

REVIEW

Eosinophil degranulation: an evolutionary vestige or a universally destructive effector function?

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Many of the prevailing hypotheses regarding eosinophil effector functions trace their origins back to the 1879 observations of Paul Ehrlich who described the rich magenta eosin-staining granules within what became known as eosinophils [1]. Specifically, the intervening 100+ years bear witness to the remarkable adherence to a fundamental paradigm that has since arisen and is inescapably still taught in medical schools and reviewed now and again in the literature [2]: *Eosinophils are granulocytes and therefore their dominant effector functions are mediated by granules*. Indeed, the hyperbole surrounding this issue is astonishing as many texts and discussions often lead observers to believe that the release of the contents of these granules by degranulation is the only effector function of significance. Given this hyperbole, a logical place to begin a discussion of the importance of degranulation is to develop a definition of eosinophil effector functions inclusive of all potential activities mediated by this leucocyte. The definition we are proposing here is not a moment of 'Einsteinian' thought, but arises from observations reported in the literature and the many conversations we have had with innumerable colleagues: Our proposal is that eosinophil effector functions constitute *any* eosinophil-mediated activity that modulates the entropy of disease or is required for physiological homeostasis. Interestingly, modulations related to disease pathogenesis are most closely associated with the historical view of eosinophil degranulation that prevails today. That is, degranulating eosinophils contribute to the inflammatory responses and tissue damage resulting from helminth infections, solid tumour growth, fungal infections, and the immunohistopathologic events associated with allergic diseases such as asthma or atopic dermatitis. In each case, the assumption is that eosinophils execute effector functions primarily through the release of toxic cationic secondary granule proteins. We believe that the acceptance of this description as the singularly significant function of degranulation underestimates its importance as well as the value of other activities mediated by eosinophils. In particular, the recent recognition that eosinophils are likely to be regulatory cells contributing in significant ways to immune responses, tissue remodelling, and/or homeostasis suggests that the potentially toxic activities of the granule proteins may represent only a small fraction of their overall impact, and in that, a distortion of their normal function [3].

The goal of this review is to examine the importance of eosinophil degranulation from a 'thinking outside of the box' perspective in an attempt to shed new light on an old problem. In particular, we will pose and answer the questions 'What is the evolutionary origin of eosinophils and degranulation and why is degranulation viewed as the ultimate eosinophil effector function?' More importantly, 'Does the available data support an alternative to the prevailing hypothesis that degranulation is a destructive effector function?' We do not expect to adequately answer these questions and many of our answers may eventually be shown to be incorrect. However, as noted by the 19th century naturalist Charles Darwin: 'False views, if supported by some evidence, do little harm, for everyone takes salutary pleasure in proving their falseness!'

Question #1: What is the evolutionary origin of eosinophil degranulation?

In many ways, an eosinophil-like cell represents an evolutionarily ideal venue by which mammals (indeed all vertebrates) could mediate responses to potential pathogens. In extant species, this cell is secretory, primarily tissue dwelling, and often associated with areas of the body that interface the environment (e.g. the submucosal regions of the gastrointestinal tract [3]). In addition, eosinophils are able to leave circulation and quickly traverse peripheral tissues [4]. Moreover, their priming and activation does not necessarily require prior exposure to a pathogen [5], and eosinophils entering circulation/tissues are 'pre-loaded' with granule components to be released into the extracellular milieu or directly onto potential pathogens [6]. The release of these pre-formed granules and/or granule contents further implies a rapid and effective means of localized defence. That is, these cells have a defined repertoire of effector functions to be utilized under circumstances dictated by environmental cues without the necessity of additional cellular differentiation events or *de novo* gene expression. Furthermore, the appearance of an eosinophil-like amoeboid granulocyte in invertebrate species without lymphocyte functions is consistent with its evolution as part of an early innate host response system [7]. However, degranulation also appears to be an effector function with some species specificity. Some characteristics, including the presence of select secondary granule proteins and the genes that encode them, display a degree of evolutionary conservation among different species, but many others do not. In some

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animals, such as humans, rapid and extensive degranulation may have a significance not present in other species (e.g. mice). Alternatively, species differences may suggest that the mode by which this effector function is executed is of limited significance: If eosinophil degranulation is universally important, granule-specific proteins (ESGPs) and the process of degranulation itself would have been conserved virtually intact among all animals.

In spite of the belief that eosinophil degranulation evolved as a cytotoxic host defence mechanism, several lines of evidence suggest that its origins do not reflect the importance and selective advantage this activity(ies) may have.

(i) *The potential cytotoxic activity mediated by eosinophil degranulation during acquired immune responses is currently limited to a finite set of pathogens and is likely to have evolved prior to the appearance of these pathogens.* Specifically, only in a limited number of circumstances (e.g. responses to helminth parasites [8] or some fungal pathogens [9]) does degranulation appear to be a dominant effector function. A more expansive hypothesis suggesting that degranulation is a widely used and singularly important effector function whose ability to kill conveys an evolutionarily selective advantage to the host is problematic. As noted above, the deployment of this defence is limited to a very small subset of encountered pathogens and the evolution of eosinophil degranulation appears to have occurred prior to the appearance of acquired immune responses [10]. Furthermore, host organisms with eosinophils would have been present prior to the evolution of helminth parasites (i.e. free living forms of helminths evolutionarily adopted a parasitic lifestyle because of the availability of suitable host organisms). As a consequence, these granulocytes would have had to exist prior to the emergence of these parasites, and thus, it is illogical to suggest that degranulation evolved as a host defence against a threat that did not yet present itself. Indeed, the ambiguity of studies in mice attempting to link definitively eosinophils with host defence against helminth infection may be a consequence of the lack of a relationship between degranulation and parasitocidal activities [8]. The effects of a degranulation-dominated defence strategy are also spatially restricted – the cytotoxic activities mediated by the release of toxic granule proteins would extend to the eosinophil itself. This mechanism would in effect make the eosinophil a dispensable single purpose 'throw away' cell with narrow defensive capabilities against a very small number of pathogens. The energy costs to maintain this leucocyte population would be high and metazoans constantly encounter more ubiquitous and potentially lethal pathogens (e.g. bacteria). Therefore, the dedication of even a small percentage of an organism's hematopoietic capability to generate cells continuously whose dominant activity would limit them to a one time use against a small number of pathogens seems counter-intuitive.

(ii) *Despite the conservation of sequence identity among some of the genes encoding the ESGPs of various species, a remarkable degree of divergence has occurred, suggesting the evolution of species-specific effector functions.* The eosinophil secondary granules of the mammals examined to date all contain members of the vertebrate ribonuclease A (*RNase A*) subfamily [11–14]. However, the number of encoded ribonucleases, the sequence identity of these proteins, and their ribonucleolytic activities are extraordinarily species specific.

In humans and old world primates, two ribonuclease family members are stored in the eosinophil secondary granule, eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) [11, 15], comprising approximately one-third the total mass of stored granule protein [16]. Among New World primates, the gene encoding ECP has been lost and only an EDN homologous protein is stored in the eosinophil granule [17]. The composition of the ribonucleases is even more complex in rodents such as the mouse where the eosinophil secondary granule contains six ribonucleases, representing more than 50% of the ESGPs (*our unpublished observations*). Surprisingly, these species differences are compounded by an extreme variability in the physical character and ribonucleolytic activities displayed by these proteins. The primate ECPs are highly cationic ($pI > 12$) proteins that display significant toxicity to cells but possess very weak ribonucleolytic activities [18]. The primate EDN homologous proteins on the other hand are not nearly as cationic ($pI \sim 9$) and/or toxic but have ribonuclease activities that rival pancreatic ribonuclease (i.e. more than 1000-fold higher than ECP). In comparison, all of the six mouse eosinophil granule ribonucleases are similar to EDN and are only slightly cationic ($pI \sim 9$) with robust ribonuclease activities. Furthermore, the physical characteristics of the eosinophil major basic proteins (MBPs) stored in the granules of different species also appear to vary greatly, including pI differences of several orders of magnitude [19, 20]. In summary, the variability in both the composition and character of ESGPs from different groups of animals suggests that eosinophil degranulation has evolved species-specific functions as opposed to a ubiquitously conserved method of killing a limited number of pathogens (e.g. helminth parasites).

(iii) *Significant species differences appear to determine the unique circumstances during which eosinophil degranulation occurs.* Given the hypothesized use of degranulation as a dominant effector function mediating pathogen killing, a likely assumption would be that its occurrence is conserved between species mounting similar inflammatory responses. However, examination of data from human patients and animal models of disease shows that degranulation events vary dramatically between species. The contrast between the mammalian orders Primata and Rodentia is both well characterized and particularly dramatic. Studies of asthma patients have shown that extensive eosinophil degranulation accompanies the accumulation of these cells in the lung [21–23]. In addition, studies of other eosinophil-associated diseases (e.g. helminth [24] or fungal [25] infections) similarly show that degranulation is a prominent and common phenomenon in humans (e.g. immunodetection of ECP is often used as a diagnostic indicator in a variety of clinical settings). Evidence of degranulation also extends to *in vitro* studies in which it has been shown that human eosinophils degranulate in response to many specific and non-specific stimuli [5]. All of these observations stand in contrast to what is observed in mice. For example, extensive degranulation of mouse eosinophils is a rarely reported phenomenon [26] and is not observed in established asthma model systems [27–31] or with *in vitro* assays using isolated eosinophils [31]. Indeed, the overwhelming consensus from the literature is that mouse eosinophils display, at best, only nominal evidence of granule protein release under a surprisingly wide variety of circum-

stances, suggesting that degranulation is not a dominant effector function in these animals. Consistent with this conclusion is the lack of observed effects on the allergen-induced responses displayed by knockout mice deficient in the abundant eosinophil granule proteins MBP-1 [27] or eosinophil peroxidase (EPO) [28]. However, it is noteworthy that although nominal in character, some level of eosinophil degranulation does occur in most mouse models of disease. In the case of pulmonary models, this low level of degranulation is limited to eosinophils recruited to the airway lumen [32]. Moreover, evidence of significant degranulation in the mouse only correlates with responses to some parasite [26, 33] and fungal (H. Kita, personal communication) pathogens, implying that considerable variability exists and that unique circumstances are needed to elicit this phenomenon in the mouse.

(iv) Finally, the focus on the destructive effects mediated by ESGPs ignores the growing body of literature showing the ability of eosinophils to modulate immune responses. The abilities of eosinophils to modulate inflammation within their resident tissue microenvironments through activities unrelated to the release of toxic cationic granule proteins have led to hypotheses suggesting that these granulocytes are potent regulators of local immune responses. Collectively, data suggesting that eosinophil activities are potentially immunoregulative fall into two broad areas:

Eosinophil-dependent cytokine production

Several studies have noted that eosinophils are capable of both synthesizing and storing within the secondary granule cytokines traditionally characterized as either T helper type 1 (Th1), T helper type 2 (Th2), or acute-phase responses [34, 35]. In particular, eosinophils appear to be potentially important sources of the Th2 modulating cytokines IL-4 and IL-13. For example, *in situ* hybridization studies have shown that eosinophils infiltrating the lung following allergen challenge express the Th2-phenotype-modulating transcription factor GATA-3 [36]. The ability of eosinophils to modulate local Th2 cytokine levels is also demonstrable in mouse models of parasite infection. These leucocytes can produce copious amounts of IL-4 in response to *Schistosoma mansoni* antigens and thus have the potential to significantly affect acquired immune responses [37, 38]. In addition, Locksley and colleagues have shown that Th2-driven responses in the lung occurring as a consequence of the migrating intestinal helminth, *Nippostrongylus brasiliensis*, appear to be mediated by effector cells such as eosinophils [39].

Eosinophil-induced modulations of peripheral T cell activities

The ability of eosinophils to elaborate Th2-mediated responses may not necessarily be restricted to eosinophil autonomous events and may include potential interactions with T lymphocytes. That is, although the relationship between T cells and eosinophils is all too often viewed from a hegemonic T lymphocyte perspective (i.e. the relationship is necessary for T cells to activate eosinophils), several studies have instead shown that eosinophils may have significant effects on T cells. In particular, pulmonary studies using allergen provocation mouse models suggest that eosinophils recruited to the lung

may have direct effects on induced Th2 responses by modulating T cell activities [40]. Still other studies have suggested that eosinophils are capable of modulating T cell activities through the secretion of Th1 polarizing cytokines such as IFN- γ [41] as well as through direct cell-cell interactions potentially modulating the balance of Th1–Th2 immune responses (e.g. through eosinophil-derived indoleamine 2,3-dioxygenase production of kynurenine [42]). Antigen presentation is also a potentially significant mechanism by which eosinophils modulate local T cell responses and, thus, the tissue microenvironment. In mouse models of pulmonary allergen challenge, eosinophils have been shown to traffic to draining lymphoid tissues where they express major histocompatibility complex Class II activation markers such as CD80/86, and are capable of presenting antigens to lymphocytes [43]. Interestingly, subsequent studies have demonstrated that eosinophils are poor antigen-presenting cells to naive T cells [44], and thus instead of a systemic response, antigen presentation by eosinophils may be a restricted and localized tissue response activating T cells previously exposed to antigen.

Question #2: Why is degranulation viewed as the ultimate eosinophil effector function?

The prevailing belief that eosinophil degranulation is the dominant effector function that is also destructive in character surreptitiously arose from the fulfillment of the same three independent criteria that police invoke during a murder investigation: *Motive*, *Opportunity*, and *Means*.

Motive

Eosinophils are generally perceived as part of a host defence strategy against helminth parasites. However, large non-phagocytosable multicellular parasites present a particularly difficult challenge to the vertebrate B cell- and T cell-dominated immune system that has evolved as a very targeted defence against ubiquitous threats such as bacteria and viruses. Thus, by and large this system is ill-equipped for host defence against pathogens larger than single cells (i.e. multicellular parasites), requiring a shift from target-specific Th1 immune responses to physiology modulating Th2 responses and the recruitment of effector cells (e.g. eosinophils) with localized non-specific killing capabilities. Although the goal of this non-specific strategy is the death of invading pathogens, all cells in affected areas, pathogens and host cells alike, often become the targets of this defence mechanism. The considerable collateral damage to surrounding host tissues at first glance appears to be counter-productive; however, this damage itself may be hypothesized to contribute to host defence by isolating the intended pathogens and by inducing the recruitment of other inflammatory cells to these sites. In this paradigm, the destructive role of eosinophils in allergic diseases such as asthma is simply the dysregulation of this defence strategy in the lungs in response to a perceived threat (i.e. an inhaled allergen).

Opportunity

Although a rare cell in circulating blood (generally 1–3% of total white blood cells in most mammalian species), clinical

observations of patients, and studies of animal models, place eosinophils at the 'scene of the crime'. A myriad studies show that high numbers of eosinophils accumulate in areas of helminth infection and in the lungs of asthma patients concomitant with the tissue pathologies often associated with these diseases. Thus, eosinophils are tissue-dwelling cells that are rapidly recruited to sites characterized by cell stress/death and tissue remodelling events where they often are the dominant leucocyte present.

Means

Despite their association with parasite and host cell death and tissue damage, the establishment of a means of killing (i.e. the definition of a destructive eosinophil effector function) had been problematic for most of the last century. However, a solution to this problem arose from the pioneering work of Gerald Gleich and colleagues in the 1970s and 1980s [23, 45, 46] demonstrating the toxic character of the abundant ESGPs. These studies showed that the abundant ESGPs are a collection of proteins with varied, seemingly unrelated, activities that include ribonucleases, a peroxidase, and proteins displaying homology with C-type lectins but otherwise no known enzymatic activities (i.e. eosinophil MBPs [47]). However, the common unifying feature of this collection of proteins was their cationic character and their robust cellular toxicity [6]. Thus, together with motive and opportunity, a circumstantial case for the dominant and destructive character of eosinophil degranulation quickly developed in the literature in support of earlier observations linking eosinophils and the killing of helminths [48–51]. Moreover, this activity also provided a unique mechanism to explain the airway epithelial damage and cell death occurring in asthma patients; inflammatory responses to a perceived threat lead to the dysregulated recruitment of eosinophils in the lung and, in turn, cytotoxic activities through the release of toxic cationic secondary granule proteins that lead to epithelial cell desquamation and death [46].

Despite their apparent fulfillment of the prerequisite criteria as destructive inflammatory cells, the possibility exists that eosinophils are not the cause of cell stress/death and tissue damage. Instead, these leucocytes may simply be first responders to signals released by stressed and/or dying cells [52]. That is, clinical studies linking these phenomena may have mistakenly assumed that the release of granule proteins was the cause of the destruction observed. Unfortunately, the lack of kinetic studies in patients or animal models unambiguously defining the timeline between tissue damage and eosinophil recruitment leaves the issue of linkage unresolved. In addition, examples of patients with tissue eosinophilia without extensive pathology (e.g. Löffler's syndrome patients [53]) may suggest that other cells/mechanisms are responsible for the tissue damage/cell death often attributed to eosinophils alone.

Question #3: Does the available data support an alternative to the prevailing hypothesis that degranulation is a destructive effector function?

A hypothesis defining the scope of potential non-destructive roles for eosinophil degranulation must reflect the observa-

tion that many aspects of this process are conserved among all mammals (perhaps all vertebrates) and therefore provides a selective advantage. Moreover, this hypothesis needs to provide an explanation for the limited number of situations in which eosinophilia is the dominant response. Namely, what is the underlying commonality between the diverse, and yet unique, circumstances that lead to recruitment and accumulation of tissue eosinophils? As noted earlier, the eosinophil (as well as degranulation) likely appeared early in the evolution of animals, before the appearance of helminth parasites. As a consequence, their origin would be independent of acquired immune responses associated with host defence mechanisms against multicellular pathogens. Instead, we propose that eosinophils, and in particular eosinophil degranulation, are a first response system that evolved in metazoans as part of a necessary remodelling strategy needed in the larger more complex body forms of these organisms. This remodelling capability also appears to accompany (and is perhaps linked to) Th2-like inflammatory responses and may represent the unifying commonality inducing the unique recruitment/accumulation of eosinophils in a limited number of circumstances. In this hypothesis, eosinophil degranulation has two critical and evolutionarily selective functions that are elicited by cell stress/tissue damage in the context of appropriate inflammatory signals (i.e. Th2-like responses): Immunoregulation of the local microenvironment necessary for tissue remodelling and catabolic activities leading to clearance of macromolecular debris.

This hypothesis argues for the evolution of eosinophils and degranulation as a solution to the logistics of increased size and complexity associated with large multicellular organisms. That is, in primitive metazoan body forms (e.g. coelenterates, hydrarians and cnidarians) systemic responses generally affect all tissues within an organism and tissue interfaces with the environment are extensive enough to allow passive diffusion-based clearance/remodelling mechanisms. Thus, a cell capable of mediating immunoregulation and clearance/remodelling would be unnecessary. However, as the body plans of more evolved metazoans grew in size and complexity, effectively isolating tissues from systemic responses and limiting tissue access to the external environment (i.e. direct clearance mechanisms), an evolutionarily selective need arose for an amoeboid secretory cell capable of quickly and efficiently traversing outlying tissues as part of mechanisms that link local defence responses with systemic activities. In addition, this cell would provide the necessary tissue remodelling activities and macromolecular debris clearance associated with cell stress/tissue damage. This suggests that an amoeboid granulocyte and degranulation evolved as a first response to cell stress/tissue damage, functioning as a regulator of host defence by translating and amplifying local danger signals [54] in those unique circumstances in which significant tissue remodelling and macromolecular debris clearance were also required. Once recruited to areas of stressed and/or dying cells, these tissue granulocytes would receive additional signals in their local environment leading to degranulation. The importance of translating/amplifying local danger signals in large multicellular organisms is self-evident; as areas of the body become more isolated from systemic circulation, tissue damage and assaults from pathogens would evade centralized immune responses leaving

the organism vulnerable to ubiquitous threats. The simultaneous need in some circumstances for tissue remodelling and catabolic clearance of macromolecular debris is less obvious, but no less important. The larger and more complex a body plan, the greater the need for mechanisms of tissue remodelling and clearance of the macromolecular debris that results from injury, disease, or simply for the maintenance of homeostasis. In other words, circumstances would have arisen in larger organisms that required a unique need for specialized remodelling strategies and debris clearance mechanisms. Thus, the hypothesis that an eosinophil-like cell and degranulation are parts of an early metazoan remodelling/clearance mechanism provides an explanation for many of the evolutionary differences observed among animals. An amoeboid granulocyte with characteristics of an eosinophil would be present in the earliest vertebrates, and in many invertebrate species because of a fundamental need of metazoan homeostasis. Moreover, degranulation would be species-specific, reflecting unique tissue remodelling and/or debris clearance needs of a given organism (e.g. because of different environmental pressures faced by fish vs. land vertebrates). Finally, the co-evolution of warm-blooded vertebrates and multiple highly specialized leucocytes, including the eosinophil, occurred as a logistical solution to the unique challenges resulting from the more complex responses associated with the dramatically higher rate of metabolism and cell turnover occurring in these animals. That is, in a simple organism the defensive and phagocytic strategies of neutrophils as well as the remodelling/clearance and secretory activities of eosinophils were likely contained within a single primitive amoeboid granulocytic cell. However, as a need for more complex and efficient mechanisms arose, separate unique lineages each evolved from this early amoeboid granulocyte (e.g. neutrophils (host defence) and eosinophils (tissue remodelling)), providing specific activities that conveyed selective advantages to animals with larger and more complex body plans.

In addition to providing an explanation for the evolutionary origin and importance of eosinophil degranulation, this alternative hypothesis is supported by the available data regarding ESGPs and the consequences of degranulation. For example, the identification of cytokines/chemokines in the secondary granules of eosinophils implies that degranulation may have significant and direct immunoregulatory capabilities that would modulate responses in the local tissue microenvironment. The catabolic activities of the ESGPs are equally provocative. Activities of proteins stored in the granule include several hydrolytic enzymes and proteases that have been shown to be capable of degrading extracellular matrix components [55–58]. We would also argue that instead of focusing on the toxic and cationic character of the abundant ESGPs, attention should shift to the functional characteristics of these proteins as contributors to clearance mechanisms and/or their potential immunomodulating agonist activities. In the case of *EPO*, this secreted peroxidase would provide a means of oxidative degradation of proteins and other macromolecules released by dying cells [56, 59]. This same activity would also provide a degree of bactericidal activity to areas of tissue damage and has been suggested as a means of immunoregulation through the inactivation of primary mediators of inflammation [60]. In addition, because ~25 pg of total RNA are released by each dying cell of the

body [61], a vital need exists for the clearance of this abundant macromolecular debris, hence the need for secreted ribonucleases. Moreover, these ribonucleases have also been shown to display additional activities whose functions may contribute to tissue remodelling [55]. Finally, the secreted cationic MBPs have been assumed to be the principal killing molecules released by eosinophils via mechanisms linked either to their highly cationic character and/or unusual amino acid composition [58]. However, because other proteins just as cationic as the MBPs (e.g. histones or seminal vesicle proteins) do not elicit cell death, MBPs may provide unique catabolic functions based on their unusual biochemistry and their ability to bind to almost anything encountered (our unpublished observations and G. Gleich, personal communication). We suggest that because damage and cell death would generate a great deal of 'cellular garbage' which ultimately needs to be cleared to allow for tissue remodelling, the ability of MBP to bind to almost anything would be an ideal property for a secreted protein whose function was to act as a marker of debris, targeting this material for removal by phagocytic leucocytes such as monocytes/macrophages and neutrophils. In this paradigm, MBP is released and functions as a tag to identify the 'cellular garbage' resulting from cell death and tissue damage. Thus, the massive release and binding of MBP into the extracellular matrix within the lungs of asthmatics do not elicit tissue damage and cell death but instead are a first response to stressed and/or dying cells leading to the tagging of debris that needs to be processed by subsequent waves of recruited inflammatory cells. The immunoregulatory cell agonist activities of MBP on other recruited phagocytic leucocytes [62, 63] and effects on resident tissue-dwelling cells critical to remodelling [64] support this hypothesis by enhancing debris clearance and eliciting tissue remodelling responses, including the stimulation of angiogenesis [65].

The maintenance of the same repertoire of abundant ESGPs among different species suggests that eosinophil degranulation has a similar role(s) in these animals. However, why do any differences arise and, in particular, why are there multiple forms of degranulation (e.g. piecemeal vs. cytolytic release of granules) and why is there apparently such a difference in the ability of eosinophils to degranulate between mice and humans? We believe that the answers to these questions reflect situational needs within any organism as well as species differences for both local immunoregulation and clearance activities. For example, a piecemeal pattern of degranulation (i.e. as opposed to the release of granules by cytolysis) represents a mechanism for the differential and/or directional release of granule contents dependent on local inflammatory/remodelling needs [66]. Thus, eosinophils are capable of responding to a wide spectrum of tissue microenvironmental signals by either the seemingly unregulated release of clusters of free eosinophil granules (*cfegs*) that subsequently disassociated to release the complete complement of granule contents or the selective release of specific granule contents, reserving the remaining components for future and/or different needs. In the case of species-specific degranulation events, differences may have arisen from the environmental niche a given animal occupies (e.g. the needs of cold-blooded vertebrates whose rate of cell turnover and metabolic activities are low would be different than the needs

of a bird or mammal). In addition, groups of animals may have evolved divergent strategies leading to differences in eosinophil effector functions or even the loss of particular activities. The observed lack of extensive degranulation in the lungs of mouse asthma models, for example, may reflect physiological differences between some rodents and primates. Mice are obligatory nasal breathers and thus have evolved elaborate turbinates that scrub the inspiratory airflow to a much higher degree than any primate, including humans. As a consequence, some physiological and immunological responses occurring in the mouse lung are likely to be different as many damaging exacerbants never reach the airspaces. That is, although eosinophils are recruited to the lung because of their 'detection' of signals released by stressed lung epithelial cells in the context of a Th2-dominated immune response, they do not display extensive degranulation because of the lack of additional signals mediated by dead and/or dying cells and an absence for the need of extensive tissue remodelling. Instead, degranulation is a nominal piecemeal process reflecting the lower levels of damage resulting from any one given pulmonary assault in the mouse. Interestingly, this lack of significant degranulation in the lungs of mice does not correlate with a diminished importance of the eosinophil as a causative agent of pulmonary pathology following allergen provocation [67]. The implication is that eosinophil effector functions other than degranulation may be the important activities linking these leucocytes with the lung dysfunction in asthma patients. The need for additional signals in the mouse to elicit significant degranulation suggests that in situations in which eosinophil recruitment was accompanied by significant cell turnover and extensive tissue remodelling, degranulation would be a prominent effector function. Indeed, as shown in Fig. 1, the inflammatory responses and tissue remodelling

events associated with solid tumour growth in the mouse are accompanied by extensive and widespread eosinophil degranulation.

A final issue to address is whether this alternative hypothesis of degranulation (i.e. as an immunoregulating event leading to remodelling and debris clearance) and the classical view of degranulation (i.e. a host defence mechanism mediated by cytotoxic activities) are mutually exclusive. We would argue that they are not! Over the millions of years of evolution since the origin of the eosinophil cell type, selective pressures on ESGPs and degranulation are likely to have led to the acquisition of additional activities beyond remodelling/clearance and immunoregulation. For example, although our hypothesis suggests that the evolutionary origins of the specialized mammalian eosinophil were in response to selective pressures for tissue remodelling and debris clearance, nothing would have prevented the ESGPs from developing additional activities (e.g. helminth killing activities) as a consequence of other evolutionary selective forces; that is, degranulating eosinophils are located in the areas occupied by helminths and the additional killing capability may have given the host organism a selective advantage over these pathogens. Similar arguments could be made for the additional proposed abilities linked with a number of other ESGPs. For example, eosinophil-associated ribonucleases have been suggested as a strategy to destroy single-stranded RNA viruses [68]. However, eosinophils that were originally recruited to sites of viral infection because of the induced tissue damage and cell death may have subsequently acquired anti-viral activities that were selectively advantageous to the host. Eosinophil MBPs have also been the subject of many studies, including their potential role in guinea-pig models of asthma-regulating neuromuscular activities in the lung through interactions with pre-synaptic M2 receptors [69].

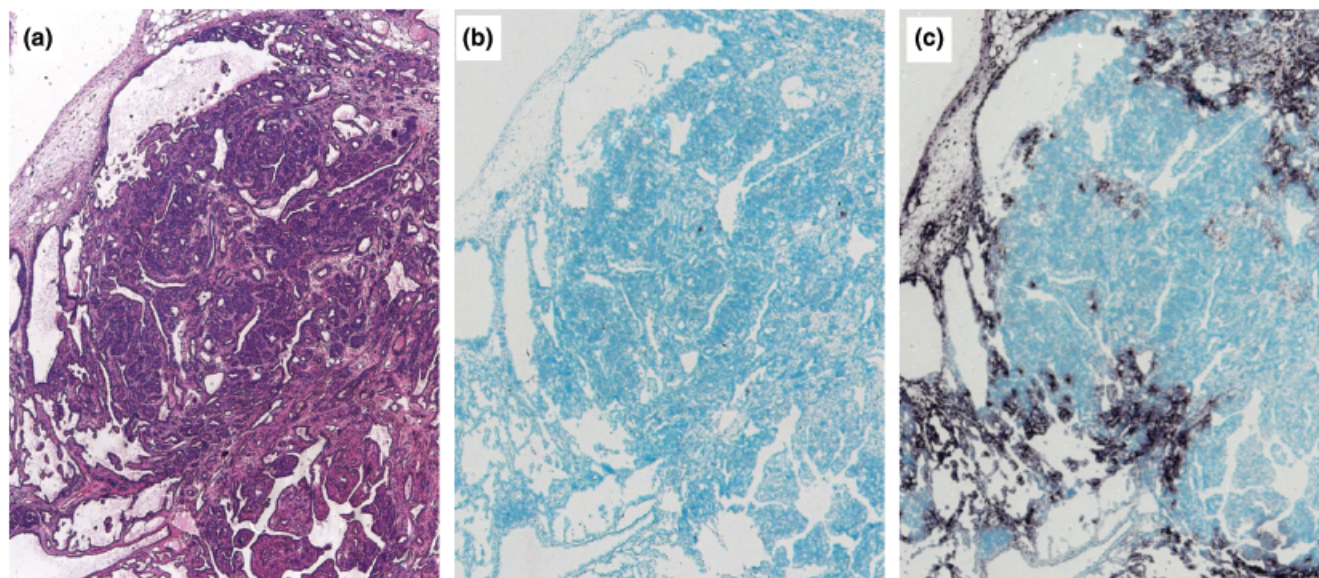


Fig. 1. Eosinophil recruitment and degranulation within the acellular necrotic and capsule regions of spontaneous mammary gland tumours arising in the PyVmt(MMTV) transgenic mice. (a) Haematoxylin–eosin-stained sections reveal an internal tumour structure which includes viable regions, areas of focal cell death, and a capsule region surrounding much of the tumour. Serial sections from the same tumour subjected either to immunohistochemistry using a control rabbit pre-immune antiserum (b) or a polyclonal anti-mouse eosinophil major basic protein antiserum (c). These panels reveal no background non-specific staining, yet the presence of many eosinophils (dark red/purple staining cells in (c) vs. (b)) as well as evidence of eosinophil degranulation in both the capsule regions and the focal areas of tumour necrosis (dark red/purple staining of the extracellular matrix areas).

Once again, although the origins of eosinophil recruitment to the lungs of these mammals may have been in response to specific cell stress and remodelling signals, selective forces on the evolution of pulmonary smooth muscle function (e.g. in response to a perceived threat, and/or on remodelling issues associated with post-inflammation repair) may also have led to the development of eosinophil–nerve interactions. The proposed roles for MBPs in these and other mammals may simply reflect these interactions. Moreover, several seemingly unrelated recent observations each lend additional support to this alternative hypothesis of degranulation: (i) studies correlating eosinophil recruitment and degranulation in the lungs of asthma patients with induced changes in the pulmonary extracellular matrix (i.e. tissue remodelling) [70]; (ii) the demonstration that eosinophils may be required for tissue morphogenesis at homeostatic baseline (e.g. mammary gland duct development [71]) highlights the potential importance of the remodelling/immunoregulatory role of degranulation as well as its fundamental importance in otherwise healthy individuals; (iii) studies using granule protein knockout mice in a parasite infection model that show the release of granule proteins has an effect on host immune responses suggesting a regulatory role as opposed to, and/or in addition to, a destructive role in host defence (A. Hoerauf, personal communication).

In summary, the hypothesis presented and the classical view of eosinophils and degranulation are not necessarily in conflict or mutually exclusive, and ideas derived from both perspectives are probably relevant. More importantly, this discussion suggests that although eosinophil degranulation is a widespread and conserved phenomenon, its specific role(s) as an effector function is not a dogmatic fact but remains an enigmatic activity whose true importance has yet to be fully appreciated.

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