

Correspondence

Lytic eosinophils produce extracellular DNA traps as well as free eosinophil granules

To the Editor:

We read with interest the report by Gevaert et al¹ on eosinophils in nasal polyps. For decades the senior authors of this article¹ have championed roles of eosinophil death through apoptosis. They now report that apoptosis was practically absent in the highly eosinophilic polyps. This concession needs underscoring because it agrees with reports questioning the importance of eosinophil apoptosis in human diseased airway tissues *in vivo*.^{2,3} Regarding eosinophil death through lysis, Gevaert et al¹ assert “that cell lysis did not occur under the conditions of EET formation.” This arguably is a misleading statement.

Referring to their previous reports, Gevaert et al maintain that extracellular eosinophilic traps (EETs) reflect mitochondrial DNA (containing 100 times less DNA than nuclei) ejected from live eosinophils. However, careful analyses by transmission electron microscopy of 500 eosinophils in nasal polyps demonstrate that around 10% of polyp eosinophils exhibit lysis (no apoptosis).³ The lysis is characterized by chromatolysis and cell membrane rupture, allowing extracellular release of nuclear and cytosolic contents.³ The number of lytic eosinophils in nasal polyps³ agrees with eosinophil numbers reported by Gevaert et al to form EETs. Furthermore, using various approaches, including transmission electron microscopy, release of EETs exclusively from lytic eosinophils has been compellingly demonstrated in nasal tissues/secretions.^{4,5} There is concomitant release of EETs and free eosinophil granules (FEGs), the latter exhibiting regulated release of toxic proteins such as major basic protein (MBP).⁴

Gevaert et al use a low-resolution light microscopy graph to claim lack of lysis, but high-resolution micrographs to illustrate EETs. The high resolution is fortunate because the EETs (Figs 2-6) conspicuously appear together with MBP-positive FEGs, indicating that lysis has occurred.¹ Gevaert et al also seem impressed by the FEGs to the extent that arrowheads in Figs 2, D, and 4, B, purported to indicate EETs, actually point at punctate staining of MBP-positive FEGs.

Gevaert et al could state that they confirm the findings by Harlin et al's group⁶ on association between MBP-secreting FEGs and epithelial derangement in rhinitis. Unknowingly, they also confirm compelling observations by Prince et al⁷ on the capacity of *Staphylococcus aureus* to very rapidly induce eosinophil lysis. So, association between *S aureus* and lysis products such as FEGs and EETs is expected. However, Gevaert et al may have missed a

fine opportunity of addressing the question whether *S aureus*-induced eosinophil lysis causes epithelial derangement in their tissue culture experiments?

Molecular mechanisms related to so-called non-lytic EET formation¹ also overlap with those affecting primary lysis in well-controlled experiments. Association between IL-5 and EET formation¹ is thus of interest because this cytokine together with other factors is known to cause primary lysis of eosinophils.⁴ Finally, blocking nicotinamide adenine dinucleotide phosphate-oxidase activity inhibits eosinophil lysis⁴ and is now reported by Gevaert et al to inhibit the EET formation that they unduly claim to occur without lysis.

We conclude that lysis of eosinophils is a major, active mechanism causing the release of secretion-competent FEGs as well as EETs, both of which may associate with central features in diseased airways including microbial pathogens and epithelial derangement.

Carl Persson, PhD

Shigeharu Ueki, MD, PhD

From Laboratory Medicine, Clinical Pharmacology, University Hospital, Lund, Sweden.
E-mail: carl.persson@med.lu.se.

Disclosure of potential conflict of interest: S.Ueki receives grant support from MEXT/JSPS, Research Grant on Allergic Disease and Immunology from the Japan Agency for Medical Research and Development, the Charitable Trust Laboratory Medicine Research Foundation of Japan, Novartis Pharma, the Japan Allergy Foundation, and Phyzer, and serves as a consultant for Boehringer Ingelheim. C. Persson declares no relevant conflicts of interest.

REFERENCES

1. Gevaert E, Zhang N, Krysko O, Lan F, Holtappels G, De Ruyck N, et al. Extracellular eosinophilic traps in association with *Staphylococcus aureus* at the site of epithelial barrier defects in severe airway inflammation. *J Allergy Clin Immunol* 2017;139:1849-60.e6.
2. 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;189:628-33.
3. Uller L, Andersson M, Greiff L, Persson CG, Erjefalt JS. Occurrence of apoptosis, secondary necrosis, and cytolysis in eosinophilic nasal polyps. *Am J Respir Crit Care Med* 2004;170:742-7.
4. 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;121:2074-83.
5. Ueki S, Konno Y, Takeda M, Moritoki T, 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;137:258-67.
6. 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;81:867-75.
7. 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* 2013;7:e31506.

<http://dx.doi.org/10.1016/j.jaci.2017.05.047>