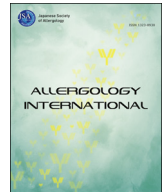




Contents lists available at ScienceDirect

Allergy International

journal homepage: <http://www.elsevier.com/locate/alit>

Letter to the Editor

ETosis-derived DNA trap production in middle ear effusion is a common feature of eosinophilic otitis media

Dear Editor,

Eosinophilic otitis media (EOM) is a recently recognized intractable middle ear disease in Japan and has been focused on in Western country.¹ EOM is characterized by the strong infiltration of eosinophils in highly viscous ear effusion (eosinophilic mucin) and in the middle ear mucosa.^{2,3} Unlike conventional chronic otitis media, EOM is generally resistant to all pharmacological therapies except for systemic steroid treatment. Surgical techniques are often warranted to improve the drainage pathway so as to remove obstructive eosinophilic mucin. Patients with EOM have a high risk of conductive hearing loss in the early stage, and half of all patients develop an increased bone-conduction threshold, leading to deafness in 6% of cases.^{2,4}

The high levels of toxic eosinophil granule proteins observed in the eosinophilic mucin are suggested to be pathogenic and to cause epithelial damage.⁴ Eosinophil degranulation is mediated by several mechanisms, namely exocytosis, piecemeal degranulation, and cytolysis. Cytolysis is a well recognized pathology in diverse eosinophil-associated diseases, including EOM,⁴ and was suggested to represent a full activation of eosinophils,⁵ although its precise mechanism is not understood. A recent finding showed that extracellular trap cell death (ETosis), a novel form of cell death, mediates eosinophil cytolytic degranulation.⁶ Eosinophil ETosis (EETosis) is characterized by its striking final morphology: the release of intact granules and web-like chromatin structures (DNA traps/extracellular traps: ETs) through the breakdown of nuclear and plasma membranes. It does not feature apoptotic signatures such as phosphatidylserine exposure on the cell surface and DNA fragmentation. We have previously reported the presence of eosinophil ETs (EETs) in the middle ear secretions obtained from a few EOM patients.⁷ To clarify whether the presence of cytolysis and ETs is common in EOM, we conducted a case series study.

All patients met the diagnostic criteria of Iino *et al.*² and were seen by experienced ENT doctors at Yamagata City Hospital Saiseikan, Yamagata University Faculty of Medicine, and Tohoku Medical and Pharmaceutical University between February 2010 and August 2017. Informed consent was obtained under protocols approved by the Institutional Review Board. Clinical features of the patients with EOM are summarized in Table 1. The patients were 4 men and 4 women who ranged in age from 25 to 72 years. Audiometry was performed by audiologists using a pure-tone audiometer (AA-76, Rion, Tokyo, Japan). All patients had comorbid chronic

rhinosinusitis with nasal polyps and asthma. Seven of them had allergic rhinitis. Five showed eosinophilia (mean 11.6%, range 1.2%–32.9%) and elevated serum IgE levels (mean 604.6 IU/ml, range 104–1348 IU/ml). All patients had hearing impairment; three had sensorineural hearing loss and the others had conductive hearing loss. All patients had perforated tympanic membranes with eosinophilic mucin and granulation in the middle ear cavity without bacterial infections.

We microscopically evaluated eosinophil morphology in middle ear effusions. Effusion smears were air-dried and stained with hematoxylin–eosin. The presence of cytolytic eosinophils was defined relative to intact eosinophils as either a predominance of eosinophils with cytolytic degranulation (++) or as less-than-intact eosinophils (+). We observed massive infiltration of eosinophils showing predominant cytolytic degranulation in all samples. The result indicates that eosinophils that migrate into the middle ear cavity undergo cytolysis to release their toxic granules.

Live intact eosinophils can release mitochondrial DNA traps.⁸ To detect nuclear-derived DNA traps, we immunostained cells for linker-histone H1.⁷ In brief, we fixed middle ear effusion smears in 4% paraformaldehyde, then blocked and permeabilized them with 3% bovine serum albumin and 1% saponin containing PBS for 30 min. The slides were then incubated first with primary mouse anti-human histone H1 monoclonal antibody (AE-4, 2 µg/ml, Abcam, 3 h at room temperature) and then with Alexa-488 conjugated antibody (goat anti-mouse IgG; Invitrogen, Carlsbad, CA, USA; 1:300, 30 min at room temp.). Control antibodies and DNA stain (Hoechst 33342, Invitrogen) were used in each experiment. Abundant filamentous DNA, co-localized with histone H1, was observed in the ear effusions from all patients (Fig. 1 shows example). These results reinforce our previous findings that ETosis-derived DNA traps represent major extracellular structural components scaffolding the eosinophilic mucin and increasing their viscosity.⁷ The occurrence of EETosis is most likely to be common in EOM patients and to contribute to pathogenesis. Indeed, EET structures can be degraded by lysates and elastase derived from neutrophils.⁴ Interestingly, the viscosity of secretions from a patient with EOM was shown to be reduced by bacterial infection.⁴ This may allow us to speculate that the presence of neutrophils may decrease the viscosity.

EETosis that mediates lytic degranulation is an activation-induced programmed cell death. IL-5, a well known eosinophil survival-promoting cytokine, paradoxically induces EETosis in conjunction with platelet activating factor in low-serum/albumin culture conditions.⁶ Indeed, higher concentrations of IL-5 have been detected in the middle ear effusions of EOM patients.⁹ Other

Peer review under responsibility of Japanese Society of Allergy.

<https://doi.org/10.1016/j.alit.2017.11.007>1323–8930/Copyright © 2017, Japanese Society of Allergy. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Table 1
Clinical and pathological characteristics of EOM patients.

Clinical characteristics											Pathological characteristics of ear effusion			
Case	Age	Sex	Asthma	Allergic rhinitis	CRSwNP	Blood Eo (%)	Serum IgE (IU/mL)	Perforation	Hearing loss	Hearing levels (dB)	Viscous mucin	Eo	Cytolytic Eo	Histon +ve ETs
1	25	M	+	+	+	15.9	1010	+	md	45.0	+	+++	++	+
2	34	M	+	+	+	8.4	104	+	md	36.7	+	+++	++	+
3	48	F	+	+	+	32.9	184	+	sn	42.5	+	++	++	+
4	29	M	+	–	+	2.7	364	+	cd	32.5	+	++	++	+
5	54	F	+	+	+	1.2	975	+	cd	30.0	+	+++	++	+
6	72	F	+	+	+	1.3	1348	+	cd	40.0	+	+	++	+
7	52	M	+	+	+	9.8	154	+	sn	36.7	+	+++	++	+
8	29	F	+	+	+	17.1	698	+	sn	43.5	+	+++	++	+

CRSwNP, chronic rhinosinusitis with nasal polyps; Eo, eosinophils; IgE, immunoglobulin E; md, mixed hearing loss; cd, conductive hearing loss; sn, sensorineural hearing loss; ETs, extracellular DNA traps. Hearing level (dB): Air conduction hearing level (dB).

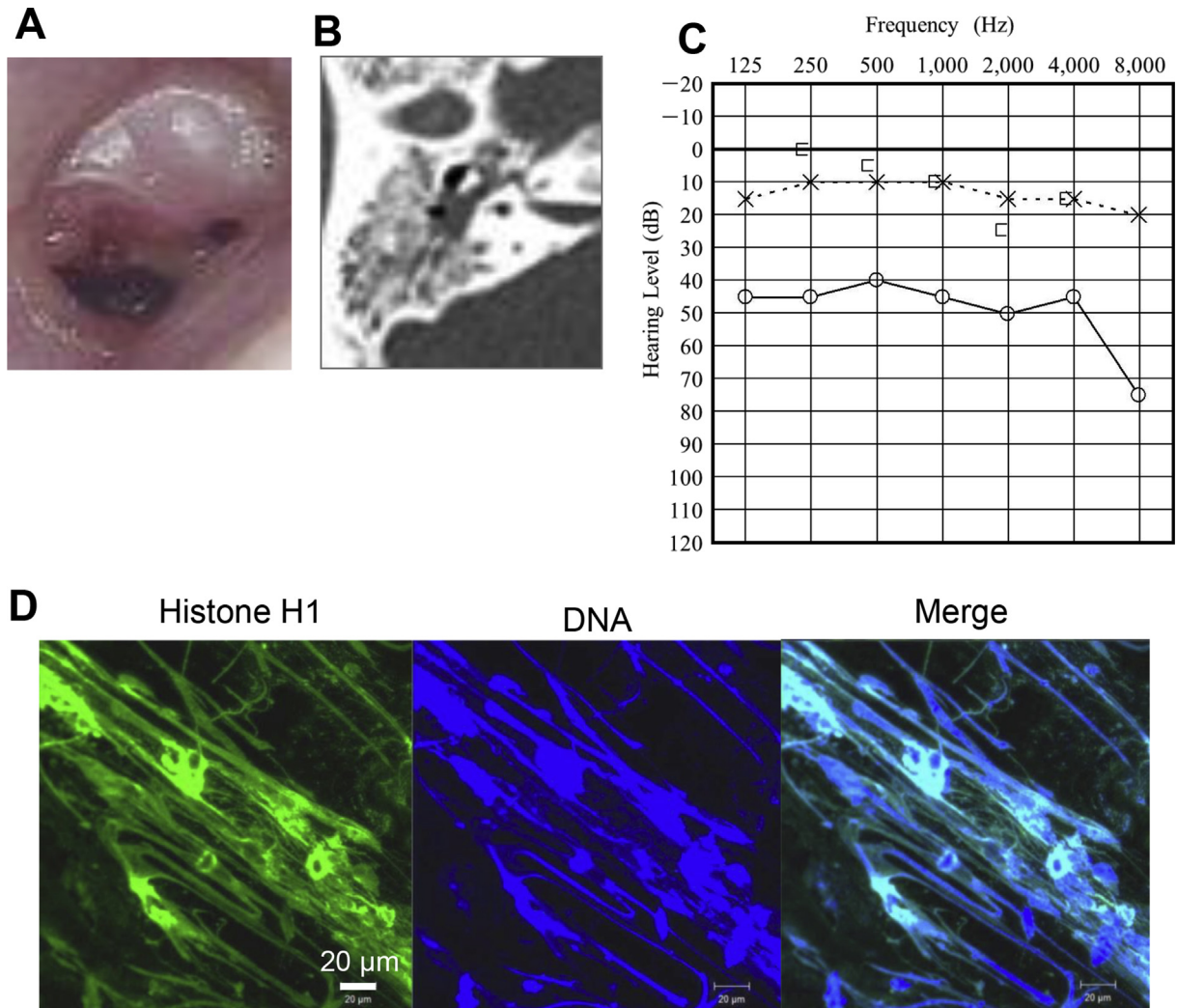


Fig. 1. Eosinophilic otitis media affecting a 25-year-old man (case 1) referred to our department because of antibiotic-resistant right middle ear effusion. (A) Otoendoscopic image of perforation of tympanic membrane associated with granulation and highly viscous discharge on admission. (B) CT scan shows soft tissue density mass in the meso-tympanic cavity. (C) Audiogram shows right conductive hearing loss of 25–45 dB across air–bone gap. (D) Section of highly viscous effusion was stained with anti-human nuclear histone H1 monoclonal antibody (green) and DNA stain (Hoechst 33342; blue) and analyzed under a Carl Zeiss LSM510 laser scanning confocal microscope. Images show web-like formation of histone, DNA, and both merged. The middle ear effusion and granulation declined rapidly after treatment with 30 mg/day oral prednisolone.

stimuli, including immobilized immunoglobulins, calcium ionophores, fungi, and phorbol myristate acetate, also induce EETosis.^{6,10} Difficult-to-remove, highly viscous eosinophilic mucin might be associated with disease severity.^{4,7} The mechanisms that regulate cytolysis/EETosis and DNA traps in EOM patients are worth further exploring, and their control might be important for developing future therapies.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research (25462675, 16K08926, 17K17611, 17K11363) from the Japan Society for the Promotion of Science and the Ministry of Health, Labor, and Welfare of Japan. We express our sincere thanks to Mrs. Yuko Ohta and Mrs. Noriko Tan for outstanding technical assistance.

Conflict of interest

The authors have no conflict of interest to declare.

Nobuo Ohta ^{a,b,*}, Shigeharu Ueki ^c, Yasunori Konno ^c,
Makoto Hirokawa ^c, Toshinori Kubota ^{b,d},
Sachiko Tomioka-Matsutani ^b, Takahiro Suzuki ^b, Yusuke Ishida ^e,
Tasuku Kawano ^f, Tomomitsu Miyasaka ^f, Tomoko Takahashi ^f,
Tatsutoshi Suzuki ^g, Isao Ohno ^h, Seiji Kakehata ^d,
Shigeharu Fujieda ⁱ

^a Division of Otolaryngology, Tohoku Medical and Pharmaceutical University, Sendai, Japan

^b Yamagata City Hospital Saiseikan, Yamagata, Japan

^c Department of General Internal Medicine and Clinical Laboratory Medicine, Akita University Graduate School of Medicine, Akita, Japan

^d Department of Otolaryngology Head and Neck Surgery, Yamagata University Faculty of Medicine, Yamagata, Japan

^e Division of Anatomy and Cell Biology, Tohoku Medical and Pharmaceutical University, Sendai, Japan

^f Division of Pathophysiology, Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, Sendai, Japan

^g Department of Otolaryngology Head and Neck Surgery, Kitasato University Faculty of Medicine, Sagami, Japan

^h Center for Medical Education, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, Sendai, Japan

ⁱ Division of Otorhinolaryngology – Head & Neck Surgery, Department of Sensory and Locomotor Medicine, Faculty of Medical Science, University of Fukui, Fukui, Japan

* Corresponding author. Division of Otolaryngology, Tohoku Medical and Pharmaceutical University, 1-12-1 Fukumuro, Miyagino-ku, Sendai 983-8512, Japan.
E-mail address: noohta@hosp.tohoku-mpu.ac.jp (N. Ohta).

References

1. Lara-Sánchez H, Vallejo LA. Eosinophilic otitis media. *N Engl J Med* 2017;**376**:e10.
2. Iino Y, Tomioka-Matsutani S, Matsubara A, Nakagawa T, Nonaka M. Diagnostic criteria of eosinophilic otitis media, a newly recognized middle ear disease. *Auris Nasus Larynx* 2011;**38**:456–61.
3. Ohta N, Ishida A, Kurakami K, Suzuki Y, Kakehata S, Ono J, et al. Expressions and roles of periostin in otolaryngological diseases. *Allergol Int* 2014;**63**:171–80.
4. Ueki S, Ohta N, Takeda M, Konno Y, Hirokawa M. Eosinophilic otitis media: the aftermath of eosinophil extracellular trap cell death. *Curr Allergy Asthma Rep* 2017;**17**:33.
5. Kita H. Eosinophils: multifaceted biological properties and roles in health and disease. *Immunol Rev* 2011;**242**:161–77.
6. 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.
7. 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.
8. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozłowski E, et al. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. *Nat Med* 2008;**14**:949–53.
9. Iino Y, Kakizaki K, Katano H, Saigusa H, Kanegasaki S. Eosinophil chemoattractants in the middle ear of patients with eosinophilic otitis media. *Clin Exp Allergy* 2005;**35**:1370–6.
10. Muniz VS, Silva JC, Braga YAV, Melo RCN, Ueki S, Takeda M, et al. Eosinophils release extracellular DNA traps in response to *Aspergillus fumigatus*. *J Allergy Clin Immunol* 2017 Sep 21. <https://doi.org/10.1016/j.jaci.2017.07.048>. pii: S0091-6749(17)31477-X.

Received 16 October 2017

Received in revised form 6 November 2017

Accepted 19 November 2017

Available online xxx