Eosinophils Extracellular Traps in Respiratory Diseases

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Abstract

After the discovery that neutrophils are able to form extracellular traps from nuclear and mitochondrial DNA, which pick up microorganisms, releasing the contents of their granules to eliminate these microorganisms, several studies have been conducted with other cells of innate immunity in order to find out if the same process happened. Among the cells that release these extracellular traps are eosinophils, which is the focus of this review. Here it is reported how eosinophils are able to form these traps, compared with the traps formed by neutrophils, which until then are the most studied. This review focuses on the role of this extracellular traps of eosinophils in eosinophilic respiratory diseases, or that secondarily recruit eosinophils. These traps can aggravate the condition of patients, causing chronicity of the disease or may be beneficial, depending on the pathology in question. Eosinophils and neutrophils can act together releasing their traps, aiding in the fight against pathogens. Thus, the role of extracellular traps of eosinophils is a current issue and, therefore, deserves attention, especially in respiratory diseases, since it may be a possible therapeutic target.

Keywords: Eosinophils; Extracellular Tramps; DNA; Granules; Respiratory Diseases

Abbreviations

Atg: Autophagy-Related Gene; BALF: Bronchoalveolar Lavage Fluid; Co1: Cytochrome C Oxidase Subunit I; COPD: Chronic Obstructive Pulmonary Disease; Cyb: Cytochrome B; DPI: Diphenyleneiodonium Chloride; ECP: Esinophil Cationic Protein; EETs: Eosinophil Extracellular Traps; EPO: Eosinophil Peroxidase; ETs: Extracellular Traps; ETosis: Formation of Eosinophils Extracellular Traps; IFN: Interferon; IL: Interleukin; LPS: Lipopolysaccharide; ND1: NADH Dehydrogenase Subunit 1; NETosis: Formation of Neutrophils Extracellular Traps; NETs: Neutrophils Extracellular Traps; PI3K: Phosphoinositide 3-Kinase; PMA: Phorbol Myristate Acetate; ROS: Reactive Oxygen Species; TLSP: Thymic Stromal Lymphopoietin

Introduction

In 2004, Brinkmann., *et al.* demonstrated a new mechanism of neutrophil action, the release of their DNA and histones to the extracellular medium, with the goal of forming networks, which act as traps for microorganisms. NETs (neutrophils extracellular traps), as they were called, also release the contents of their granules, to degrade the microorganisms [1]. The released DNA can be nuclear, in an irreversible process, culminating in the death of the neutrophil, in a different process of necrosis and apoptosis, or the DNA can be mitochondrial, in an independent death process [1-4]. There are many studies involving these processes and today it is already well understood the mechanisms that lead to the activation of the formation of NETs, which is called NETosis, and when it is formed, that is, when the neutrophil cannot kill the microorganism by phagocytosis or simple degranulation, as in the case of very large microorganisms [5].

After this process was seen in neutrophils was investigated whether it occurred in other cells of innate immunity. The formation of extracellular traps (ETs) was also seen in macrophages, basophils and eosinophils [6-9]. The events leading to activation of ETs in these other cell types are not well elucidated, nor the factors that lead to their formation.

In this review are approached the mechanisms of activation of EETs (eosinophils extracellular traps) and their mechanisms of action, which are distinct from the NETs, with an emphasis on pulmonary diseases such as asthma [10,11], rhinosinusite [12] and chronic obstructive pulmonary disease (COPD) [13], since most of these diseases are eosinophilic and in addition to sepsis, which indirectly causes an increase of eosinophils in the lung parenchyma [14].

The ETTs can act many times with NETs, so the joint involvement of neutrophils and eosinophils is also reported. It is also questioned whether EETs have a beneficial or harmful role in various respiratory diseases, demonstrating that they behave in different ways according to the pathology or microorganism that they are facing.

Historic

The first report that, like neutrophils, eosinophils release nuclear DNA traps, overcoming the nuclear and plasma membrane, in response to stimuli such as IgG, IgA, cytokines with platelet activating factor, calcium ionophores and phorbol myristate acetate (PMA), was in 2013 [15]. These extracellular traps also act as traps and postulated that the formation of eosinophils extracellular traps (EETosis) could occur in several eosinophilic pathologies, such as rhinosinusitis [12]. They evaluated sinus tissue of patients with allergic sinusitis and skin of patients with the eosinophilic syndrome. They observed that for the formation of the extracellular tramps there was the death of eosinophils, which was different from apoptosis and necrosis, in addition to condensed chromatin compaction. EETosis occurred progressively from 30 to 120 minutes and was ROS dependent, being inhibited by diphenyleneiodonium chloride (DPI) which is an irreversible inhibitor of NADH/NADPH [12]. However, an earlier study demonstrated that mitochondrial eosinophil DNA can form EETs very rapidly in response to bacteria, lipopolysaccharide (LPS), C5a and eotaxin, without eosinophil death, which can be confirmed with the presence of genes like Co1 (cytochrome c oxidase subunit 1), ND1 (NADH dehydrogenase subunit 1) and Cyb (cytochrome b) [8]. In this paper they also found that there are other stimuli for the release of TSEs such as interleukin (IL) -5, interferon (IFN)-γ, calcium ionophores, thymic stromal lymphopoietin (TLSP), PMA and other factors dependent on the generation of reactive oxygen species (ROS) that were also reported later [8,12,16,17].

Mechanisms of EETs formation

When EETosis depends on death, i.e. traps are nuclear DNA, death occurs rapidly and is mediated by ROS through NADPH oxidase. The nucleus loses the bi-lobular form, there is disintegration of the nuclear envelope, the chromatin reaches the cytoplasm and breaks the membrane together with the DNA network, with the release of the granules [12]. However, in addition to what most authors report, EETs can be produced independently of ROS, the blocking of EETs is possible via tyrosine kinase or CD11b [14].

A molecular mechanism seen for the beginning of the nuclear NETosis is that migration of chromatin is accompanied by the migration of elastase and myeloperoxidase of the granules to partially degrading the histones [17,18]. There are no studies analyzing the mechanisms of eosinophil nuclear DNA release, however, there is a divergence regarding the type of histone that is responsible for chromatin decondensation. According to Ueki., *et al.* eosinophil DNA traps is originated from H1 histones that form 30 nm chromatin fibers, much thicker than neutrophils [12], but according to Muniz., *et al.* the eosinophils extracellular traps come from histones H3 [14].

The interaction between the anionic surface of the microorganisms and the cationic components of ETs has been assumed [19]. EETs form hydrophobic bonds that aid the adhesion of microorganisms and thus function as traps, as well as NETs [12]. Unlike NETosis, eosinophils do not release the contents of their granules along with DNA traps, they remain intact around the traps [20,21], causing the increased viscosity of the secretions where these eosinophils are located [23]. Eosinophil granules express binding sites for cytokines, chemokines and eicosanoid receptors on their surface [24,25]. The stimulated granules activate cell signaling mechanisms that stimulate the secretion

of cationic proteins such as eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), IL-6, complement factor, chemokines and cytokines [14,24,26,27]. These granules, once trapped in the extracellular networks, generate chronic inflammation [23]. Interestingly, the presence of neutrophils in the area of EETs, there is a decrease in viscosity, as can be seen in otitis media of bacterial origin [23].

In NETs autophagy is a feature of great relevance [28,29]. Autophagy was also evaluated in the formation of EETs. Germic., *et al.* used for the first-time mice with neutrophils and eosinophils deficient of autophagy-related (Atg) 5, gene essential for the formation of the autophagosome. No Atg5-deficient mice exhibited abnormalities in EETS formation after physiological activation or by PMA. Human and mouse neutrophils and eosinophils were also treated with inhibitors of phosphoinositide 3-kinase (PI3K), blocked ROS and consequently EETs. Autophagy inhibitors such as bafilomycin A1 and chloroquine had no effect, indicating that EETs work differently from NETs [30].

Few studies have pointed out the percentage of eosinophils that undergo EEtosis, and even if they report, the amount of eosinophils that release extracellular networks may vary according to the pathology, its severity and the presence of other cells that release their traps, such as neutrophils, macrophages and basophils, for example. In a study that evaluated biopsy samples from patients with eosinophilic esophagitis, it was reported that 80% of eosinophils had evidence of cytolysis, with extravasation of DNA [31].

EETs in respiratory diseases

Most of the respiratory disorders there is the recruitment of eosinophils and thus, most of the studies on EETs involves respiratory diseases. In respiratory allergies, it has been reported that few eosinophils undergo necrosis or apoptosis [32,33]. Free eosinophilic granules have been seen after cytolysis of eosinophils in various respiratory disorders such as asthma, rhinitis, and rhinosinusitis [15,26,32,35,36].

In a murine model of ovalbumin-induced allergic asthma, ETTs production in bronchoalveolar lavage fluid (BALF) was observed both *in vivo* and in vitro [10].

EETs are formed in the airways of patients with allergic asthma [16,22]. In addition to the presence of EETs, the presence of NETs in airway biopsies can be observed, and in this case, the DNA released was mitochondrial and did not generate eosinophil or neutrophil death [16]. The role of EETs is not clear in allergic asthma, but there are some hypotheses. EETs in asthma could damage the pulmonary parenchyma [16] and is in accordance with what has been reported in another study, where increased IL-5, IFN-γ and eotaxin was seen in BALF of asthmatic patients [22]. EETs also release cytotoxic cationic proteins, which may exacerbate airway injury and tissue remodeling [22], but EETs may have protective function, preventing the distribution of cytotoxic proteins by lung tissue, and may be useful in protecting the airway of microorganisms [16,22]. As there were NETs present in asthmatic patient samples, the performance may have been beneficial, since neutrophils decrease the viscosity of secretions and there may be a lower deposition of mucus in the airways [16,23]. It is necessary that the role of TSEs be further investigated in allergic asthma.

Eosinophil traps act as adhesives, which capture the granules of intact eosinophils and microorganisms, but the effects of this arrangement can persist because there is no proteolytic activity in EETs [12].

The presence of EETs was also seen in bronchial mucus in patients with eosinophilic allergic bronchopulmonary aspergillosis. *Aspergillus fumigatus* induces the release of EETs in vitro but has no fungicidal or fungistatic function [14]. Probably the TSEs promote the capture of *A. fumigatus* so that other inflammatory cells or mediators take action.

Ueki., *et al.* used eosinophil-rich secretions from patients with eosinophilic otitis media and chronic eosinophilic rhinosinusitis and found that eosinophilic traps were clustered, eosinophils were elongated with cytolytic release of granules and presence of much cellular debris. ETTosis was ROS-dependent and signaling was via calcium. *In vivo* and *in vitro* studies have pointed out that the DNA released was nuclear rather than mitochondrial. They also reported that these traps support eosinophilic secretions and increase their viscosity, which contributes to the persistence/chronicity of the disease. The authors also suggest that EETs could be a possible therapeutic target [12].

In the sepsis model, the eosinophil has a protective effect associated with ETTosis [14]. The phagocytic capacity of eosinophils is much smaller than that of other cells that specialize in phagocytosis [37], but it was reported that eosinophils have the ability to enhance the elimination of *Pseudomonas aureginosa* [38], since in the sepsis model there is DNA infiltration and deposition of extracellular DNA in the presence of microorganisms [8].

Eosinophils in co-culture with *S. aureus* release ETTs that are efficient in capturing and killing bacteria, but when eosinophils were co-cultured with *S. epidermalis*, it was necessary to add TSLP, demonstrating that eosinophils act differently according to the type of microorganism they are facing [8,39].

Periostin, which facilitates the infiltration of eosinophils into the lung tissue of allergic patients and with eosinophilic esophagitis [11] was found at high levels in tissues of patients with high levels of EETS production, but periostin was not found when eosinophils were co-cultured with *S. aureus* [40], which was expected, since there is no need for periostin when there is culture in vitro, as there is no tissue infiltration in this case.

COPD is mainly due to the presence of neutrophils, and the neutrophil elastase contributes to the pulmonary lesions observed in the disease [41]. In addition, it was reported that the presence of eosinophils has no clinical relevance [34], but it is now known that eosinophils are the main cause of chronic cough in middle-aged COPD patients associated with airway hyperresponsiveness or airflow obstruction [13]. It was investigated and found that the formation of EETs and NETs occurred in the sputum of healthy patients who stopped smoking for at least six months and patients with COPD. It has been observed that even in patients who have stopped smoking for at least six months, there is EETosis that triggers NETosis. Symptomatic patients with low risk of disease exacerbation produce EETs and patients at high risk of exacerbation produce EETs and NETs. They also observed that EETosis was associated with cell injury and it was seen that the extracellular traps remain even in patients who do not smoke more but maintains the COPD frame. The cell debris of eosinophils that have undergone EETosis and the chromatin from this process accumulates in the airways and causes recruitment of neutrophils and the NETs can form from this process [13].

Conclusion

Like neutrophils, eosinophils can release their DNA traps, which may also be of nuclear or mitochondrial origin, in a process that depends on or not on death, respectively. Unlike neutrophils, eosinophils do not release the contents of their granules into their traps, but they trap microorganisms. These granules increase the viscosity of airway secretions that can contribute to the chronicity of lung diseases, where eosinophils are recruited, with EETs being a possible therapeutic target. However, neutrophils can be recruited after ETTosis to eliminate the microorganism and cell debris from eosinophils through the formation of NETs. The NETs, in turn, decreases the viscosity of secretions, improving the patient's condition with chronic lung disease. Some authors further propose that EETs can have beneficial effects by limiting injury to the lung parenchyma, as in the case of sepsis. More studies should be conducted in the most diverse lung diseases, in order to know if the EETs are beneficial or not in each one of the pathologies studied and what should be the clinical management that installs after the formation of the ETs.

Conflict of Interest

The author declares no financial or commercial conflict of interest.

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