Published in final edited form as:

Curr Allergy Asthma Rep. 2016 July ; 16(8): 54. doi:10.1007/s11882-016-0634-5.

Eosinophil ETosis and DNA Traps: a New Look at Eosinophilic Inflammation

Shigeharu Ueki¹, Takahiro Tokunaga², Shigeharu Fujieda², Kohei Honda³, Makoto Hirokawa¹, Lisa A. Spencer⁴, and Peter F. Weller⁴

¹Department of General Internal Medicine and Clinical Laboratory Medicine, Akita University Graduate School of Medicine, 1-1-1, Hondo, Akita 010-8543, Japan

²Department of Otorhinolaryngology, Head and Neck Surgery, University of Fukui, Fukui, Japan

³Department of Otorhinolaryngology, Head and Neck Surgery, Akita Graduate School of Medicine, Akita, Japan

⁴Divisions of Allergy and Inflammation and Infectious Diseases, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Abstract

The traditional paradigm of eosinophils as end-stage damaging cells has mainly relied on their release of cytotoxic proteins. Cytokine-induced cell survival and secretion of granular contents from tissue-dwelling eosinophil are thought to be important mechanisms for eosinophilic inflammatory disorders, although the occurrence of cytolysis and its products (i.e., free extracellular granules) has been observed in affected lesions. Recent evidence indicates that activated eosinophils can exhibit a non-apoptotic cell death pathway, namely extracellular trap cell death (ETosis) that mediates the eosinophil cytolytic degranulation. Here, we discuss the current concept of eosinophil ETosis which provides a new look at eosinophilic inflammation. Lessons from eosinophilic chronic rhinosinusitis revealed that ETosis-derived DNA traps, composed of stable web-like chromatin, contribute to the properties of highly viscous eosinophilic mucin and impairments in its clearance. Intact granules entrapped in DNA traps are causing long-lasting inflammation but also might have immunoregulatory roles. Eosinophils possess a way to have post-mortem impacts on innate immunity, local immune response, sterile inflammation, and tissue damage.

Correspondence to: Shigeharu Ueki.

This article is part of the Topical Collection on *Otitis*

Electronic supplementary material The online version of this article (doi:10.1007/s11882-016-0634-5) contains supplementary material, which is available to authorized users.

Conflict of Interest Drs. Ueki, Tokunaga, Fujieda, Honda, Hirokawa, Spencer, and Weller declare no conflicts of interest relevant to this manuscript.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent With regard to the authors' research cited in this paper, all procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration.

Keywords

Cytolysis; Eosinophilic chronic rhinosinusitis; Eosinophils; ETosis; DNA traps; Neutrophils

---

Introduction

Human eosinophils, bone marrow-derived leukocytes notably associated with allergic, anthelmintic parasite and other immune responses, contain approximately 200 cytoplasmic crystalloid granules per cell [1]. These phospholipid bilayer-enveloped granules are central to the functional responses of eosinophils in that granules house preformed stores of four major granule proteins, including eosinophil cationic protein (ECP), major basic protein (MBP), and eosinophil peroxidase (EPO) [2••], which are highly cationic proteins directly toxic to extracellular microorganisms and parasites as well as host tissue. Eosinophil-derived cationic granule proteins can function by disrupting the integrity of lipid bilayers, exhibit neurotoxic properties and RNase activities, and/or participate in generating reactive oxidants and radical species [3].

In addition to the known mechanisms of extracellular secretion of granule proteins (i.e., exocytosis and piecemeal degranulation) [4•, 5], a distinct mode of eosinophil degranulation has been observed arising from “cytolysis” or “lytic degranulation” of eosinophils that deposits intact membrane-bound granules extracellularly (free eosinophil granules (FEGs), reviewed by Persson and Uller) [6••]. By their unique ultrastructure, FEGs have been recognized in the airways and tissues in association with diverse disorders, including allergic asthma, rhinitis, chronic rhinosinusitis, dermatitis, helminth infections, hypereosinophilic syndrome, and urticaria [2••, 7]. None of the understood types of necrosis (e.g., complement attack, severe hypoxia, and viral lysis) or crush artifacts have not been able to explain the mechanisms [8]. Moreover, Saffari et al. reported that approximately 80 % of eosinophils recognized by electron microscopy in biopsy specimens from patients with eosinophilic esophagitis exhibit evidence of cytolysis [7]. In contrast, in vivo studies showed only limited signs of apoptosis or of necrosis secondary to apoptosis in tissue eosinophils, even upon resolution of eosinophilia [6••, 9]. Eosinophil lysis in the inflamed locus is paradoxical, because eosinophilic disorders are driven by eosinophil-poietic, survival-promoting cytokines including IL-3, IL-5, and GM-CSF.

Although the mechanism is unknown, lysis of eosinophils has been suggested to represent a state of “ultimate activation” [10] or “full activation” [11] of eosinophils. Our recent study revealed that activated eosinophils undergo a distinct pathway of suicidal cell death, extracellular trap cell death (ETosis). ETosis involves the cytolytic release of intact granules also in conjunction with filamentous chromatin structures [2••]. This article discusses the role(s) and significance of eosinophil ETosis (EETosis) of the eosinophilic inflammation.

Lessons from Chronic Rhinosinusitis: Pathogenesis of Eosinophilic Mucin

Chronic rhinosinusitis (CRS) is a heterogeneous disease characterized by local inflammation of the upper airways and sinuses which persists for at least 12 weeks [12]. CRS is a complex disease that presents as several variants caused by different cellular and molecular

mechanisms [13, 14]. CRS can be classified into CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). In Western countries, CRSwNP displays intense eosinophilic infiltration. In contrast, in Asia, CRSwNP has been primarily associated with neutrophil infiltration, although the numbers of cases of CRSwNP with eosinophil infiltration have increased in recent years [12, 15]. The presence of mucosal eosinophilia is correlated with disease severity and clinically distinct subsets of CRS [15–17]. The pathophysiology of tissue eosinophilia is incompletely known, but Th2 cytokines including IL-5 and eotaxins play an indispensable role in eosinophil-selective accumulation, similar to other allergic diseases [12, 18]. Of note, approximately 30 % of tissue eosinophils in CRSwNP exhibit ultrastructural evidence of cytolysis [19].

The Japan Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis Study (JESREC Study), a multi-center retrospective study, showed that nasal polyps frequently recur after surgery in cases of CRSwNP accompanied by eosinophil infiltration [20••]. In order to differentiate this refractory phenotype, the JESREC Study identified CRSwNP with eosinophil infiltration as eosinophilic CRS (ECRS). The diagnostic criterion of ECRS is shown in Table 1. Indeed, mucosal eosinophilia of 70 eosinophils per high-power field or higher was significantly correlated with recurrence of polyps after surgery. The recurrence of ECRS was significantly associated with the following clinical features: (1) comorbidity of asthma, (2) aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) intolerance, (3) eosinophilia in peripheral blood, and (4) ethmoid-dominant shadow in CT. CRS was classified into four groups (i.e., non-ECRS, mild ECRS, moderate ECRS, and severe ECRS), which were significantly correlated with the rate of recurrence.

The clinical differences between ECRS and non-ECRS are summarized in Table 2. Particularly, thick, glue-like secretion is a noteworthy hallmark of ECRS. Massive infiltration of eosinophils and FEGs in the viscous secretion is also observed in other persistent eosinophilic sinusitis including allergic fungal sinusitis, eosinophilic otitis media, and most cases of CSwNP in Western countries [21, 22]. The secretions, often collected at the time of surgery, are called “eosinophilic mucin” or “allergic mucin” [23]. The consistency has been described as being similar to peanut butter, cottage cheese, or axle grease [22, 24]. The appearance of eosinophilic mucin clearly differs from secretions associated with neutrophil-dominant sinusitis [25]. With their unique characteristics, several researchers described “eosinophilic mucin rhinosinusitis” for an entity with histologic features similar to allergic fungal sinusitis but without detection of fungus [26].

The presence of eosinophilic mucin is associated with enhanced disease severity [27]. The difficult-to-remove secretion could be pathogenic, because local concentrations of MBP in the secretions far exceed those levels necessary to mediate epithelial damage [28]. Immunohistologic analyses of CRS specimens showed an association between the presence of extracellular deposition of MBP and damage to sinus mucosa [29]. Ponikau et al. reported that lytic eosinophil degranulation was observed much more readily in the mucin than in tissue; therefore, tissue eosinophils and also eosinophils within the luminal side mucin may contribute to epithelial damage [28]. Resulting epithelial damage may then inhibit the effectiveness of ciliary beat, thereby decreasing mucus transport and perpetuating a cycle of thickening secretions [30].

Although eosinophils induce mucin glycoprotein production in airway epithelial cells [31], the viscosity of secretions is not simply the result of overproduction of mucin. Unlike normal conditions, chronic inflammatory airway disorders exhibit an increased content of immune cells and large polymers including DNA in the airway secretions [32]. Histologic analyses suggested that eosinophils migrate from tissue into the mucus to form distinct and characteristic clusters [28, 33, 34]. In a detailed histologic study, Granville et al. described the hematoxylin and eosin staining of eosinophilic mucin in allergic fungal sinusitis as “the tightly clustered eosinophils often showed degenerative changes, such that their nuclei appeared smudged, elongated, and, in some cases, formed collections of strongly basophilic nuclear debris” [35]. Schubert et al. described the morphology as “large concretions of strongly staining pyknotic eosinophils” [36].

Because it is difficult to demonstrate the nuclear structure using thin slice sections from paraffin-embedded materials, fixed eosinophilic mucin was directly stained with the DNA-specific dye SYTOX; and 3D reconstruction images were obtained from the confocal Z-stacks. Supplementary material 1: Video 1 shows an example of DNA staining of eosinophilic mucin from an ECRS patient. The spreading of filamentous DNA indicated that DNA fragmentation characteristic of apoptosis is absent in luminal eosinophils. Abundant DNA that has spread and aggregated is present in the eosinophilic mucin, forming a sticky scaffold for clustered eosinophils and their debris including FEGs [37••]. Immunostaining of eosinophilic mucin revealed that they are nuclear-derived chromatin structures: DNA traps [37••].

Eosinophil ETosis

Nuclear-derived DNA traps were not produced by apoptotic or necrotic eosinophils but from ETosis [2••, 38]. ETosis is a NADPH-oxidase-mediated active form of rapid non-apoptotic cell death first recognized in neutrophils (neutrophil ETosis (NETosis)) [38]. Stimuli-activated blood-derived human neutrophils and eosinophils can undergo a similar sequence of ETosis [2••, 38]. In contrast to apoptosis, caspase activation, nuclear condensation, and DNA fragmentation do not occur in ETosis. Rather, the nucleus shows dynamic changes, including the loss of nuclear lobulation and chromatin decondensation. Finally, the nuclear membrane disintegrates followed by plasma membrane rupture, releasing whole cellular contents including fibrous chromatin structures. Importantly, ETotic eosinophils and released granules do not expose phosphatidylserine on the external leaflet of the membrane [2••], suggesting escape from swift clearance by phagocytic macrophages if they are produced in the tissue [39, 40].

In vitro, activated human eosinophils rapidly (30–120 min) undergo EETosis in response to various stimuli, including immobilized IgG and IgA [2••, 37••, 41], which have been used to investigate the mechanisms of eosinophil degranulation as defined models for parasites [11]. Since the majority of eosinophils normally reside within mucosal tissues interfacing with the environment [42, 43], it is conceivable that crosslinking of IgA receptors induces EETosis and contributes to mucosal immunity. High concentration of monosodium urate induces rapid eosinophil death [44] and DNA traps [45], suggesting that endogenous danger signals also elicit EETosis. Platelet-activating factor is a less potent EETosis inducer,

although it paradoxically induces EETosis in combination with well-known survival factors for eosinophils, IL-5 or GM-CSF [2••]. Calcium ionophore and phorbol myristate acetate (PMA), known to induce NETosis [38, 46], are also potent inducer of EETosis [2••, 47]. Closer examination of eosinophil death induced by immobilized plasma proteins [48•], glass adhesion and coagulation [49], crosslinking Siglec-8 [50], or exposure to *Staphylococcus aureus* supernatant [51] might implicate these as eosinophil cytolytic stimuli as well [4•, 6••]. Of interest, neutrophils exhibit size-sensing properties to selectively undergo NETosis [52], although it is not known whether such mechanisms are present in eosinophils. Much remains to be clarified regarding the induction of EETosis.

Stimuli-induced extracellular calcium flux is required for EETosis to proceed [2••, 37••]. Of note, ETosis is not only inhibited by a NADPH oxidase inhibitor, but also by catalase that extracellularly converts hydrogen peroxide into water and dioxygen [2••, 38]. Presence of serum, albumin, and red blood cells that can scavenge reactive oxygen species is also inhibitor of ionophore-induced EETosis [2••]. These experimental observations suggest that cellular activation is well regulated in eosinophils transiting the blood stream and EETosis is mainly occurring within inflamed tissue or after luminal entry [53]. Simultaneously, the oxidant-antioxidant imbalance in allergic diseases [54, 55] might favor the development of EETosis. Tight regulation of ETosis appears to be critical for minimizing unnecessary autoimmunity and sterile inflammation [56].

Eosinophil DNA Traps

DNA traps consisting of filamentous chromatin structures have been shown to trap and kill extracellular microbes including Gram-positive and negative bacteria, fungi, and viruses [37••, 57–59]. To date, DNA trap formation is considered to be a common mechanism of the innate immune system in vertebrates [39]. Recently, Robb et al. reported that even invertebrate cells are capable of ETosis, suggesting an ancient and evolutionarily conserved mechanism [60].

Eosinophil DNA traps were first described by Simon and colleagues, as eosinophil extracellular traps (EETs) [61]. Cytokine-primed eosinophils, subsequently stimulated with lipopolysaccharide, eotaxin, complement factor 5a (C5a), or thymic stromal lymphopoietin (TSLP) [62], eject net-like structures composed of mitochondrial DNA and granule-derived proteins that exhibit anti-microbial properties within the extracellular space. The mitochondrial DNA release was reported to take place in viable eosinophils in a catapult-like manner in seconds. However, the mechanism by which the small mitochondrial genome is launched against the resistance of fluid or expelled in solid tissue is unknown [63, 64].

In contrast to active projection of mitochondrial DNA from live eosinophils in EETs, ETosis-mediated DNA release was accompanied by cell death, and DNA traps spread by a passive process [37••]. In the solid tissue section, eosinophil DNA traps with disintegrated nuclear membrane can be detected by electron microscopy [2••, 4•]; however, the detailed structure to be demonstrated might be challenging because extracellular spaces severely restricts the distribution of DNA traps [39]. It is possible that tissue EETosis has been overlooked as chromatolytic cytolysis by limited resolution of microscope.

Nevertheless, in highly inflamed, elastic tissue, this might not be the case. An eosinophilic abscess, flame figure in Wells syndrome (eosinophilic cellulitis), is histologically described as “secondary to disintegration of eosinophils and consists of aggregates of eosinophilic granules and nuclear fragments” [65]. Wouters et al. recently found that the mesh-like structures observed in flame figures stain positively with propidium iodide, indicating the presence of DNA [66]. The origin of the DNA is likely nuclear since they are co-localized with immunostaining for histone H2A. In addition, the protein content assessed by mass spectrometry displayed that histone H2 was the third most abundant protein within flame figures. Extracellular histones, concentrated in DNA traps, have potent antimicrobial effects but are also toxic to host cells [63, 67, 68]. In addition, extracellular host DNA has been recently recognized as a damage-associated molecular pattern (DAMP) and involved in various immune processes [69].

In fluid phase such as culture media, the blood stream, exudates, or secretions, expanding filamentous DNA traps are easily visualized [39, 70]. Eosinophil DNA traps extending from the originating cells were often entangled, stacking onto the adjacent cells, culture plates, or cell debris [2••, 37••]. Cohesion of eosinophil DNA traps and extracellular particles is contact-dependent manner, extremely enhanced by passive forces generated by shear flow [37••, 70]. A hypothesis for adhesiveness of DNA traps readily seen was charged-dependent interaction [39, 71, 72], although hydrophobic interaction is shown to play a major role in eosinophil DNA traps [37••]. It is noteworthy that a large number of bacterial and fungal pathogens depend on hydrophobic interactions for the initial adhesion of pathogens to tissues, leading to colonization, invasion, or tissue destruction [73]. The adhesiveness of DNA traps in fluid phase is mainly regulated by the hydrophobicities of the targets and the surrounding surface-active agents [37••]. However, host/pathogen-derived nucleases [74] and proteases (discussed later) also affect the structure and stability of DNA traps; therefore, trapping capacity might be more complex in in vivo settings.

Attributes of Eosinophil ETosis and DNA Traps: Comparison to Neutrophil ETosis

In NETosis, the granule membranes disappear followed by translocation of neutrophil elastase (NE) and myeloperoxidase (MPO) to the nucleus, where they bind to chromatin and promote nuclear decondensation [38, 75, 76]. This intracellular degranulation process requires the necessary time for these proteins to bind to chromatin prior to neutrophil rupture and is considered essential for formation of DNA traps loaded with antibacterial granule proteins. Immunostaining images have shown that neutrophil DNA traps were uniformly stained with anti-NE or anti-MPO antibodies [38, 75].

A main difference observed in eosinophil DNA traps is their association with intact granules (either individually or in clusters), as opposed to the free granule-derived proteins observed in neutrophil DNA traps. The release of intact granules is a common feature of EETosis regardless of the stimulus [2••]. Rapid plasma membrane disintegration and limited prior degranulation during the process of EETosis appear to cause the lack of mixing of nuclear

chromatin and the contents of granules. As a result, both intact granules and granule protein-free DNA traps are generated.

DNA traps containing intact crystalloid granules and lacking liberated granule proteins are consistent with clinical observations and are potentially an important feature of EETosis because cell-free eosinophil granules remain secretion-competent organelles [77].

Remarkably, eosinophil granules themselves express ligand-binding competent cytokine, chemokine, and eicosanoid receptors on the exterior surface of the phospholipid bilayer membrane [48•, 78]. Stimulation of granules activates intragranular signaling mechanisms that elicits secretion of selected granule-derived proteins, including ECP, EPO, IL-4, and IL-6 [48•]. A portion of the EETosis-derived free granules responded to eotaxin to secrete ECP [2••]. Therefore, the release of FEGs in tissue, secondary to EETosis, is more than simply granule protein deposition causing chronic inflammation but may also have immunoregulatory roles.

The excess production of DNA traps, from either eosinophil or neutrophil origin, contributes to the increased viscosity of the secretions [37••]. What then is the cause of the different viscosities of secretions observed in ECRS and non-ECRS? Characteristics of DNA traps and proteases from different cell types are one potential explanation; eosinophil DNA traps are composed by less-pliable, more stable, and more condensed chromatin. Neutrophil DNA traps consist of 5–10 nm-stacked cylindrical nucleosome and 25–50-nm globular domains [57, 79]; in contrast, eosinophil DNA traps consist mostly of 25–35-nm chromatin fibers [2••]. Core histones, the most abundant proteins in DNA traps [79], are packaging nearly 2 m of DNA into a small nucleus and are targets for abundant enzymes [80]. NETosis releases their own proteases including NE, which degrade histones and promote relaxation of chromatin, resulting in increased susceptibility to nuclease [37••, 81]. Interestingly, the viscosity of eosinophilic mucin from a patient with eosinophilic otitis media is reduced by bacterial infection [21], suggesting that the presence of neutrophils decreases the viscosity. Thus, proteases appear to play an important role in the structure and stability of neutrophil DNA traps. Eosinophils exhibit far lower protease activities compared to neutrophils; therefore, eosinophil DNA traps may escape from proteolytic clearance and remain intact over time [37••].

Conclusions

Eosinophils have been considered end-stage effector cells involved in host protection against parasite infection and Th2-type inflammation. Eosinophil cytolysis is an activated phenotype that has been observed in biological specimens from patients with disease, and deposition of FEGs is readily observed in the affected tissues or secretions from these patients [3].

However, the clinical importance of eosinophil lysis has been underappreciated, in part because of a lack of understanding of the causative mechanism and etiology. The current concept of EETosis provides a new perspective on eosinophilic inflammation. Figure 1 shows a CT image of an ECRS patient and a schematic diagram of tissue infiltrating eosinophils and their fate. Blood-derived eosinophils accumulate in inflamed tissue where some of them undergo EETosis to form FEGs, others further transmigrate into the sinus cavity and release DNA traps as their fate, forming a sticky framework of eosinophilic

mucin. Unlike apoptosis, EETosis releases intact cellular contents, with long-lasting effects. Dysregulation or excessive EETosis and DNA traps might be novel therapeutic targets for eosinophilic diseases. For instance, disruption of DNA traps with DNase effectively decreases the viscosity of secretions [37•, 82]. Further studies are warranted to clarify the precise pathophysiological roles in vivo, but it is noteworthy that mouse models might have several limitations for studying eosinophil cytolysis [8, 11].

EETosis provides short-lived, non-dividing eosinophils a mechanism whereby they might continue to maintain stimulus-dependent secretory functions within eosinophil-enriched organs and tissues even after eosinophil death [77, 83]. DNA traps, providing a larger adhesive surface than the cell itself, are central to encapsulation reactions mounted against non-self agents together with intact granules. It would be reasonable for the innate role of eosinophil DNA traps to function as a cordon in the airway and/or intestinal lumen or abscess around the non-phagocytosable parasites or fungal hyphae [34, 52, 77]. Moreover, cytolytic ETosis results in the release of granule proteins, cytokines, sticky chromatin, DAMPs, and lipid mediators [84] and contributes to the rheological properties of secretions, the local immune response, sterile inflammation, and tissue damage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was funded in part by Research Grant on Allergic Disease and Immunology from Japan Agency for Medical Research and Development, Charitable Trust Laboratory Medicine Research Foundation of Japan, and JSPS KAKENHI 16 K08926 (SU), Grants-in-Aid for Scientific Research from the Japanese Ministry of Health, Labor and Welfare H27, H28-Research on measures for intractable disease-general-004 (SF), NIH R37-AI020241 (PFW), R01-AI051645 (PFW), and R01-HL095699 (LAS).

Abbreviations

CRS	Chronic rhinosinusitis
CRSwNP	CRS with nasal polyps
CRSsNP	CRS without nasal polyps
DAMP	Damage-associated molecular pattern
ECP	Eosinophil cationic protein
ECRS	Eosinophilic chronic rhinosinusitis
EETs	Eosinophil extracellular traps
EPO	Eosinophil peroxidase
ETosis	Extracellular trap cell death
EETosis	Eosinophil extracellular trap cell death

FEG	Free eosinophil granule
GM-CSF	Granulocyte-macrophage colony-stimulating factor
MBP	Major basic protein
NETosis	Neutrophil extracellular trap cell death
PMA	Phorbol myristate acetate
TSLP	Thymic stromal lymphopoietin

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Moqbel, RLP., Adamko, DJ., Odemuyiwa, SO. Biology of Eosinophils. 7th. Philadelphia: Elsevier Science; 2009.
2. Ueki S, Melo RC, Ghiran I, Spencer LA, Dvorak AM, Weller PF. Eosinophil extracellular DNA trap cell death mediates lytic release of free secretion-competent eosinophil granules in humans. *Blood*. 2013; 121:2074–2083. [PubMed: 23303825] First report showing EETosis-mediates cytolysis and production of intact free granules.
3. Kita H. Eosinophils: multifunctional and distinctive properties. *Int Arch Allergy Immunol*. 2013; 161(Suppl 2):3–9.
4. Spencer LA, Bonjour K, Melo RC, Weller PF. Eosinophil secretion of granule-derived cytokines. *Front Immunol*. 2014; 5:496. [PubMed: 25386174] Up-to-date review of mechanisms of eosinophil secretory functions.
5. Melo RC, Liu L, Xenakis JJ, Spencer LA. Eosinophil-derived cytokines in health and disease: unraveling novel mechanisms of selective secretion. *Allergy*. 2013; 68:274–284. [PubMed: 23347072]
6. Persson C, Uller L. Theirs but to die and do: primary lysis of eosinophils and free eosinophil granules in asthma. *Am J Respir Crit Care Med*. 2014; 189:628–633. Up-to-date review for current knowledge for eosinophil lysis and free eosinophil granules.
7. Saffari H, Hoffman LH, Peterson KA, Fang JC, Leiferman KM, Pease 3rd LF, et al. Electron microscopy elucidates eosinophil degranulation patterns in patients with eosinophilic esophagitis. *J Allergy Clin Immunol*. 2014; 133:1728–1734. e1721. [PubMed: 24439077]
8. Persson CG, Erjefalt JS. Eosinophil lysis and free granules: an in vivo paradigm for cell activation and drug development. *Trends Pharmacol Sci*. 1997; 18:117–123. [PubMed: 9149540]
9. Erjefalt JS, Greiff L, Andersson M, Matsson E, Petersen H, Linden M, et al. Allergen-induced eosinophil cytolysis is a primary mechanism for granule protein release in human upper airways. *Am J Respir Crit Care Med*. 1999; 160:304–312.
10. Persson CG, Erjefalt JS. “Ultimate activation” of eosinophils in vivo: lysis and release of clusters of free eosinophil granules (Cfegs). *Thorax*. 1997; 52:569–574. [PubMed: 9227728]
11. Kita H. Eosinophils: multifaceted biological properties and roles in health and disease. *Immunol Rev*. 2011; 242:161–177. [PubMed: 21682744]
12. Kato A. Immunopathology of chronic rhinosinusitis. *Allergol Int*. 2015; 64:121–130. [PubMed: 25838086]
13. Akdis CA, Bachert C, Cingi C, Dykewicz MS, Hellings PW, Naclerio RM, et al. Endotypes and phenotypes of chronic rhinosinusitis: a PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol*. 2013; 131:1479–1490. [PubMed: 23587334]

14. Takabayashi T, Kato A, Peters AT, Suh LA, Carter R, Norton J, et al. Glandular mast cells with distinct phenotype are highly elevated in chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol.* 2012; 130:410–420. e415. [PubMed: 22534535]
15. Nakayama T, Yoshikawa M, Asaka D, Okushi T, Matsuwaki Y, Otori N, et al. Mucosal eosinophilia and recurrence of nasal polyps - new classification of chronic rhinosinusitis. *Rhinology.* 2011; 49:392–396. [PubMed: 21991563]
16. Soler ZM, Sauer D, Mace J, Smith TL. Impact of mucosal eosinophilia and nasal polyposis on quality-of-life outcomes after sinus surgery. *Otolaryngol Head Neck Surg.* 2010; 142:64–71. [PubMed: 20096225]
17. Nakayama T, Asaka D, Yoshikawa M, Okushi T, Matsuwaki Y, Moriyama H, et al. Identification of chronic rhinosinusitis phenotypes using cluster analysis. *Am J Rhinol Allergy.* 2012; 26:172–176. [PubMed: 22449557]
18. Saito H, Honda K, Asaka C, Ueki S, Ishikawa K. Eosinophil chemotaxis assay in nasal polyps by using a novel optical device EZ-TAXIScan: Role of CC-chemokine receptor 3. *Allergol Int.* 2016 in press.
19. Armengot M, Garin L, Carda C. Eosinophil degranulation patterns in nasal polyposis: an ultrastructural study. *Am J Rhinol Allergy.* 2009; 23:466–470. [PubMed: 19807977]
20. Tokunaga T, Sakashita M, Haruna T, Asaka D, Takeno S, Ikeda H, et al. Novel scoring system and algorithm for classifying chronic rhinosinusitis: the JESREC Study. *Allergy.* 2015; 70:995–1003. [PubMed: 25945591] First large scale epidemiological multi-center retrospective study for eosinophilic rhinosinusitis in Japan.
21. Iino Y, Tomioka-Matsutani S, Matsubara A, Nakagawa T, Nonaka M. Diagnostic criteria of eosinophilic otitis media, a newly recognized middle ear disease. *Auris Nasus Larynx.* 2011; 38:456–461. [PubMed: 21251776]
22. Adelson RT, Marple BF. Fungal rhinosinusitis: state-of-the-art diagnosis and treatment. *J Otolaryngol.* 2005; 34(Suppl 1):S18–S23. [PubMed: 16089236]
23. Slavin RG. Sinusitis: viral, bacterial, or fungal and what is the role of Staph? *Allergy Asthma Proc.* 2006; 27:447–450. [PubMed: 17176777]
24. de Shazo RD, Swain RE. Diagnostic criteria for allergic fungal sinusitis. *J Allergy Clin Immunol.* 1995; 96:24–35. [PubMed: 7622760]
25. Collins MM, Nair SB, Der-Haroutian V, Close D, Rees GL, Grove DI, et al. Effect of using multiple culture media for the diagnosis of noninvasive fungal sinusitis. *Am J Rhinol.* 2005; 19:41–45. [PubMed: 15794073]
26. Ferguson BJ. Eosinophilic mucin rhinosinusitis: a distinct clinico-pathological entity. *Laryngoscope.* 2000; 110:799–813. [PubMed: 10807359]
27. Healy DY, Leid JG, Sanderson AR, Hunsaker DH. Biofilms with fungi in chronic rhinosinusitis. *Otolaryngol Head Neck Surg.* 2008; 138:641–647. [PubMed: 18439472]
28. Ponikau JU, Sherris DA, Kephart GM, Kern EB, Congdon DJ, Adolphson CR, et al. Striking deposition of toxic eosinophil major basic protein in mucus: implications for chronic rhinosinusitis. *J Allergy Clin Immunol.* 2005; 116:362–369. [PubMed: 16083791]
29. Harlin SL, Ansel DG, Lane SR, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. *J Allergy Clin Immunol.* 1988; 81:867–875. [PubMed: 3286721]
30. Hamilos DL. Host-microbial interactions in patients with chronic rhinosinusitis. *J Allergy Clin Immunol.* 2014; 133:640–653. e644. [PubMed: 24290275]
31. Burgel PR, Lazarus SC, Tam DC, Ueki IF, Atabai K, Birch M, et al. Human eosinophils induce mucin production in airway epithelial cells via epidermal growth factor receptor activation. *J Immunol.* 2001; 167:5948–5954. [PubMed: 11698473]
32. Voynow JA, Rubin BK. Mucins, mucus, and sputum. *Chest.* 2009; 135:505–512. [PubMed: 19201713]
33. Braun H, Buzina W, Freudenschuss K, Beham A, Stammberger H. 'Eosinophilic fungal rhinosinusitis': a common disorder in Europe? *Laryngoscope.* 2003; 113:264–269. [PubMed: 12567080]

34. Watanabe K, Misu T, Ohde S, Edamatsu H. Characteristics of eosinophils migrating around fungal hyphae in nasal discharge. *Ann Otol Rhinol Laryngol*. 2004; 113:200–204. [PubMed: 15053201]
35. Granville L, Chirala M, Cernoch P, Ostrowski M, Truong LD. Fungal sinusitis: histologic spectrum and correlation with culture. *Hum Pathol*. 2004; 35:474–481. [PubMed: 15116329]
36. Schubert MS, Goetz DW. Evaluation and treatment of allergic fungal sinusitis. I. Demographics and diagnosis. *J Allergy Clin Immunol*. 1998; 102:387–394. [PubMed: 9768578]
37. Ueki S, Konno Y, Takeda M, Moritoki Y, Hirokawa M, Matsuwaki Y, et al. Eosinophil extracellular trap cell death-derived DNA traps: Their presence in secretions and functional attributes. *J Allergy Clin Immunol*. 2016; 137:258–267. [PubMed: 26070883] Demonstration of clinical and experimental properties of eosinophil DNA traps.
38. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, et al. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol*. 2007; 176:231–241. [PubMed: 17210947]
39. Brinkmann V, Zychlinsky A. Beneficial suicide: why neutrophils die to make NETs. *Nat Rev Microbiol*. 2007; 5:577–582. [PubMed: 17632569]
40. Serhan CN, Chiang N, Dalli J. The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. *Semin Immunol*. 2015; 27:200–215. [PubMed: 25857211]
41. Weiler CR, Kita H, Hukee M, Gleich GJ. Eosinophil viability during immunoglobulin-induced degranulation. *J Leukoc Biol*. 1996; 60:493–501. [PubMed: 8864134]
42. Shamri R, Xenakis JJ, Spencer LA. Eosinophils in innate immunity: an evolving story. *Cell Tissue Res*. 2011; 343:57–83. [PubMed: 21042920]
43. Ueki S, Nishikawa J, Yamauchi Y, Konno Y, Tamaki M, Itoga M, et al. Retinoic acids up-regulate functional eosinophil-driving receptor CCR3. *Allergy*. 2013; 68:953–956. [PubMed: 23742077]
44. Kobayashi T, Kouzaki H, Kita H. Human eosinophils recognize endogenous danger signal crystalline uric acid and produce proinflammatory cytokines mediated by autocrine ATP. *J Immunol*. 2010; 184:6350–6358. [PubMed: 20483787]
45. Schorn C, Janko C, Latzko M, Chaurio R, Schett G, Herrmann M. Monosodium urate crystals induce extracellular DNA traps in neutrophils, eosinophils, and basophils but not in mononuclear cells. *Front Immunol*. 2012; 3:277. [PubMed: 22969769]
46. Wang Y, Li M, Stadler S, Correll S, Li P, Wang D, et al. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J Cell Biol*. 2009; 184:205–213. [PubMed: 19153223]
47. Fukuda T, Ackerman SJ, Reed CE, Peters MS, Dunnette SL, Gleich GJ. Calcium ionophore A23187 calcium-dependent cytolytic degranulation in human eosinophils. *J Immunol*. 1985; 135:1349–1356. [PubMed: 3925008]
48. Neves JS, Perez SA, Spencer LA, Melo RC, Reynolds L, Ghiran I, et al. Eosinophil granules function extracellularly as receptor-mediated secretory organelles. *Proc Natl Acad Sci U S A*. 2008; 105:18478–18483. [PubMed: 19017810] First describe the cell-free eosinophil granules posses independent secretory functions.
49. Muniz-Junqueira MI, Barbosa-Marques SM, Junqueira Jr LF. Morphological changes in eosinophils are reliable markers of the severity of an acute asthma exacerbation in children. *Allergy*. 2013; 68:911–920. [PubMed: 23745834]
50. Kano G, Almanan M, Bochner BS, Zimmermann N. Mechanism of Siglec-8-mediated cell death in IL-5-activated eosinophils: role for reactive oxygen species-enhanced MEK/ERK activation. *J Allergy Clin Immunol*. 2013; 132:437–445. [PubMed: 23684072]
51. Prince LR, Graham KJ, Connolly J, Anwar S, Ridley R, Sabroe I, et al. *Staphylococcus aureus* induces eosinophil cell death mediated by alpha-hemolysin. *PLoS One*. 2012; 7:e31506. [PubMed: 22355374]
52. Branzk N, Lubojemska A, Hardison SE, Wang Q, Gutierrez MG, Brown GD, et al. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. *Nat Immunol*. 2014; 15:1017–1025. [PubMed: 25217981]
53. Malm-Erfjelt M, Greiff L, Ankerst J, Andersson M, Wallengren J, Cardell LO, et al. Circulating eosinophils in asthma, allergic rhinitis, and atopic dermatitis lack morphological signs of degranulation. *Clin Exp Allergy*. 2005; 35:1334–1340. [PubMed: 16238793]

54. Nadeem A, Siddiqui N, Alharbi NO, Alharbi MM. Airway and systemic oxidant-antioxidant dysregulation in asthma: a possible scenario of oxidants spill over from lung into blood. *Pulm Pharmacol Ther.* 2014; 29:31–40. [PubMed: 24929073]
55. Ito W, Kobayashi N, Takeda M, Ueki S, Kayaba H, Nakamura H, et al. Thioredoxin in allergic inflammation. *Int Arch Allergy Immunol.* 2011; 155(Suppl 1):142–146. [PubMed: 21646810]
56. Branzk N, Papayannopoulos V. Molecular mechanisms regulating NETosis in infection and disease. *Semin Immunopathol.* 2013; 35:513–530. [PubMed: 23732507]
57. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science.* 2004; 303:1532–1535. [PubMed: 15001782]
58. Urban CF, Reichard U, Brinkmann V, Zychlinsky A. Neutrophil extracellular traps capture and kill *Candida albicans* yeast and hyphal forms. *Cell Microbiol.* 2006; 8:668–676. [PubMed: 16548892]
59. Saitoh T, Komano J, Saitoh Y, Misawa T, Takahama M, Kozaki T, et al. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. *Cell Host Microbe.* 2012; 12:109–116. [PubMed: 22817992]
60. Robb CT, Dyrinda EA, Gray RD, Rossi AG, Smith VJ. Invertebrate extracellular phagocyte traps show that chromatin is an ancient defence weapon. *Nat Commun.* 2014; 5:4627. [PubMed: 25115909]
61. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozlowski E, et al. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. *Nat Med.* 2008; 14:949–953. [PubMed: 18690244]
62. Morshed M, Yousefi S, Stockle C, Simon HU, Simon D. Thymic stromal lymphopoietin stimulates the formation of eosinophil extracellular traps. *Allergy.* 2012; 67:1127–1137. [PubMed: 22764833]
63. Remijsen Q, Kuijpers TW, Wirawan E, Lippens S, Vandenabeele P, Vanden Berghe T. Dying for a cause: NETosis, mechanisms behind an antimicrobial cell death modality. *Cell Death Differ.* 2011; 18:581–588. [PubMed: 21293492]
64. Yipp BG, Petri B, Salina D, Jenne CN, Scott BN, Zbytnuik LD, et al. Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. *Nat Med.* 2012; 18:1386–1393. [PubMed: 22922410]
65. Brehmer-Andersson E, Kaaman T, Skog E, Frithz A. The histopathogenesis of the flame figure in Wells' syndrome based on five cases. *Acta Derm Venereol.* 1986; 66:213–219. [PubMed: 2426897]
66. Wouters J, Waelkens E, Vandoninck S, Segaert S, van den Oord JJ. Mass Spectrometry of Flame Figures. *Acta Derm Venereol.* 2015; 95:734–735. [PubMed: 25613159]
67. Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semeraro F, et al. Extracellular histones are major mediators of death in sepsis. *Nat Med.* 2009; 15:1318–1321. [PubMed: 19855397]
68. Saffarzadeh M, Juenemann C, Queisser MA, Lochnit G, Barreto G, Galuska SP, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. *PLoS One.* 2012; 7:e32366. [PubMed: 22389696]
69. Marichal T, Ohata K, Bedoret D, Mesnil C, Sabatel C, Kobiyama K, et al. DNA released from dying host cells mediates aluminum adjuvant activity. *Nat Med.* 2011; 17:996–1002. [PubMed: 21765404]
70. McDonald B, Urrutia R, Yipp BG, Jenne CN, Kubes P. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe.* 2012; 12:324–333. [PubMed: 22980329]
71. Oehmcke S, Morgelin M, Herwald H. Activation of the human contact system on neutrophil extracellular traps. *J Innate Immun.* 2009; 1:225–230. [PubMed: 20375580]
72. Wartha F, Beiter K, Normark S, Henriques-Normark B. Neutrophil extracellular traps: casting the NET over pathogenesis. *Curr Opin Microbiol.* 2007; 10:52–56. [PubMed: 17208512]
73. Doyle RJ. Contribution of the hydrophobic effect to microbial infection. *Microbes Infect.* 2000; 2:391–400. [PubMed: 10817641]
74. Arazna M, Pruchniak MP, Demkow U. Neutrophil extracellular traps in bacterial infections: strategies for escaping from killing. *Resp Physiol Neurobiol.* 2013; 187:74–77.

75. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol.* 2010; 191:677–691. [PubMed: 20974816]
76. Metzler KD, Goosmann C, Lubojemska A, Zychlinsky A, Papayannopoulos V. A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during NETosis. *Cell Rep.* 2014; 8:883–896. [PubMed: 25066128]
77. Muniz VS, Baptista-Dos-Reis R, Neves JS. Functional extracellular eosinophil granules: a bomb caught in a trap. *Int Arch Allergy Immunol.* 2013; 162:276–282. [PubMed: 24136351]
78. Neves JS, Radke AL, Weller PF. Cysteinyl leukotrienes acting via granule membrane-expressed receptors elicit secretion from within cell-free human eosinophil granules. *J Allergy Clin Immunol.* 2010; 125:477–482. [PubMed: 20159258]
79. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. *PLoS Pathog.* 2009; 5:e1000639. [PubMed: 19876394]
80. Peterson CL, Laniel MA. Histones and histone modifications. *Curr Biol.* 2004; 14:R546–R551. [PubMed: 15268870]
81. Papayannopoulos V, Staab D, Zychlinsky A. Neutrophil elastase enhances sputum solubilization in cystic fibrosis patients receiving DNase therapy. *PLoS One.* 2011; 6:e28526. [PubMed: 22174830]
82. Wagener JS, Kupfer O. Dornase alfa (Pulmozyme). *Curr Opin Pulm Med.* 2012; 18:609–614. [PubMed: 22990660]
83. Rosenberg HF, Foster PS. Reply to eosinophil cytolysis and release of cell-free granules. *Nat Rev Immunol.* 2013; 13:902.
84. Persson C, Uller L. Primary lysis of eosinophils as a major mode of activation of eosinophils in human diseased tissues. *Nat Rev Immunol.* 2013; 13:902.

Fig. 1.

CT image of an ECRS patient represented ethmoid-dominant shadow and polyps in bilateral nasal cavity. The characteristic symptoms of ECRS are hyposmia or anosmia, glue-like viscous rhinorrhea, and nasal congestion [20••, 37••]. The fate of eosinophils is pathologically important in chronic sinusitis with tissue eosinophilia. EETosis-derived FEGs are causing long-lasting inflammation but also might have immunoregulatory roles. In

mucosal tissue, extracellular spaces restrict the distribution of sticky DNA traps, although the expanding DNA traps contribute to the toxic eosinophilic mucin in the sinus cavity

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Diagnostic criterion of ECRS

Factor		JESREC score
Disease side: bilateral		3
Nasal polyp		2
CT shadow: ethmoid maxillary		2
Eosinophils of peripheral blood		
2 < 5 %		4
5 < 10 %		8
10 % <		10
Factor		Refractory score
All of two factors are applied		1
•Eosinophils of peripheral blood >5 %		
•CT shadow: ethmoid $\geq$ maxillary		
At least one factor is applied		1
•Comorbid of asthma		
•Aspirin intolerance		
•NSAIDs intolerance		
Final diagnosis	JESREC score	Refractory score
Non-ECRS	10	–
Mild ECRS	11	0
Moderate ECRS	11	1
Severe ECRS	11	2

CT computed tomography, *ECRS* eosinophilic chronic rhinosinusitis, *NSAIDs* non-steroidal anti-inflammatory drugs

Table 2

The feature of ECRS and non-ECRS

	ECRS	Non-ECRS
Susceptible age	Adult	All age
Nasal polyps	Site: middle nasal meatus, olfactory cleft Bilateral and multiple Eosinophilic infiltration	Site: middle nasal meatus Unilateral and monostotic Neutrophilic infiltration
Main symptom	Hyposmia and anosmia	Nasal congestion, rhinorrhea, headache
Rhinorrhea	Glue-like, very viscous	Mucous, purulent
Lesion location	Ethmoid sinus	Maxillary sinus
Treatment	Systemic steroid	Antibiotics
Recurrence after surgery	Often	Rare
Others	Comorbid of asthma Comorbid of aspirin intolerance Eosinophilia	

CT computed tomography, *ECRS* eosinophilic chronic rhinosinusitis, *NSAIDs* non-steroidal anti-inflammatory drugs