

EDITORIAL

Clinical & Experimental Allergy

Eosinophilic inflammation: life, death and apoptosis

This editorial discusses the findings of the paper by Farahi et al. [18] pp. 673–687.

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Apoptosis, or programmed cell death, is a controlled outcome of cell maturation and senescence. Human eosinophils undergo apoptosis spontaneously, upon withdrawal of cytokine support, primarily IL-5, via mechanisms that involve expression and activation of the Bcl-xL, Bax and Bid. Apoptosis can also be induced in eosinophils via ligation of cell surface Fas, and the Ig-like receptor, Siglec 8, among other cell surface proteins. Apoptotic eosinophils become involuted, with pyknotic nuclei, and they retain rather than extrude potentially destructive cationic granule components. Apoptotic eosinophils are detected and can undergo phagocytosis by macrophages and can also be cleared by airway epithelial cells. This is but a brief summary of many more complete studies, which are the subject of many excellent and detailed reviews [1–4].

While eosinophil apoptosis has been carefully considered in well-defined tissue culture studies, less certain is the contribution of specific pro-apoptotic pathways to physiologic or pathophysiologic inflammation and/or disease pathogenesis *in vivo*. Several prominent studies have addressed the role of apoptosis in the resolution of eosinophilic inflammation in tissue explants and in *in vivo* settings. Apoptotic eosinophils have been detected and identified in sputum isolated from the airways of asthmatics in several independent reports [5–7]. Duncan et al. [6] reported a correlation between eosinophil apoptosis and asthma severity, and Xu et al. [7] noted that the fraction of apoptotic eosinophils correlated inversely with the levels of IL-5 and eotaxin. Simon et al. [8] examined eosinophil-enriched nasal polyps, a finding characteristic of individuals with rhinitis and asthma. Antibody-

mediated blockade of IL-5 within the tissue resulted in eosinophil apoptosis *in situ*. Similarly, in two recent mouse model studies utilizing the traditional ovalbumin (OVA) sensitization and challenge to generate pulmonary and airway eosinophilia, Tumes et al. [9, 10] documented associations between the propensity to undergo apoptosis and the expression of apoptosis-related transcripts and protection from the negative sequelae of allergic inflammation. It is important to recognize that, while these experiments are all meaningful and significant, there remains no direct proof regarding apoptosis as a definitive biologic control mechanism for resolving eosinophilic inflammation *in vivo*.

From this perspective, Uller and colleagues [11–17] have published a series of papers that highlight an alternative interpretation, specifically, that apoptosis is not a crucial or even a prominent mechanism promoting the resolution of eosinophilic inflammation *in vivo*. Among their findings, they report that nasal polyp tissue includes at baseline primarily viable and cytolytic, not apoptotic eosinophils [15], and they posit that eosinophil clearance in human and mouse models occurs not via apoptosis, followed by phagocytosis, but via transepithelial migration of viable cells.

The featured manuscript by Farahi et al. [18] entitled 'Effects of the cyclin-dependent kinase (CDK) inhibitor R-roscovitine on eosinophil survival and clearance' can be best understood in light of this ongoing controversy. R-roscovitine (also known as Seliciclib or CYC202) is a substituted purine analogue [2-(R)-(1-Ethyl-2-hydroxy-ethylamino)-6-benzylamino-9-isopropylpurine] that induces apoptosis via its interactions with CDKs, conserved proteins with crucial roles in regulating progression through the cell cycle [19]. R-roscovitine induces apoptosis in multiple cell types, including neutrophils [20, 21]. In this manuscript, the authors evaluate the impact of R-roscovitine on isolated human eosinophils: they confirm the expression of specific CDKs and R-roscovitine-mediated induction of apoptosis as reported previously

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by Duffin et al. [22], and go on to document transition to secondary necrosis, overriding pro-survival signals provided by IL-5 or GM-CSF, and enhancing eosinophil phagocytosis. Interestingly, despite its impact on the survival of isolated human eosinophils, the intraperitoneal administration of R-roscovitine had no impact on tissue or airway eosinophilia in mice in response to OVA sensitization and challenge. This final result is of course quite relevant, given the potential interest in exploring R-roscovitine and CDK inhibitors in general as a novel class of anti-asthma therapeutic strategy [21, 22].

One immediate question emerging from this work concerns the identification of CDKs expressed in mouse eosinophils, the similarities and potential different responses with respect to their human counterparts. Although the authors did show that R-roscovitine could induce apoptosis in eosinophils in mixed granulocyte populations from mouse peripheral blood and spleen, a more in-depth study of the characteristics, dose response and ability to overcome pro-survival cytokine stimulation, similar to that presented here for human eosinophils, would be crucial supporting data for this finding. This is especially relevant, given the limited dose-response range over which human eosinophils respond to this inhibitor [18], and the fact that human and mouse eosinophils differ quite substantially from one another in a number of salient features. These features include the nature of cell surface receptors and cationic granule proteins, the propensity to degranulate and the nature of physiologic chemotactic responses (reviewed in [23]). While apoptotic responses have been studied quite extensively in human eosinophils, the literature on mouse eosinophil

apoptosis is more limited, and the findings are sometimes conflicting [24, 25]. There are several methods available for obtaining large numbers of relatively pure eosinophils from mice [26–28]; any one of these would be an excellent starting point for a parallel evaluation of R-roscovitine responses and for determining the crucial parameters necessary for the interpretation of the findings from a more extensive investigation into the mouse model.

Likewise, it is important to recognize that, regardless of what is ultimately concluded of R-roscovitine and/or other similar pharmacologic agents, questions regarding the physiologic relevance of apoptosis and eosinophilic inflammation will remain. As such, R-roscovitine, administered in some other fashion or via some other dosing regimen, may be reasonably effective at reducing inflammation by promoting eosinophil apoptosis, even if induction of apoptosis is ordinarily of minimal importance in promoting clearance under normal circumstances. In other words, the induction of eosinophil apoptosis need not be a physiologically relevant event in order to be a pharmacologically meaningful one. For this reason, a follow-up study with a further, more substantial assessment of dose response and with a clear understanding of the specifics of the *in vivo* pre-clinical model would be a most worthwhile undertaking.

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