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Letter to the Editor

Mucus plugging in allergic bronchopulmonary aspergillosis: Implication of the eosinophil DNA traps

Dear Editor,

Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disorder caused by exaggerated hypersensitivity to *Aspergillus* species, namely *Aspergillus fumigatus*. It is characterized by poorly controlled asthma, recurrent pulmonary infiltrates, and bronchiectasis often with thick, viscous eosinophilic mucus plugging.¹ Although the mechanism of this hyperdense mucus generation is poorly understood, the severity of this mucus plugging, which may represent severe inflammation, is associated with recurrent relapse.²

Extracellular trap cell death (ETosis) of human leucocytes, particularly neutrophils and eosinophils, is a distinct programmed cell death pathway.³ It is characterized by release of filamentous chromatin structures (DNA traps) in contrast to cell apoptosis.⁴ DNA trap formation is considered to be an innate immune reaction by ensnaring microorganisms, although its excess production could be pathogenic.⁵ For instance, in thick and viscous airway fluids from patients with cystic fibrosis, large numbers neutrophil-derived DNA traps are present (also called neutrophil extracellular traps; NETs).^{4,5} Notably, treatment by inhalation of recombinant human DNase improved lung function,⁶ indicating the pathogenicity of NETs.

In contrast, the clinical significance of eosinophil ETosis-derived DNA traps is less well studied. We recently revealed their involvement in high viscosity of sinus secretions, a characteristic in refractory eosinophilic chronic rhinosinusitis (ECRS) and eosinophilic otitis media (EOM).⁴ Here, we report a case of ABPA, which had bronchiectasis with viscous mucus plugging containing abundant DNA traps. The patient demonstrated a rapid clinical response and decrease in DNA traps to systemic corticosteroid.

A 64-year-old Japanese man was referred to our hospital for cough, sputum, and chest pain. He had a 10-year history of asthma and had experienced the above symptoms for 6 months before admission. Laboratory examination revealed an elevated blood eosinophil count (WBC: 6800/ μ L, eosinophil: 1120/ μ L) and serum total IgE (1628 IU/mL). Testing for specific IgE and precipitating antibodies against *A. fumigatus* was positive. Chest X-ray and computed tomography (CT) revealed infiltration with bronchial wall thickening, central bronchiectasis, and mucoid impaction with middle and lower lobe predominance. Pulmonary function testing showed reduced forced expiratory volume in 1 s and a force vital capacity ratio (FEV₁/FVC) of 67.9%. From these findings, he was diagnosed with ABPA, fulfilling the diagnosis criteria¹. He was

treated with prednisolone 40 mg/day for 4 weeks, followed by 30 mg/day. His symptoms dramatically improved following prednisolone treatment. He was discharged on the 35th day. The prednisolone dose was gradually reduced at the outpatient clinic, and tapered off 3 months after discharge. One month after prednisolone was tapered off, FEV₁/FVC improved to 76.2%, and both eosinophil count and serum total IgE decreased (WBC: 5500/ μ L, eosinophil: 610/ μ L, serum IgE: 643 IU/mL), indicating clinical improvement after prednisolone treatment.

Before and 34 days after corticosteroid treatment, fiberoptic bronchoscopy was performed and bronchial secretions were collected. Papanicolaou staining of bronchial secretions before treatment were analyzed using Keyence BZ-X700 microscopy (Osaka, Japan). Fully-focused high-power image showed massive eosinophil accumulation in the mucus, and chromatolytic nucleus from lytic cells elongated and aggregated to form basophilic filaments (Fig. 1A, arrows). Clusters of free eosinophil granules localized with mesh-like structures are also evident in Z-stack images (Supplementary Video 1). Fibrous structures from bronchial secretions were stained with anti-human histone H1 mAb (Abcam, Cambridge, UK) and Alexa Fluor 488-conjugated antibody (Invitrogen, Carlsbad, CA, USA) for secondary incubation. Hoechst 33342 (Invitrogen) was used for DNA staining. Images were obtained using a confocal microscope (LSM780; Zeiss, Oberkochen, Germany). Sample preparation, staining, and microscopic imaging were performed as previously described.⁴ Co-localization of histone H1 and DNA indicated that the fibrosis structures were nuclear-derived chromatin fibers, i.e., ETosis-derived DNA traps⁴ (Fig. 1B). Additionally, bronchial lavage fluid (BALF) from right middle lobe bronchus were stained with cell-impermeable DNA-specific dye SYTOX Green (Invitrogen). Before treatment, BALF contained considerable amounts of elongated fibrous DNA, indicating an abundance of DNA traps (Fig. 1C). Conversely, after treatment, BALF showed a decreased fibrous DNA and a small number of inflammatory cells with pigmented nuclei, indicating improvement of inflammation and presence of apoptotic cells. Chest CT findings, initially showing central bronchiectasis with mucous plugging, were also improved.

Since eosinophils are terminally differentiated, non-dividing cells, it is critical to understand the fate of eosinophils at inflammatory foci. DNA traps are produced as an active form of cell death (ETosis), but not from apoptosis or necrosis.^{3,4} As a result of eosinophil ETosis, intact, free granules are liberated (i.e., lytic degranulation) and often associated with DNA traps.^{3,4} Our observations indicated that eosinophils might undergo ETosis after luminal entry and produce DNA traps in bronchial secretions as a structural component of mucus plugs. Importantly, compared with NETs,

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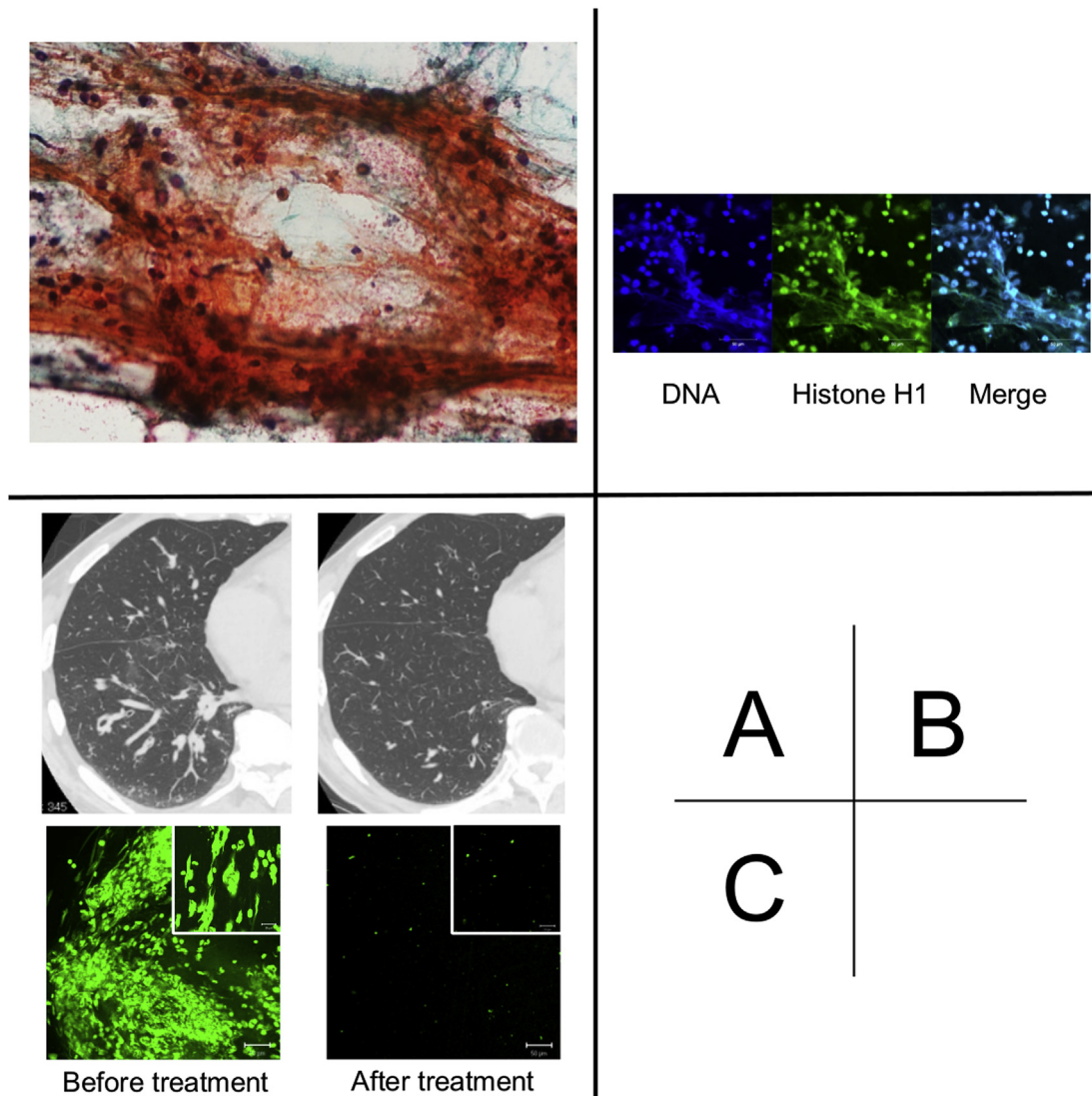


Fig. 1. (A) Papanicolaou staining (scale bar: 10 μ m) and (B) DNA and histone H1 co-localization in bronchial secretions before treatment (scale bar: 50 μ m). Chromatolytic cells aggregated to form basophilic filaments (A, arrows, see also [Supplementary Video 1](#)). (C) Chest CT images (upper panels) and bronchial lavage fluid (BALF) stained with cell-impermeable DNA dye SYTOX Green (lower panels) before and after treatment (scale bars: 50 μ m). Inserts in the lower panels indicate higher magnification (scale bars: 20 μ m).

eosinophil DNA traps are composed of stable and condensed chromatin fibers, and thus might contribute to higher viscosity secretions as observed in ECRS.⁴ Analysis of bronchoscopically obtained secretions before and after corticosteroid treatment demonstrated a drastic decrease in DNA traps, parallel to clinical improvement. It is noteworthy that *A. fumigatus* directly activates human eosinophils to induce ETosis (Muniz VS *et al.*, in press). Since persistent airway colonization of *A. fumigatus* is a common feature of ABPA, we speculate that *A. fumigatus* activates luminal eosinophils to produce DNA traps, which may contribute to the pathogenesis of bronchiectasis with mucus plugging.

The rheological properties of mucus and mucociliary transport system are known to function as a self-cleaning mechanism for respiratory tract.⁷ In active state of ABPA, we speculate that eosinophils, supplied from blood, accumulate into bronchial lumen and undergo ETosis. Free eosinophil granules contribute to increase the local concentrations of toxic eosinophil granule proteins. Resulting epithelial damage may then inhibit the effectiveness of ciliary beat, thereby decreasing mucus transport and perpetuating a cycle of thickening secretions.⁸ Corticosteroid is known to reduce

excess airway infiltration of eosinophils by inhibiting the production of chemoattractants from diseased airway.⁹ In addition, corticosteroid induces eosinophil apoptosis and DNA fragmentation.¹⁰ Thus, reduced number of migrating eosinophils and apoptotic eosinophils might contribute to lesser mucus plugging. In this context, regulation of excess eosinophil ETosis (induction of apoptosis) or removal of DNA traps could be a novel therapeutic modality for this disease entity.

To our knowledge, this is the first report to describe the existence of DNA traps in bronchial secretions in ABPA and confirm DNA trap decrease is associated with clinical improvement after corticosteroid treatment. Our observation aids in understanding the mechanisms of mucus plugging in ABPA and clinical effects of corticosteroids. Massive infiltration of eosinophils and their lytic degranulation in the thick, tenacious, and viscous secretion has been commonly observed in ABPA, ECRS, and EOM but also in other eosinophilic diseases such as severe asthma.⁸ Further study on pathophysiological roles of eosinophil ETosis-derived DNA traps in allergic diseases may benefit future therapy targeting eosinophilic inflammation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.alit.2017.08.002>.

Conflict of interest

The authors have no conflict of interest to declare.

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References

1. Agarwal R, Chakrabarti A, Shah A, Gupta D, Meis JF, Guleria R, et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy* 2013;**43**:850–73.
2. Agarwal R. High attenuation mucoid impaction in allergic bronchopulmonary aspergillosis. *World J Radiol* 2010;**2**:41–3.
3. 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.
4. Ueki S, Konno Y, Takeda M, Moritoki Y, 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.
5. Brinkmann V, Zychlinsky A. Beneficial suicide: why neutrophils die to make NETs. *Nat Rev Microbiol* 2007;**5**:577–82.
6. Yang CL, Chilvers M, Montgomery M, Nolan SJ. Dornase alfa for cystic fibrosis. *Paediatr Respir Rev* 2017;**21**:65–7.
7. King M. Experimental models for studying mucociliary clearance. *Eur Respir J* 1998;**11**:222–8.
8. Ueki S, Tokunaga T, Fujieda S, Honda K, Hirokawa M, Spencer LA, et al. Eosinophil ETosis and DNA traps: a new look at eosinophilic inflammation. *Curr Allergy Asthma Rep* 2016;**16**:54.
9. Schwiebert LM, Beck LA, Stellato C, Bickel CA, Bochner BS, Schleimer RP. Glucocorticosteroid inhibition of cytokine production: relevance to antiallergic actions. *J Allergy Clin Immunol* 1996;**97**:143–52.
10. Druilhe A, Letuve S, Pretolani M. Glucocorticoid-induced apoptosis in human eosinophils: mechanisms of action. *Apoptosis* 2003;**8**:481–95.

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