <i>S. aureus</i> and killing is temperature dependent [32]
	<i>H. influenzae</i>	MOI of 100 required for secretion of ECP [23]
	<i>C. perfringens</i>	MOI of 100 required for secretion of ECP [23]
	RSV	Reduced viral infectivity (minimally) [49]
EPO	<i>E. coli</i>	More EPO released at MOI of 1 than 100 and blockade of fMLF receptor inhibits respiratory burst [23]; EPO is bactericidal when combined with H ₂ O ₂ and iodide, chloride, or bromide [33]; Inhibition of EPO through azide significantly impairs killing [31]
	<i>S. aureus</i>	More bacteria results in more EPO released and blockade of fMLF receptor inhibits release of EPO and respiratory burst [23]
	<i>H. influenzae</i>	More EPO released at MOI of 1 than 100 and blockade of fMLF receptor inhibits respiratory burst [23]
	HIV-1	Purified EPO is virucidal to HIV-1 [56]
EDN	RSV	Reduced viral infectivity [54]
	Parainfluenza virus	Reduced viral infectivity [54]
	<i>A. alternata</i>	Kills in a CD11b-mediated contact-dependent manner [24]; Release of EDN was dependent on organism concentration [39]
	<i>P. notatum</i>	Release of EDN was dependent on organism concentration [39]