A gap in cell death knowledge: is necroptosis of eosinophils involved in allergic airway inflammation?

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Abstract

Introduction

Eosinophils are continually targeted in allergic airway inflammatory disease therapies. The presence of their granules in airway tissues remains unexplained, yet implies the involvement of necrosis. The latter’s definition has evolved into a highly regulated and distinct signalling pathway leading to physiological inflammation, known as necroptosis. Even though necroptosis is a recently introduced concept, we currently recognize its role as an alternative mechanism in the absence of apoptosis. Furthermore, necroptosis seems to act as a host-defence strategy against viral infections, which consequently associates itself with eosinophils’ role in airway viral clearance. The aim of this review is to discuss if necroptosis of eosinophils is involved in allergic airway inflammation.

Conclusion

The investigation of necroptosis in eosinophils is currently an area which lacks research, yet harbours great promise for drug development.

Introduction

Allergic airway inflammatory diseases remain prevalent in many populations today. Their management remains a burden on health care systems; moreover, the diseases affect the patients’ quality of life.

Although therapies triggering eosinophils, in particular corticosteroids, have greatly improved allergic and asthmatic symptoms in airways, disease management is not optimal in every case. Human studies within this field continue to unveil unexplained phenomena involving eosinophils in persisting inflammation. With recent studies suggesting that the regulation of cell death pathways is implicated in the progression of allergic inflammation, one may wonder if necrosis may be the missing link in therapeutic approaches. Physiological necrosis was first observed over a decade ago, yet we recently started to understand its regulated mechanisms within the immune system as well as its pathological relevance. The purpose of this review is to bridge our knowledge of eosinophil biology in allergic airway disease with cutting-edge discoveries in programmed cell death (PCD) pathways, in order to incite research towards better alternatives for treating eosinophilic airway inflammation. Even though concepts discussed in this review may be applicable to other eosinophilic pathologies, this paper will be limited to the area of eosinophilic allergic airway inflammation.

Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

The ever-changing concept of ‘necrosis’

Necrosis is largely characterized by morphological traits, such as an increase in cell volume, swelling of organelles, among loss of plasma membrane integrity, among other traits. The term ‘necrosis’ was classically understood as an unregulated form of cell death that induces inflammation. Stemming from this perspective, necrosis is commonly known as the opposite of apoptosis, the classical PCD pathway which promotes the resolution of inflammation. Nowadays, the concept of necrosis has adopted some complexity as well as a new name, necroptosis, as to indicate its regulated nature. Based on current scientific literature, necrosis may be subdivided into three distinct definitions: cytolysis, secondary necrosis and necroptosis. Cytolysis is a general term used to describe cells which have died through lysis, thus expelling their intracellular contents due to non-specific external factors which have compromised the integrity of the plasma membrane. Similarly, secondary necrosis also fits the latter description, yet is said to occur within a cell that has first been in the apoptotic state. In contrast, necroptosis is a newer concept which describes a highly regulated death signalling pathway specific for this pro-inflammatory process. It is now known that necroptosis can be initiated, similarly to apoptosis, by ‘extrinsic stimulus’, such as tumour necrosis factor (TNF) and Fas ligand (FasL), or ‘intrinsic stimulus’, such as DNA damage, depending on the cell’s state. Pertaining to host-defence, it has also been shown that necroptosis can be specifically triggered through recognition of pathogen-associated molecular patterns (PAMPs) by toll-like receptors (TLRs) and sensors of DNA as well as RNA. Considering its involvement in inflammation, necroptosis is beginning to be studied...
in some immune cells, such as T cells\textsuperscript{17}. Even though eosinophils have been well established as effector cells in allergic inflammatory reactions, necroptosis remains a mystery within this cell type.

**Eosinophils in allergic airway inflammation: revealing hints of necroptosis**

Over the years, eosinophils have been shown to play a variety of roles ranging from homeostasis, pathogen clearance and pathology. Its pro-inflammatory abilities are largely related to its granules and granule contents. In addition to a plethora of immunological and physiological molecules (i.e. chemokines, cytokines and growth factors), eosinophil granules contain cationic proteins, such as major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPX)\textsuperscript{13–16}. Combined with traditional intracellular adjuvants, also known as damage-associated molecular patterns (DAMPs) (i.e. HMGB1, heat shock proteins, DNA and RNA), these cytotoxic molecules render eosinophils extremely potent inducers of inflammation in the event of their cytolysis. In fact, cytolysis is now recognized as a separate mode of degranulation in eosinophils\textsuperscript{17–19}.

Apart from the basic biology of eosinophils, their abnormal recruitment and infiltration into lung tissues, which lead to airway inflammation and tissue damage, have become hallmarksof asthma. Although the exact mechanism is not yet fully described, various researchers previously proposed that the increased total amount of eosinophils observed in asthmatics caused improper airway eosinophilia. More recently, studies in this field have shown that delayed-apoptosis or insufficient apoptosis of eosinophils is an important factor in the pathology of asthma\textsuperscript{20–22}. As previously mentioned, immune cells involved in allergic inflammation tend to lack proper cell death regulation.

Moreover, studies on airway tissues, even as old as the study by Parrot and Leyden in the 1800s, report the presence of free extracellular eosinophil granules (Cfegs). Cfegs can equally be found in human whole-mount airway preparations\textsuperscript{17,23–25}. Although the study of Cfegs in wild-type mouse models have not been reported due to lack of degranulation and cytolysis, eosinophil cytolysis has been observed in rats, dogs, primates and guinea pigs. In fact, cytolysis of mucosal tissue eosinophils was induced within 1 h of an allergen challenge in guinea pigs. These findings suggest a necrotic type of eosinophil cell death rather than apoptotic, where intracellular components would remain in apoptotic bodies for proper clearance\textsuperscript{26}.

In support of this, a recently developed IL-5/human eosinax-2 double transgenic mouse model demonstrates eosinophil-dependent inflammation as well as granule deposition\textsuperscript{27}. It has also been reported that ‘asthma-like’ insults, such as airway inflammation, induces rapid death of eosinophils, which causes granule deposition in these tissues\textsuperscript{28}. Likewise, eosinophil cytolysis has been often observed and imaged in various tissues subdued to eosinophilic inflammation\textsuperscript{17,24}. Interestingly, apoptosis has been viewed as the primary mode of demise for eosinophils\textsuperscript{13,28–32}, although classic studies, such as work done by Kodama and co-workers, fail to demonstrate apoptotic eosinophils in these tissues\textsuperscript{33}. In addition, the ambiguous outcomes in asthma symptom management from various anti-interleukin-5 (anti-IL-5) therapies, which specifically target the eosinophil apoptotic pathway, could be interpreted as supporting that an alternative PCD pathway is playing a role in airway inflammatory pathologies\textsuperscript{29,35}.

Overall, eosinophils are distinct immune cells that are highly susceptible to dying in the absence of pro-survival cytokines\textsuperscript{13,14,16,29,32,36,37}, which validates that death signalling pathways are at the heart of their biology and, consequently, our understanding of eosinophilic pathologies. Therefore, deepening our understanding of PCD pathways, primarily necroptosis in relation to apoptosis, is necessary in improving eosinophil-targeting therapies.

**Cross-talk between apoptosis and necroptosis**

While the subject of eosinophil cytolysis and necrosis has been largely pushed aside within scientific literature\textsuperscript{17}, eosinophil apoptosis has received major attention. It is currently accepted that apoptosis is an important component for the resolution of inflammation. Thus, most popular drugs targeting eosinophil seek to increase the initiation of apoptosis (e.g. corticosteroids and anti-IL-5 therapies)\textsuperscript{17,28,38,39}. In fact, most of our knowledge of eosinophil apoptosis stems from studies on the role of IL-5 in eosinophilpoiesis and eosinophil survival\textsuperscript{12,35,40}. Extensive knowledge of eosinophil apoptosis can indeed help us understand eosinophil necroptosis. We now know that apoptosis and necroptosis share many components for signalling complexes in which receptor interacting serine/threonine-protein kinase 3 (RIPK3) recruitment and activation causes it to complex with RIPK1, which specifically determines the cell’s fate\textsuperscript{41}. In fact, it has been shown in pancreatic tissues that a switch from apoptosis to necroptosis can occur through induction of RIPK3\textsuperscript{42}. Also, overexpression of RIPK3 explicitly induces necroptosis\textsuperscript{43}. Interestingly, the inhibition of caspase-8 (casp8), a molecule necessary for the extrinsic apoptotic pathway, results in increased expression of RIPK3\textsuperscript{44}. This finding may be suggestive of a cause–effect relationship between delayed apoptosis and increased necroptosis, which would...
explain the presence of improper apoptosis in airway inflammatory pathologies. Also, there is increasing evidence that both PCD pathways may inhibit each other.6,41

In general, our familiarity with apoptosis–necroptosis cross-talk remains basic, yet expansion in this field of research is very promising. So far, we understand that necroptosis may serve as a backup PCD pathway in the event of caspase-dependent apoptosis inhibition.4

The role of necroptosis within the immune system

The role of necroptosis as an immunological process is currently gaining more interest. Although there is still much to learn about necroptosis, examining its inflammatory function during immune responses as a whole can help us clarify its mechanisms in relation to pathology. Even if necroptosis is a pro-inflammatory event, there is increasing evidence that such a cytolytic death may be a natural process for immune cells.4 When various mouse tissues were screened for the expression of necroptosis-related genes using zVAD.fmk, a necroptosis inducer and non-specific caspase inhibitor, clusters showing increased expression were found particularly in immune as well as neuronal cells. More specifically, out of 119 mouse tissue samples, 83% were primary immune cells. Furthermore, necrostatin-1 (Nec-1), an inhibitor of necroptosis, was able to block zVAD.fmk-induced death as well as spontaneous death in macrophages.8 Although the effects of zVAD.fmk and Nec-1 were not directly studied using eosinophils, one may extrapolate that eosinophils be intrinsically more prone to necroptosis as well.

The marked presence of necroptosis in immune cells may be explained through immune cell activation. More specifically, necroptosis is implicated in certain immune cellular processes, such as mitochondrial and lysosomal alterations leading to production of reactive oxygen species (ROS), nitric oxide (NO) and other similar compounds.6,41 In fact, Hitomi and colleagues were able to link RIPK1-dependant necroptosis to increased ROS production by which they suggest that necroptosis may be driven by ROS production in T cells.8 Overall, the production of these compounds in immune cells might explain why necroptosis may be more prominent in these cell types.

It is presently well known that allergic responses are inappropriate events which consist of immune cells mimicking a host-defence strategy. Because necroptosis is pro-inflammatory in nature, one may wonder the primary function of having such a regulated mechanism. Therefore, the function of necroptosis may be explained through pathogen clearance. There is a marked association between viral infections, such as vaccinia virus, herpes virus and cowpox, and ‘necrosis-like’ cell death.4,45,46 Also, necroptosis seems to be intrinsically initiated by viruses that block apoptosis through virally encoded caspase inhibitors.4 Specifically, the inhibition of casp8, which results in the apoptotic pathway in host cells,47,48 In this way, necroptosis may be an evolutionary back-up signalling pathway to clear pathogens in the absence of apoptosis. More convincingly, cytomegalovirus (CMV) actually possesses a gene which encodes a necroptosis inhibitor.49 It seems this virus has already evolved a mechanism in order to counter the necrototic host-defence strategy. Such a virulence factor is suggestive that necroptosis is an important pathway for immune defence and should be studied more closely. The latter explanation may pertain to allergic airway disease since eosinophils are known to promote viral clearance in the lung through ligand binding of various TLRs.50,51 Indeed, certain viruses, such as respiratory syncytial virus (RSV), have been shown to induce eosinophil degranulation.52 As previously alluded, viral-specific pathogen-recognition receptors (PRRs) may directly trigger necroptosis. Furthermore, it has been well established that respiratory viruses, including RSV, are associated with asthma exacerbations.53,54 Taking into account both facts as well as the knowledge of viruses with anti-apoptotic abilities, one may assume that RSV could trigger eosinophil recruitment into lungs as well as cause the switch from apoptosis to necroptosis, in order to trigger potent inflammation as a mechanism of viral clearance. Such an explanation would be in line with worsening of asthma symptoms during respiratory viral infections, such as asthma exacerbations.

If major findings in the necrotic signalling pathway were to be merged with our knowledge of eosinophil functions related to allergic airway inflammatory diseases, a conceptual mechanism can take shape, as depicted in Figure 1. Theoretically, eosinophils which are recruited into airway tissues may be triggered by viral particles directly through PRRs, or indirectly by cytokines, such as through the TNFα receptor, or interactions with other immune cells, through the Fas receptor, in order to induce necroptosis. The activation of eosinophils causes ROS production, which may further induce necroptosis. It may also be possible that direct viral infection of eosinophils causes a state of anti-apoptosis through the inhibition of casp8, which results in an upregulation of RIPK3. The latter would form a complex with other death signalling proteins, including RIPK1, rendering it a necrototic signalling complex. In the context of allergy, an allergen may initiate a very similar cascade of events. Finally, the induction of necroptosis would result in plasma membrane disruption...
causing intracellular components to leak out. Some researchers have previously suggested that eosinophil cellular debris might be expelled from the lung tissue through luminal entry, where eosinophils may be further expelled from the body by mucociliary movement and coughing. Clegs would be deposited within the lung tissue, which could extend the inflammation. In order to confirm this regulated pro-inflammatory mechanism, various supporting evidence is needed. Although the presence of Clegs in airway tissues is a well-established phenomenon, there is no direct evidence that necroptosis of eosinophils is a factor in allergic airway inflammation, even though current knowledge strongly implies that necroptosis may be the missing link in understanding how eosinophils potently promote inflammation during allergic responses. More than 25 years ago, Fukuda and team had noted that eosinophils may be programmed to lyse upon stimulation under specific conditions. Although their research efforts alluded to necroptosis, it cannot be denied that this question was asked prior to its time. It is very reasonable to postulate that necroptosis is gravely influencing the pro-inflammatory capabilities of eosinophils through controlling the release of their intracellular cytotoxic elements as an independent degranulation mode.

**Conclusion**

Presently, the topic of eosinophil PCD pathways is a field with many unanswered questions although it is a very promising area for drug development. More specifically, there is no direct evidence that necroptosis of eosinophils is a factor in allergic airway inflammation, even though current knowledge strongly implies that necroptosis may be the missing link in understanding how eosinophils potently promote inflammation during allergic responses. More than 25 years ago, Fukuda and team had noted that eosinophils may be programmed to lyse upon stimulation under specific conditions. Although their research efforts alluded to necroptosis, it cannot be denied that this question was asked prior to its time. It is very reasonable to postulate that necroptosis is gravely influencing the pro-inflammatory capabilities of eosinophils through controlling the release of their intracellular cytotoxic elements as an independent degranulation mode.

**Abbreviations list**

- DAMPs, damage-associated molecular patterns
- ECP, eosinophil cationic protein
- EDN, eosinophil-derived neurotoxin
- EPX, eosinophil peroxidase
- FasL, Fas ligand
- MBP, major basic protein
- PAMPs, pathogen-associated molecular patterns
- PCD, programmed cell death
- PRR, pathogen-recognition receptor
- ROS, reactive oxygen species
- RSV, respiratory syncytial virus
- TNF, tumour necrosis factor

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**References**


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