

The regulatory role of eosinophils in viral, bacterial, and fungal infections

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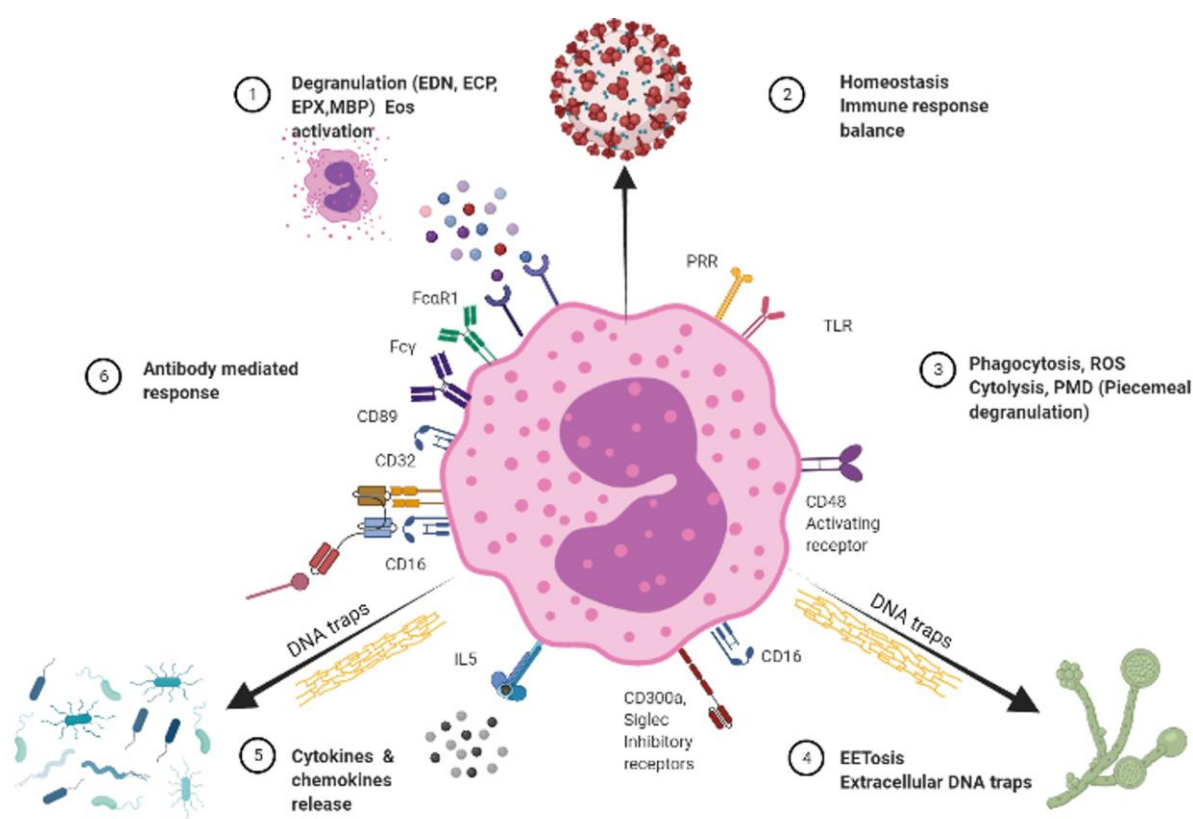
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Summary

Eosinophils are innate immune cells typically associated with allergic and parasitic diseases. However, in recent years, eosinophils have also been ascribed a role in keeping homeostasis and in fighting several infectious diseases. Indeed, these cells circulate as mature cells in the blood and can be quickly recruited to the infected tissue. Moreover, eosinophils have all the necessary cellular equipment such as pattern recognition receptors (PRRs), pro-inflammatory cytokines, anti-bacterial proteins, and DNA traps to fight pathogens and promote an efficient immune response. This review summarizes some of the updated information on the role of eosinophils' direct and indirect mediated interactions with pathogens.

Keywords: Eosinophils, Bacteria, Virus, Fungus, Infections

Graphical abstract



Abbreviations

PRRs	Pattern recognition receptors
GI	Gastrointestinal tract
GM-CSF	Granulocyte-macrophage colony stimulating factor
MHC	Major histocompatibility complex
IL-5	Interleukin 5
DC	Dendritic cells
MBP	Major basic protein
ECP	Eosinophil cationic protein
EPO/EPX	Eosinophil peroxidase
EDN	Eosinophil-derived neurotoxin
CLC	Charcot-Leyden crystal protein
ROS	Reactive oxygen species
PAF	Platelet-activating factor
TLRs	Toll-like receptors
NOD	Nucleotide-binding oligomerization domain
RLRs	RIG-I-like receptors

CLRs	C-type lectin receptors
DAMPs	Damage associated molecular patterns
PAMPs	Pathogen associated molecular patterns
SEB	Staphylococcus enterotoxin B
IR	Ig-like inhibitory receptors
NK	Natural killer
PMD	Piecemeal degranulation
EET	Eosinophil extracellular traps
CEACAM1	Carcinoembryonic cell adhesion molecule 1
APC	Antigen-presenting cells
NO	Nitric oxide
ssRNA	Single stranded RNA
dsRNA	Double-stranded RNA
RSV	Respiratory Syncytial Virus
Tg	Transgenic
PIV	Parainfluenza virus
IAV	Influenza A Virus

PHIL	Eosinophil-deficient mice
HRV	Human rhinovirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
CR1/CD35	Complement receptor 1
<i>sCD48</i>	<i>Soluble CD48</i>
PAR-2	Protease activated receptor 2
CR3	Complement receptor type 3
ABPA	Allergic bronchopulmonary aspergillosis
GC	Glucocorticosteroids
DRESS	Drug reaction with eosinophilia and systemic symptoms

Introduction

Eosinophils were discovered in 1879 by Paul Ehrlich through eosin-acidophilic staining of their granules(1). Eosinophils are granulocytes differentiating from CD34⁺ pluripotent stem cells' progenitors in the bone marrow and are found mature in the blood, gastrointestinal tract (GI), lungs, adipose tissues, uterus and thymus(2). Under physiological conditions, eosinophils represent 1-5% of the total white blood cell population, but, in some pathological conditions such as helminth infections, allergy, and hypereosinophilic syndrome, they are significantly increased. In health, the eosinophil lifespan is about 2-5 days in blood and tissues, but this can be prolonged up to 14 days in inflamed tissues due to increased levels of growth factor and survival cytokines(3). Eosinophil differentiation, maturation, and survival are mainly promoted by interleukin 5 (IL-5), granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-3(4). As recently described in the mouse there are two subpopulations of eosinophils characterized by their distinct roles: resident eosinophils and inflammatory eosinophils that are present in steady state and in inflamed tissues respectively. For example, tissue resident eosinophils facilitate metabolic homeostasis by maintaining activated macrophages in adipose tissues(5), contribute to control dendritic cells (DC) activation in the intestinal immune system(6), and maintain lung homeostasis in mice(7) (**Fig. 1**). Moreover, in normal mice the resident lung eosinophils had low expression of major histocompatibility complex (MHC) class II and were weak stimulators of T-cell proliferation(8). However, inflammatory eosinophils in ovalbumin-immunized mice exhibited an activated phenotype with up-regulated MHC class II expression(8). Additionally, during a house dust mite-induced airway allergy resident eosinophils found in non-asthmatic human lungs were also phenotypically different from the inflammatory

eosinophils isolated from the sputa of asthmatic patients implying that also in humans, there are two subpopulations of eosinophils with distinct roles(7).

Eosinophil functions are mediated mostly by the release of their pre-formed granules and newly formed mediators (**Fig. 2**). Eosinophil granules contain cationic proteins, e.g., major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO/EPX) and eosinophil-derived neurotoxin (EDN). Additionally, eosinophils' cytoplasm encompasses primary granules that store Charcot-Leyden crystal protein (CLC/Galectin-10), and lipid bodies(9). Moreover, eosinophils release reactive nitrogen and oxygen species (NOS and ROS), immune regulatory cytokines belonging to all immune subtypes/classes, e.g. IL-1 β (10), tumor necrosis factor-alpha (TNF- α , Th1), IL-4 (Th2)(11), and short-lived lipid mediators, e.g. cysteinyl leukotrienes, platelet-activating factor (PAF) and prostaglandins(12). This diverse battery of eosinophil products implies a larger spectrum of immune functions than just the typical eosinophil-related ones. Th1 is characteristically associated with immune response to bacteria and viruses while Th2 is associated with defense against helminths or fungi and Th17 with clearance of bacteria and fungi(13). The vast array of secreted molecules of eosinophils indicates they have both effector and regulatory immune functions against pathogenic infections. Moreover, eosinophils express an array of activating and some inhibitory membrane receptors (**Fig. 1**). These receptors recognize different exogenous ligands and activate or inhibit the eosinophils according to the balance of inhibitory and activating signals.

In this review, we will discuss some of the recent evidence for an eosinophil role in anti-bacterial, anti-viral and anti-fungal immunity, both by direct and indirect mechanisms. We will not cover in our review the activity of eosinophils in parasitic infections as it has been

extensively reviewed recently(14)(15). We will discuss the rationale for the use and/or development of eosinophil-related drugs in infections.

Eosinophil surface receptors and mechanisms in infections

Eosinophils express various cell surface receptors that regulate their survival, migration, activation, and inhibition. Also, eosinophils display receptors that recognize specific pathogenic epitopes and trigger eosinophil activation (**Fig. 1, 2**). Among these are the PRR family members including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors, RIG-I-like receptors (RLRs) and C-type lectin receptors (CLRs) which are known to detect damage associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs)(16). Expression of all TLRs, except TLR8, was found in eosinophils at mRNA or protein levels (reviewed by Kvarnhammar and Cardell(16)) and among them only TLR7 expression is accepted universally. Being a single strand RNA receptor, TLR7 expression suggests a potential role for eosinophils in the recognition of RNA viruses (**Fig. 1**).

Eosinophils express constitutively several antibody receptors, including the IgA receptor Fc α RI (CD89), and the IgG receptor Fc γ RII (CD32), whereas Fc γ RI (CD64), and Fc γ RIII (CD16) are inducible after stimulation with interferon (IFN)- γ or PAF(17) (**Fig. 2**).

Activation of Fc γ RI, Fc γ RII, and Fc γ RIII on eosinophils was found to induce their degranulation and their anti-microbial activity(18). Interestingly, it has been suggested that in asthmatic patients, IgG1 and IgG3, but not IgE, activate eosinophils via Fc γ RII, causing bronchial hyperactivity(17). Moreover, in *T. gondii* and *C. albicans* infections, Fc γ RII stimulation induces antibody-mediated degranulation of eosinophils against this parasitic and fungal pathogens(17).

IgA is the first line of defense in the resistance against infections, both by inhibition of bacterial and viral adhesion to epithelial cells and by neutralization of bacterial toxins(19)(20)(21),(22). IgA is present mostly on mucous membranes and surfaces such as the GI, respiratory and urogenital tracts where the entry and subsequent proliferation of many pathogens take place. There are two distinct subclasses of IgA based on their structural variations, termed IgA1 and IgA2, and their proportion varies at different mucosal sites(23). Several studies have demonstrated that IgA-coated particles mimicking microbes or parasites lead to eosinophil degranulation as a possible host defense response(24)(25). The IgA receptor Fc α RI on eosinophils has weak interactions with IgA immune complexes in a resting state. However, its ligand binding capacity increases after stimulation with cytokines like GM-CSF, IL-4 and IL-5(26)(27). IgA triggered NADPH oxidase activation and degranulation in eosinophils(28). Since both IgA subclasses have equivalent affinities for Fc α RI, they are similarly capable to induce eosinophil degranulation(26).

The expression of the IgE high-affinity receptor (Fc ϵ RI) and low-affinity receptor (CD23/Fc ϵ RII) on eosinophils is controversial and relatively unexplored. In hypereosinophilic patients infected with *S. mansoni*, eosinophils were shown to express both mRNA and protein for Fc ϵ RI (both α and β chain) and to have cytotoxic activity against *S. mansoni*(29)(30). However, another study that evaluated the expression of Fc ϵ RI on human eosinophils from atopic and non-atopic subjects found that this receptor is detectable mostly in atopic subjects with it being expressed only in a small minority of non-atopic subjects(31)(32). Moreover, murine eosinophils obtained from both hepatic granulomas and bone marrow seem not to express either Fc ϵ RI or Fc ϵ RII(33).

Besides Ig-receptors, eosinophils also express Ig-like receptors that are either activating or inhibitory. One notable example of an activating receptor is CD48 which belongs to the CD2

family (**Fig. 2**). Activation of CD48 either with monoclonal antibodies or with *S. aureus* and/or by some of its exotoxins such as enterotoxin B (SEB), caused eosinophil degranulation, production of cytokines, and prolonged survival mediated by distinct signal transduction(34).

In addition to activating receptors, eosinophils express Ig-like inhibitory receptors (IR) that when activated down-regulate eosinophil functions, thus being a physiological brake to excessive cell activation. These receptors are of interest for drug development as their potential to down-regulate eosinophil functions. We have shown the expression and function of the IR CD300a on human eosinophils(35). CD300a ligands are phosphatidylserine, phosphatidylethanolamine and the recently described CD148(36). Membrane of bacteria, fungi, and viruses possesses these phospholipids, and it is therefore conceivable that they can bind the CD300a receptor on eosinophils(37). Importantly, microbes and viruses have been shown to exploit host cell machinery for entry or to escape the host immune system by using phosphatidylserine(38). Moreover, Siglec-8, another IR was found to suppress eosinophils' function and to induce their death(39). α 2,3-linked sialylated glycoprotein is a ligand for Siglec-8 and its expression on adenoviruses and parainfluenza viruses was found to enhance the binding of these viruses to host cells(40) although its effect on eosinophil activity was not described. In contrast to Siglec-8, activation of Siglec-7, which we described on human eosinophils, inhibits their degranulation and cytokine release but does not induce cell death(41). In addition, eosinophils express the carcinoembryonic cell adhesion molecule 1 (CEACAM1), an IR that binds several bacterial(42)(43), viral(44), and fungal antigens(45) on T, B, and natural killer (NK) cells even though its role on eosinophils is still unknown. In general, we can conclude that while activating receptors fuel the eosinophils to react against

the pathogens, IRs will limit their responses and perhaps can be employed by the pathogens themselves to evade the eosinophil mediated responses.

Eosinophil activation mechanisms in infections

To understand the role of eosinophils in response to infections, we should consider how these cells can be activated to release pre-formed and newly synthesized mediators and which mechanisms and receptors are involved. Eosinophil activation is characterized by the release of their cytoplasmic granule content (**Fig. 2**). The release of granule-stored mediators can be led by three main mechanisms: 1) Classical compound exocytosis, in which the granule membrane fuses with the cell membrane to release granule content into the surrounding milieu. This process has been observed mostly in helminth infection *in vitro* but is not a common process *in vivo*(14); 2) Piecemeal degranulation (PMD), the most commonly observed form of degranulation, occurs in response to cytokines, such as CCL11 (eotaxin1) and IFN- γ (46). Unlike classical exocytosis, PMD is characterized by secretion of granule contents, without fusion of granule to the cell membrane but rather by the formation of spherical and tubular vesicles that traffic granule-stored proteins from the granule to the plasma membrane for secretion; 3) Cytolytic degranulation, which is characterized by the dissolution of the nucleus and cell membrane followed by the expulsion of intact granules. This type of cell activation ends with cell death that is morphologically distinct from both apoptosis and necrosis. Importantly, the intact granules released in this process act as independent organelles expressing chemokine, cytokine, and lipid receptors on their outer membrane, and therefore respond to several stimuli to release their content(47).

In addition, eosinophils, like neutrophils, can form DNA extracellular traps (commonly called eosinophil extracellular traps-EET) by a process named ETosis, usually involving eosinophil

death. Yousefi *et al*, showed that in response to LPS, eosinophils secrete EET composed of mitochondrial rather than nuclear DNA-extracellular traps (in a ROS-dependent manner), a process that does not therefore cause eosinophil death(48). Importantly, it was recently demonstrated that free cytoplasmic granules and CLC are associated with ETosis in human tissues(49)(50) (**Fig. 1, Fig. 2**).

Finally, despite not being “professional” phagocytic cells, in some instances, eosinophils can phagocytose pathogens. Moreover, eosinophils can express MHC-II and act as antigen-presenting cells (APC) and trigger an adaptive immune response(51).

Eosinophil functions in viral infections

Evidence from the last decade suggests that eosinophils may play a protective role against viral infection. Eosinophils contain and produce molecules with anti-viral activity, including EDN and ECP that are potent RNases(18). Eosinophils are also capable to produce nitric oxide (NO) by inducible NO synthase, a molecule that inhibits viral replication by multiple mechanisms(52). Moreover, eosinophils via TLR-3, 7, 9 can recognize viral single and double-stranded RNA (ssRNA and dsRNA), and DNA(16) (**Fig. 1**).

Since respiratory viruses are associated with asthma exacerbations, several studies have evaluated the role of eosinophils against respiratory viruses. Studies have shown that in the case of *Respiratory Syncytial Virus (RSV)* infection in mice, eosinophils have an anti-viral and host protective effect(53). Viral ssRNA binds to eosinophils via TLR7, inducing eosinophil degranulation and release of EPX, newly formed IFN- β and NO, and up-regulation of the cell surface receptor integrin CD11b, a marker of activated phagocytes(53). Moreover, the hypereosinophilic IL-5-Transgenic (Tg) mice can clear *RSV* significantly faster than wild-type mice when the virus is given intranasally(54). However, in a mouse

model of eosinophilic bronchiolitis induced by RSV, it was shown that eosinophil recruitment in the lung, driven by CCL11 (eotaxin1) contributed to disease severity(55). More recently it was also found that RSV induces EETs formation *in vitro*, which can probably promote airway obstruction(56). Eosinophils have been shown to increase in nasopharyngeal RSV-infected infants(57). Infectious virions and the pro-inflammatory IL-6 are released from eosinophils infected with RSV(58). ECP is the main mediator from eosinophils involved in the inactivation of RSV(59). Until now, the effect of antibodies or immune complexes on RSV-infected eosinophils has not been shown. However, Fc γ R has been demonstrated to form an immune complex with RSV and IgG, and to contribute to human RSV pathogenesis(60). Moreover, it has been demonstrated that IgG produced by RSV vaccination increased eosinophil activation possibly via activation of Fc γ R expressed by eosinophils(61). Therefore, we can hypothesize that higher eosinophil numbers and the release of mediators, especially of ECP, have a pivotal role in the immunopathology of RSV infections. Eosinophil-associated pulmonary disease was also observed post-RSV infection even after RSV vaccination(62). Interestingly, asthmatic patients treated with the anti-IL5 mAb (mepolizumab) and subsequently challenged by rhinovirus showed higher viral titer in the upper airway(63) suggesting that eosinophils possess an anti-viral role. Altogether eosinophils contribute to innate anti-viral immunity and promote clearance of RSV, but at the same time their hyperactivation may increase the disease severity.

As in RSV, eosinophils have anti-viral activity against *Parainfluenza virus (PIV)*. Human PIVs remain the second main cause of hospitalizations in children under 5 years of age suffering from a respiratory illness(64). Only RSV causes more respiratory hospitalizations for this age group. Eosinophils infected by PIV produced NO in response to the virus via TLR-7 *in vitro*(65). In the same study, eosinophil recruitment to the airways significantly

decreased *PIV* RNA in the lungs. The anti-viral effect was also seen in IL-5-Tg mice with an abundance of airway eosinophils but was lost in eosinophil-deficient (PHIL) mice. Interestingly, in *PIV*-infected eosinophils, the quantity of viral RNA remained constant, demonstrating that the eosinophils' induced reduction in infectivity was not due to the degradation of the viral RNA by eosinophil granules(65). Taken together the examples of *RSV* and *PIV* would indicate differences in eosinophil responses to different strains of viruses. In addition to their potential direct anti-viral effect, eosinophils can influence other immune cells in response to viral infections. Handzel *et al*, showed that human eosinophils incubated with *Human rhinovirus (HRV)* presented viral antigens to RV16-specific CD4⁺ T cells, causing T cell proliferation and secretion of IFN- γ (66).

In mice, *Influenza A Virus (IAV)* infected eosinophils had an altered surface expression of various cell activation markers such as Siglec-F and IL-5R α . Moreover, exposure of eosinophils to *IAV* induced the up-regulation of transcription of genes encoding for viral recognition proteins, such as Ddx58 (RIG-I), TLR-3, and IFIH1 (MDA5). Importantly, eosinophils exposed to *IAV* up regulated the expression of MHC-I and *MHC-II*, boosting CD8⁺ T cell activation and anti-viral activity(67). Human eosinophils have been demonstrated to bind to *IAV* via α -2,6 and α -2,3 sialic acid, to reduce viral titers, release IL-8 and, similarly to mouse eosinophils, to up regulate RIG-I gene(68). As we mentioned above α -2,3 and α -2,6 sialic acid are ligands for Siglec-8 and Siglec-7 respectively, therefore we hypothesized that *IAV* might use Siglec-7/8 to bind human eosinophils.

In the current scenario of COVID-19 pandemic caused by *Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)*, it was observed that patients display peripheral blood eosinophilopenia that might be associated with poor prognosis(69)(70). Therefore it has been hypothesized that certain aspects of type 2 immune response, including host production of

type 2 cytokines and accumulation of eosinophils, might provide potential protective effects against COVID-19(71). Following this, Drake *et al*, showed that eosinophils might affect directly or indirectly the viral load and replication by secretion of RNase and by the production of Th1-related cytokines rather than Th2 involved in anti-viral defense(72). However, another study has shown that gold nanoparticle adjuvanted spike protein from SARS-CoV-2 induced a strong IgG response to spike protein but was unable to curb lung eosinophilic immunopathology and failed to induce protective immune response post-viral infection(73) (**Table 1**).

Altogether, eosinophils usually appear to have anti-viral effects. In consequence, only theoretically, can we hypothesize that under specific circumstances enhancement of eosinophil numbers and/or topical administration of a selected eosinophil mediator could be considered for fighting against viral infections. The reason is because of the potential dangers connected with such an approach.

Eosinophil functions in bacterial infections

There is accumulating evidence on the complex role that eosinophils play in host defense against bacterial infections. A common clinical example of bacterial infection with eosinophil involvement is the one caused by gram-negative *Mycobacterium* that is characterized by eosinophil infiltration into the site of infection and systemic massive eosinophilia with elevation in α -defensin blood levels(74). Driss *et al*, showed *in vitro* that *M. bovis* induces TLR2 expression on eosinophils. Thereafter *M. bovis* lipomannans bind TLR2 and thereby induce eosinophils to phagocytize the bound bacteria and to release α -defensin and ROS, which contribute directly to bacterial death, and TNF α which induces other immune cells to attack and eventually kill the invading bacteria(75). In line with this, it was shown that *M.*

bovis infection in wild-type mice but not in TLR2^{-/-} mice, results in eosinophil recruitment and degranulation(76) highlighting TLR2's importance at least on mouse eosinophils. Mice infection with *M. avium*, led to a similar anti-mycobacterium response accompanied by eosinophilia and high levels of α -defensin in the blood(74). These studies showed that by secretion of the anti-microbial peptide α -defensin and other cytotoxic proteins, eosinophils play a role in the direct anti-microbial response and might contribute to inflammation by the production of TNF α .

LPS from opsonized gram-negative *E. coli* has been shown to activate eosinophils, after priming with IL-5 or IFN- γ , to release mitochondrial DNA. Mitochondrial DNA and granule proteins forming extracellular traps were able to bind and efficiently kill *E. coli in vitro*. Moreover, after cecal ligation and puncture, hypereosinophilic IL-5-Tg mice showed intestinal eosinophil infiltration and extracellular DNA deposition. This effect was associated with protection against microbial sepsis that supports the concept that eosinophils have a major function in response to this bacteria(48).

S. aureus is a gram-positive highly pathogenic bacteria usually associated with allergic diseases. Hatano *et al*, demonstrated that eosinophils can phagocytize heat-killed *S. aureus* using a mechanism that is different from the one employed by neutrophils being complement receptor 1 (CR1/CD35) dependent, while neutrophil phagocytosis is Fc γ RIII and Fc γ RII dependent(77). Moreover, eosinophils are activated by heat-killed *S. aureus* to release EDN and to produce ROS in part via PAF receptor(78). However, although eosinophil prolonged incubation with live *S. aureus* barely affects the bacteria viability(79), it does induce eosinophil cell death(80). We showed that heat-killed *S. aureus* or its three exotoxins, SEB, protein A, and peptidoglycan, activate both human and murine eosinophils by binding the CD48 cell surface receptor resulting in cell degranulation and release of EPO and ECP(34).

Importantly, we could not detect TLR2 expression on naïve and exotoxin exposed human eosinophils, therefore, ruling out its potential role in *S. aureus*/eosinophil interactions(34). The interaction of eosinophils with SEB induced the cleavage and release of CD48 (sCD48) via phospholipase C that acts as an anti-inflammatory and a decoy receptor(81). Indeed, sCD48 binds SEB and therefore decreases the stimulatory effect of this exotoxin on eosinophils as assessed by IL-8 and EPO release. When injected in mice with SEB-induced peritonitis, sCD48 inhibits inflammatory responses(81). Notably, both *M. tuberculosis* and *E. coli* were found to bind and activate mast cells via CD48(82). We therefore can postulate that besides TLR2 and TLR4, CD48 can be involved in eosinophil interactions with bacteria. Interestingly, we have recently found that CD244 (2B4), the high affinity ligand of CD48, can also bind SEB (Gaur, Ben-Zimra, Levi-Schaffer, unpublished data).

Eosinophil function can be modulated by IL-25 (IL-17E). In gram-positive *C. difficile* infection, intestinal IL-25 production was suppressed and correlated to mortality and tissue pathology in humans and mice. However, IL-25 reconstitution in mice provided microbial protection that correlated with increased mice survival and an increase in IL-4 and eosinophils but not neutrophils in the colon. Indeed, IL-25 protection was abrogated following eosinophil depletion by administration of anti-Siglec-F antibody and in PHIL mice. This eosinophil protective effect did not depend on its direct anti-bacterial activity but rather on increased strength of the gut epithelial barrier in an eosinophil dependent manner(83). Interestingly, in *C. difficile* infection eosinophils create a balance between pro-inflammatory and tolerogenic response by promoting tissue remodeling pathways for the integrity of the epithelial barrier.

C. rodentium is a gram-negative bacterium that shares several pathogenic mechanisms with *E. coli* and infects rodents. In an acute model of mouse colon infection with *C. rodentium*, the

bacterial load was higher in PHIL mice than in wild-type mice. This was observed together with an increased expression of transcripts for pro-inflammatory cytokines and anti-microbial molecules such as S1009a, CXCL1, and CXCL2, implying for an efficient eosinophil anti-bacterial activity. *In vitro*, incubation of eosinophils with *C. rodentium* induced eosinophil degranulation as measured by EPX release and EETs formation which resulted in a strong bactericidal effect(84). However, in the *C. rodentium* model, PHIL mice also showed an increased frequency of neutrophils, Th1, and Th17 cells. Hence, Arnold *et al*, suggested that together with the anti-bacterial effect, eosinophils might restrict T cell response and suppress the recruitment of immune cells in the colon during bacterial infections. Similar to *C. rodentium*, in a mouse model of chronic infection of stomach with the gram-negative *H. pylori* , resident eosinophils suppressed the Th1 immune response, decreasing T cell proliferation and activation. This resulted in an increased *H. pylori* load in the gastric mucosa of wild-type mice while this effect was abrogated in PHIL mice. Eosinophils' regulatory function and the pro-bacterial effect were found to be mediated by IFN- γ (84). In contrast to *C. rodentium*, eosinophils did not show an anti-bacterial effect against *H. pylori* (**Table 1**).

Overall, these results provide insights into the complexity of eosinophil functions in bacterial infections that depend also on the specific bacteria species. Eosinophils can have a direct bactericidal activity mediated by degranulation and extracellular traps, can behave as immunomodulators at the T lymphocyte level and can be a protective component of the epithelial barrier.

Eosinophil functions in fungal infections

Fungi are ubiquitous in the environment and cause, like other pathogens, local or systemic infections. They are especially common in immunocompromised patients, i.e., HIV, cancer patients receiving cytoreductive therapy, and transplanted patients. Examples of frequent fungal infections are vaginal candidiasis, bronchopulmonary aspergillosis, thrush, ringworm, etc. Moreover, fungi are associated with several diseases such as COPD, asthma, atopic dermatitis, psoriasis, and chronic rhinosinusitis.

The innate immune responses towards fungal pathogens are still elusive. Even though macrophages, monocytes, neutrophils, and DC are recognized effector cells against fungal infections(85), the role of other innate immune cells and especially of the eosinophils is still a mystery.

In general, as for the other pathogens including fungi, eosinophil responses vary according to the spectrum of fungal species. Fungal recognition by human eosinophils has been ascribed to CD11b domain and protease activated receptor 2 (PAR-2) receptors. Interestingly, eosinophils do not express Dectin-1(86), as we have recently reconfirmed (*Zaffran**, *Gaur** and *Levi-Schaffer unpublished data*) but exploit CD11b for fungal antigen recognition.

Human eosinophils appear to interact with *A. alternata* by specifically recognizing the cell wall component β -glucan by CD11b, followed by the release of the granular proteins EDN and MBP-1(86)(87) and killing of the fungus. In this study by Yoon *et al*, anti- β -glucan antibodies were found to bind and facilitate the recognition of *A. alternata* by eosinophils(86). Additionally, it has been demonstrated *in vitro* that blocking of complement receptor type 3 (CR3) or Fc γ RII unaltered the response of eosinophils to *A. alternata*, thus implying a direct binding of β -glucan to CD11b on eosinophils(86). Fungal derived proteases

might also be involved in eosinophil anti-fungal reactions, as aspartate proteases from *A. alternata* trigger human eosinophils to release EDN through PAR-2(88).

Regarding *C. albicans*, Ishikawa *et al*, demonstrated that human eosinophils phagocytize this fungus(89). Additionally, EETosis mediated DNA traps have been observed when human eosinophils were incubated with *C. albicans*(90) indicating a role for eosinophils in *C. albicans* elimination. This response is bolstered by our recent observation that eosinophil deficient (GATA-1) mice have a higher fungal load and higher mortality in a model of severe systemic candidiasis in comparison to wild-type mice (Zaffran*, Gaur*, and Levi-Schaffer unpublished data).

Lilly *et al*, demonstrated that in a mouse model of acute infection with *A. fumigatus*, eosinophils are an important player in the clearance of the fungus from the lung. They demonstrated that eosinophils are recruited to the lungs 24h and 48h after intratracheal challenge of *A. fumigatus* and that this was accompanied by enhanced EPX release in the lung. Furthermore, they explored the infection susceptibility in wild-type and GATA-1 mice that leads to impairment of fungal clearance in GATA-1 mice. Since GATA-1 mice had lower cytokine (IL-1 β , IL-6, IL-17A and GM-CSF) and chemokine levels compared to wild-type mice(91), it was suggested that eosinophils are the source for their production.

Fungi have been demonstrated to represent an important source of allergens(92). Exposure to the fungal allergens could worsen allergic diseases such as allergic bronchopulmonary aspergillosis (ABPA) where *A. fumigatus* colonizes the bronchial tract leading to lung dysfunction and severe asthma. Importantly, in ABPA that is accompanied with peripheral blood and airway eosinophilia an increase in total serum IgE, specific anti-fungal IgE levels, mucus obstruction, and bronchiectasis were observed(92). It is important to note that *A.*

fumigatus by itself can induce EETs from human eosinophils *in vitro*(93). Patients with eosinophilic mucus chronic rhinosinusitis have in addition high IgG3, IgM, and IgA levels. IgG3 is responsible for activating the complement system and binds to the FcγRI, RII, and RIII receptors, expressed among other cells, also on eosinophils. Therefore, it can be speculated that fungal-specific IgG3 could provide a mechanism for eosinophils antibody-dependent mediated cytotoxicity against fungi(94) (**Table 1**).

Altogether, the eosinophil response to fungi, through the release of eosinophil toxic mediators and by the formation of EETs and phagocytosis, induces a fungicidal response.

Eosinophils in infections and pharmacological interventions

Long regarded as cells of parasitic infections and allergic diseases, eosinophils have acquired in recent years a recognized status as players in several other pathological and physiological conditions and as possible modulators of the immune response. Because of the prominent damaging eosinophil effect in allergic diseases such as eosinophilic asthma, atopic dermatitis, allergic rhinitis with polyposis, and in hypereosinophilic syndrome, several monoclonal antibodies have been developed as drugs to reduce their numbers in these conditions. These new therapies have targeted principally IL-5 being the critical cytokine in eosinophil biology(95)(96)(97). Anti-IL5 antibodies(95) (mepolizumab and reslizumab) and the anti-IL5Rα antibody(97) (benralizumab) reduce eosinophil numbers and activation that leads to the severity of these diseases. For example, in a meta-analysis study, it was found that severe eosinophilic asthma patients treated with benralizumab had a lower risk of bronchitis and sinusitis suggesting the beneficial role of inhibiting eosinophils(98). However, the pivotal question of whether an anti-eosinophil therapy can increase the susceptibility to infections remains elusive. Only a few studies have been done to address this issue(11). In a study

performed on mild asthmatic patients pretreated with mepolizumab who were then infected with rhinovirus, together with reduced eosinophil activation and counts, an increase in viral titer was detected(63). Another long term safety study of mepolizumab in asthmatic subjects showed that the most frequently reported-treatment adverse effects were respiratory tract infections(99). Albeit eosinopenia was incomplete (i.e., blood eosinophil count was reduced by 78%), these results indicate an important role for eosinophils to fight respiratory infections and the risks associated by inhibiting these cells.

Moreover, glucocorticosteroids (GC), administered routinely to patients with bacterial, viral and fungal infections to counteract excessive inflammation, notably induce eosinophil cell death(100) together with several modulating effects of the drug on neutrophils and the immune system. Therefore, the increased risk of infections detected during GC treatment cannot be attributed only to the reduction of eosinophils. For example, it is known that inhaled GC reduce the frequency of exacerbations in COPD but might increase the risk of respiratory infections(101). However, COPD patients with lower blood eosinophil counts had more pneumonia events than those with higher counts(102). Despite all these considerations and the described potential of eosinophils to fight bacterial and other pathogens, currently, in clinical practice, the rule of the thumb is to inhibit inflammation, even if harming the eosinophils and to administer antibiotics to treat the infections.

Notably, hypereosinophilia caused by some infectious diseases(103) or by the antibiotic-induced drug reaction (DRESS),(104)(105) reveals the danger of uncontrolled and enhanced eosinophil numbers and activity.

Conclusions

In conclusion, eosinophils are powerful immune cells that can, on one hand, fight and take part in the clearance of several bacterial, viral, and fungal infections, while on the other hand may cause damage to host tissues. Therefore, eosinophil numbers and activity should be strictly regulated. Additionally, the potential treatment of infections by drugs that could increase eosinophil numbers and/or activity are too dangerous for patients to be envisaged. Currently, treatments that combine antibiotics to fight infections, with GC or specifically in allergic diseases with eosinophil-targeted biologics to reduce eosinophil numbers and activation provide the best rationale cure available today.

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Conflicts of Interest

The authors have no conflict of interest.

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Data Availability

Data is openly available in a public repository that issues datasets with DOIs. The data that support the findings of the studies mentioned in the manuscripts are openly available on PubMed.

Author Contributions:

Conception: PG, IZ, FLS; Figure preparation PG, TG; Assisting the literature: TG, FRA. All the authors have contributed to the writing and revision of the article. Critical revision of the article: MBZ, FLS. All authors have read, edited, and approved the final version of the article.

Table 1: Eosinophil effects on bacterial, viral, and fungal infections

Pathogen	Eosinophil Response and Effect	Mediator released	Receptors	Model system	Ref.
Eosinophil role upon bacterial infections					
<i>M. bovis</i>	• ↑ Eosinophil number and activation • Phagocytosis • ↓ Bacterial load	Degranulation (EPX, MBP, ECP, EDN) Cytokine release (TNF α) α -defensin ROS	TLR2	<i>In vitro:</i> Human peripheral blood eosinophil (PbEos) <i>In vivo:</i> Intrapleural injection of <i>M. bovis</i> in mice	75,76
<i>E. coli</i>	• Eosinophils extracellular traps (EETs) • Bacterial death	DNA traps	---	<i>In vitro:</i> murine and human eosinophils <i>In vivo:</i> Cecal ligation and puncture in	48

				mice	
<i>S. aureus</i>	• Degranulation of human and murine eosinophils • Phagocytosis of dead but not live bacteria • Eosinophil death	Degranulation (EPX, MBP, ECP, EDN) ROS	CR1/CD35 TLR2 CD48 PAF	<i>In vitro:</i> PbEos and bone marrow murine eosinophils (BMEos) <i>In vivo:</i> AD skin from patients, SEB-induced peritonitis in mice	34,77-81
<i>C. difficile</i>	• ↑ Mice survival • ↑ Eosinophils accumulation in colon • ↑ Strength of gut epithelial barrier	Unclear	IL-25 receptor	<i>In vivo:</i> <i>C. difficile</i> colon infection in mice <i>Ex vivo:</i> Human colon biopsies	83
<i>C. rodentium</i>	• Degranulation of murine eosinophils	Degranulation (ECP) EETs	---	<i>In vivo:</i> <i>C. rodentium</i> colon infection	84

	• ↓Bacterial load • ↓Anti-microbial molecules • ↓Th1 and TH17 cells • Eosinophil activation			in mice	
<i>H. pylori</i>	• ↑ Eosinophils accumulation in colon • ↓ CD4 T cell • ↑ Bacterial load	Unclear	---	<i>In vitro:</i> BMEos <i>In vivo:</i> <i>H. pylori</i> colon infection in mice	84
Eosinophil role upon viral infections					
<i>IAV</i>	• ↓ Viral titer • ↑ Siglec-F expression • ↑ viral recognition protein (RIG-1, Tlr3, MDA5)	IL-8	---	<i>In vitro:</i> pbEos and BMEos <i>In vivo:</i> Transfer of eosinophils from the lungs of allergen-	67,68

	• ↑ T cell activation & anti-viral activity			sensitized and challenged mice into influenza virus-infected mice	
RSV	• ↓ Viral load • ↑ Increase disease severity (by secretion of EETs) • ↑ Eosinophils accumulation • ↑ CD11b	EPX, ECP IFN-β NO	TLR-7 CCL11	<i>In vitro:</i> BMEos <i>In vivo:</i> Intranasal injection of RSV in mice and in OVA-asthma mice model, <i>Ex vivo</i> clinical study: Nasal fluids from patients with lower respiratory infections, Clinical study	55-64

SARS-CoV-2	• ↓ Eosinophils absolute count (EAC) • ↑ EAC correlate with recovery	Unknown	Unknown	Clinical study	69-72
Eosinophil role upon fungal infections					
A. alternata	• Degranulation of human eosinophils • Killing of the fungi	Degranulation (MBP, EDN, ECP)	β2 Integrin CD11b PAR-2	<i>In vitro:</i> pbEos infected with fungus, <i>Ex vivo:</i> Samples from patients infected with fungal allergy	86-88
A. fumigatus	• ↑ Eosinophil recruitment • Airway eosinophilia • EETs • Killing of the fungi	Cytokine and chemokine release Degranulation (EDN)	---	<i>In vitro:</i> mice pbEos <i>Ex vivo:</i> Bronchial mucus samples of patients with ABPA and Samples from	91-93

				patients infected with fungal allergy	
C. <i>albicans</i>	• Phagocytosis • EETs • Killing of the fungi	---	Dectin1	<i>In vitro</i> ; Human eosinophilic leukocytes <i>In vivo</i> model of systemic candidiasis <i>In vitro</i> : pbEos infection with fungi, <i>Ex vivo</i> : Sinus samples from rhinosinusitis patients infected with fungi	89,90

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Fig. 1: Eosinophil receptors and mechanisms involved in bacterial, viral, and fungal infections and homeostasis: The expression of a variety of receptors on the eosinophils surface that activate the cells to sense and respond against pathogens. Eosinophils can participate with different effects and relevance in several pathophysiological conditions such as infections and metabolic homeostasis. Toll-like receptor (TLR), Platelet activating factor (PAF), Eosinophil Extracellular Traps (EETs), ROS (reactive oxygen species), Protease activated receptors-2 (PAR-2), Eosinophil cationic protein (ECP), Eosinophil-derived neurotoxin (EDN), Eosinophil peroxidase (EPO), Eosinophil granule major basic protein (MBP).

Fig. 2: Eosinophil activation and response during viral, bacterial, and fungal infections: Eosinophil response during viral, bacterial, and fungal infections include degranulation and release of pre-formed and newly formed mediators and cytokines, secretion of EETs, and phagocytosis. The Eosinophil activation is mediated by activation of receptors expressed on eosinophil cell surface. 1. Degranulation, 2. Immune response, 3. Phagocytosis, 4. Eosinophil extracellular DNA traps, 5. Cytokines & chemokines release, 6. Antibody mediated response. Eosinophils (Eos), Reactive oxygen species (ROS), Piecemeal degranulation (PMD), Eosinophil extracellular DNA traps (EETosis), Toll-like receptors (TLR), Pattern recognition receptors (PRR).

Figure 1

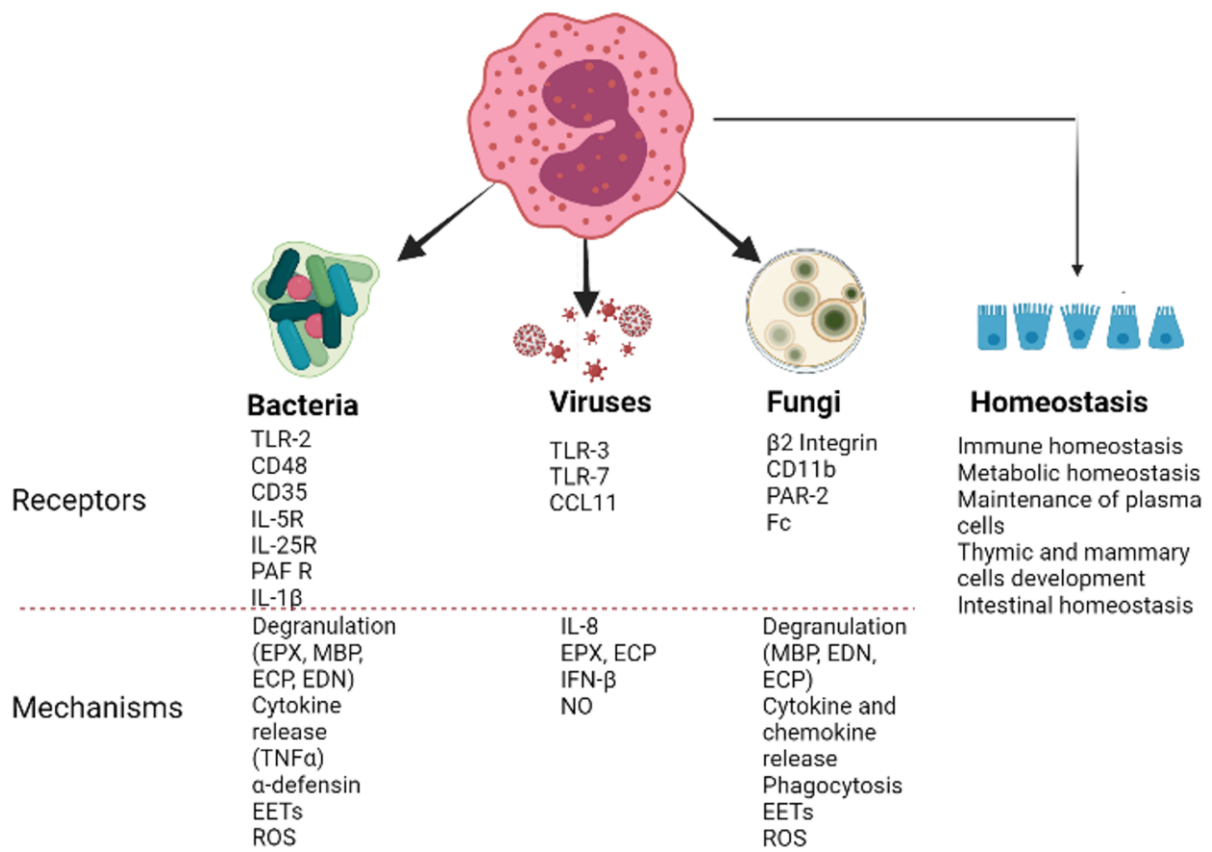


Figure 2

