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Construction of an irreversible allergic rhinitis-induced olfactory loss mouse model

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ABSTRACT

Clinical data show that part of patients with sinonasal diseases suffered from olfactory dysfunction, especially with allergic rhinitis (AR) and chronic rhinosinusitis (CRS). However, the mechanisms responsible for AR-induced olfactory loss are still largely unknown. Because of the difficulty to obtain human olfactory mucosa, an AR-induced olfactory loss animal model needs to be constructed to clarify the mechanism. The AR mouse model was induced by intraperitoneal sensitizing with ovalbumin (OVA) followed by intranasal challenge lasted from 1 to 12 weeks. For groups with recovery, mice were housed for another 4-week long without any treatment after the last intranasal challenge. Olfactory function, olfactory receptor neurons (ORNs) density and leukocytes infiltration were examined at different time points. Olfactory loss occurs immediately after 1-week intranasal challenge and deteriorates almost to anosmia after 8th week, and after that olfactory loss become irreversible. Nasal inflammation induces significant infiltration of leukocytes into olfactory epithelium (OE), which negatively correlated with the density of ORNs and mouse olfaction in a time dependent manner. The neutrophilic subtype dominates in number amongst the total infiltrated leukocytes, indicating its pivotal role in nasal inflammation-induced olfactory dysfunction. In this study, we constructed a persistent AR-induced olfactory loss mouse model, losing the ability to recover from dysfunction if the disease duration more than eight weeks, which implies that timely treatments are necessary. Meanwhile, this mouse model could provide an easy and reliable system to clarify the mechanisms of AR-induced irreversible olfactory dysfunction.
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1. Introduction

Olfaction is an ancient and critical system in the evolution which plays important roles in safety, nutrition, sexuality, mood, etc [1]. Olfactory dysfunction impairs the ability to discriminate flavors, detect harmful odors from the environment, and decreases the quality of life [2,3]. Recent researches show the prevalence of olfactory dysfunction is 3–20% in the general population [4], which may be underestimated due to the lack of attention (as compared to blindness and deafness). Risks of olfactory dysfunction include age, chronic sinonasal diseases, upper airway infections, severe head trauma and neurodegenerative diseases [5]. Chronic sinonasal diseases, especially allergic rhinitis (AR) and chronic rhinosinusitis (CRS) account for the main causes of olfactory dysfunction [6]. Morbidity of olfactory dysfunction in AR patients ranges from 20% to 40%, with a mild-to-moderate severity [7], which increases to 61%–83% in CRS patients. Nowadays AR-induced olfactory loss has attracted much attention, while the mechanisms responsible for AR-induced olfactory loss are still largely unknown and need further investigation.

Because of the difficulty to obtain and analyze human olfactory mucosa, available animal model needs to be constructed. When odor molecules bind to the receptors on the olfactory cilia, they activate the ion channels of olfactory receptor neurons (ORNs), which transduce the signals through the cribriform plate into olfactory bulb and then transfer to the olfactory center, leading to sense of smell [8]. Several animal models of olfactory loss have been developed by damaging the olfactory pathways, including viruses,

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toxins, transection of the olfactory filaments, or use transgenic mouse that exhibits olfactory ciliary loss [9–12]. It is considered that nasal obstruction prevents odor molecules from binding to the olfactory receptor, then causing olfactory loss. Recently, it is reported that loss of ORNs has been observed in CRS patients [13], which may be the main reasons for olfactory dysfunction. Despite the animal models of olfactory loss constructed by direct impairment of nasal mucosa, there are also limitations that they cannot reflect the main progress of olfactory dysfunction induced by allergic response, which is the main reason of olfactory loss in patients.

The classic animal model of AR was made by ovalbumin (OVA) sensitization and challenge [14]. After nasal challenge, this AR model can show typical symptoms of AR including sneezing and nasal rubbing, as well as increased serum IgE and eosinophil infiltration in the nasal mucosa [15]. Loss of ORNs can also be observed in an AR mouse model [16]. However, these animal studies did not show the dynamic changes of olfactory dysfunction and immunological profile within the olfactory epithelium (OE) in a long period of time. Therefore, the results of AR-induced olfactory loss in a long period of time are still unclear.

In the present study, we attempted to use the mouse model of OVA induced AR to study the olfactory changes, and then observe the dynamic changes of olfactory function in different period of time, using behavioral assessment of olfaction and histopathological analysis of olfactory epithelial, so as to construct a mouse model to mimic AR-induced olfactory loss in patients.

2. Materials and methods

2.1. Animals

Six-week-old male BALB/c mice were purchased from SLAC Laboratory Animal Co. Ltd (Shanghai, China). Mice were maintained under specific-pathogen-free animal laboratory of Changhai Hospital (Shanghai, China) with 12 h light/dark cycle, temperature of 18–22 °C, humidity of 50–60% and OVA-free food and water. The experiments were performed in accordance to the Institutional Animal Care and Use Committee (IACUC) guidelines of Second Military Medical University (Shanghai, China).

2.2. Induction of sinonasal inflammation

Mice were randomly divided into OVA groups and saline groups. In OVA groups, mice were sensitized by intraperitoneally injecting of 25 μg OVA (grade V, Sigma-Aldrich, St. Louis, MO, USA) with 50 μl Imject®Alum (Pierce Biotechnology, Rockford, USA) as adjuvant once a week for 3 weeks, and mice in saline groups received the same dosage of saline. One week after the last sensitization, mice were intranasal challenged with 20 μl OVA (50 mg/ml in saline, OVA groups) or saline only (saline groups) once a day for the duration as Fig. 1D illustrated. For groups with recovery, mice were housed for another four or eight weeks without any treatment after last intranasal challenge.

2.3. Symptom assessment and sample collection

Fifteen minutes after intranasal challenge, frequencies of sneezing and nasal rubbing were measured within a 15 min period at the corresponding time points. Twenty-four hours after the last intranasal challenge, blood samples were collected from the sinus retro-orbital rout under general anesthesia. Nasal tissues including the paranasal parts of the skull were dissected and fixed with 4% PFA. The intact nasal tissues with preserved anatomical structures were collected by carefully removing the bones for further use.

2.4. Olfactory ability test

Mouse olfactory ability was measured by the buried food test as previously described [17]. The testing food should be ensured that highly palatable to the mice. The experimental protocol consists 3 consecutive days. On day 1, mice were placed in clean cages containing a 3-cm layer of fresh cage bedding. The conventional forage was replaced with the testing cookies. Cages were checked the following morning to verify that the cookies were consumed. On day 2, food was removed leaving the mice fasted overnight. On day 3, 24 h after fasting, mice were individually introduced into a clean cage containing fresh bedding and allowed to acclimate to the cage for 5 min before testing. A piece of cookies was buried in a random corner of the cage and the mouse was reintroduced into the cage. The time that the mouse took to retrieve the cookie with its forepaws was recorded in a 15 min limit.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Blood samples were stayed 1 h at room temperature followed by 3000 rpm centrifugation for 10 min and serums were collected. OVA-specific immunoglobulin (Ig) E was measured by mouse serum anti-OVA IgE ELISA kit (Chondrex Kits, Redmond, WA, US) according to the manuals.

2.6. Histopathological analysis

Nasal tissues for histopathological analysis were fixed in 4% PFA for 48 h followed by 1-week decalcification in 10% EDTA, gradient dehydration in ethanol and then embedded with paraffin. Serial coronal sections (3-μm thick) were mounted on glass slides and stained with hematoxylin and eosin (HE). Photos were taken with Olympus BX53 microscope.

2.7. Immunofluorescence (IF) analysis

After fixation in 4% PFA overnight, nasal tissues were dehydrated overnight. Serial cryo-sections (6-μm thick) were mounted on glass slides followed by IF staining. Briefly, sections were rinsed with PBS 5 min for 3 times, permeabilized with 0.5% Triton X-100 (Pierce Biotechnology, Rockford, USA) in PBS for 15 min, blocked with 4% BSA in PBS for 1 h at room temperature followed by incubation with primary antibody for OMP (WAKO, Japan), CD45 (ab10558, Abcam), Ly6G (G11229, Servicebio) or CD3 (ab16669, Abcam) diluted in blocking buffer at 4 °C overnight. Sections were then rinsed with PBS 5 min for 3 times, incubated with Cy3 conjugated donkey anti-goat antibody (1:200, Jackson ImmunoResearch, West Grove, PA, USA), FITC conjugated donkey anti-rabbit antibody (1:400, Jackson ImmunoResearch, West Grove, PA, USA), or Cy3 conjugated donkey anti-rabbit antibody (Servicebio) for 1 h followed by Hoechst 33258 (1 μg/ml, Sigma-Aldrich, USA) for 15 min at room temperature and rinsed with PBS 5 min for 3 times. Slides were mounted with 50% glycerol in PBS and examined with a Nikon ECLIPSE NI-E microscope. TUNEL assay was performed according to the manuals of In Situ Cell Death Detection Kit (Roche). The slides were viewed with a Zeiss LSM 510 confocal laser microscope.

2.8. Immunocytochemistry

Nasal mucosa samples were fixed in PFA and then embedded in paraffin. Dewaxed 3 mm-thick sections were treated with 3% H_2O_2 for 25min to neutralize the activity of endogenous peroxidase followed by blocking with 3% BSA for 30 min at room temperature [18]. Primary antibody for MBP (ab187523, Abcam) or CD68 (GB11067, Servicebio) was added overnight at 4 °C. The next day,

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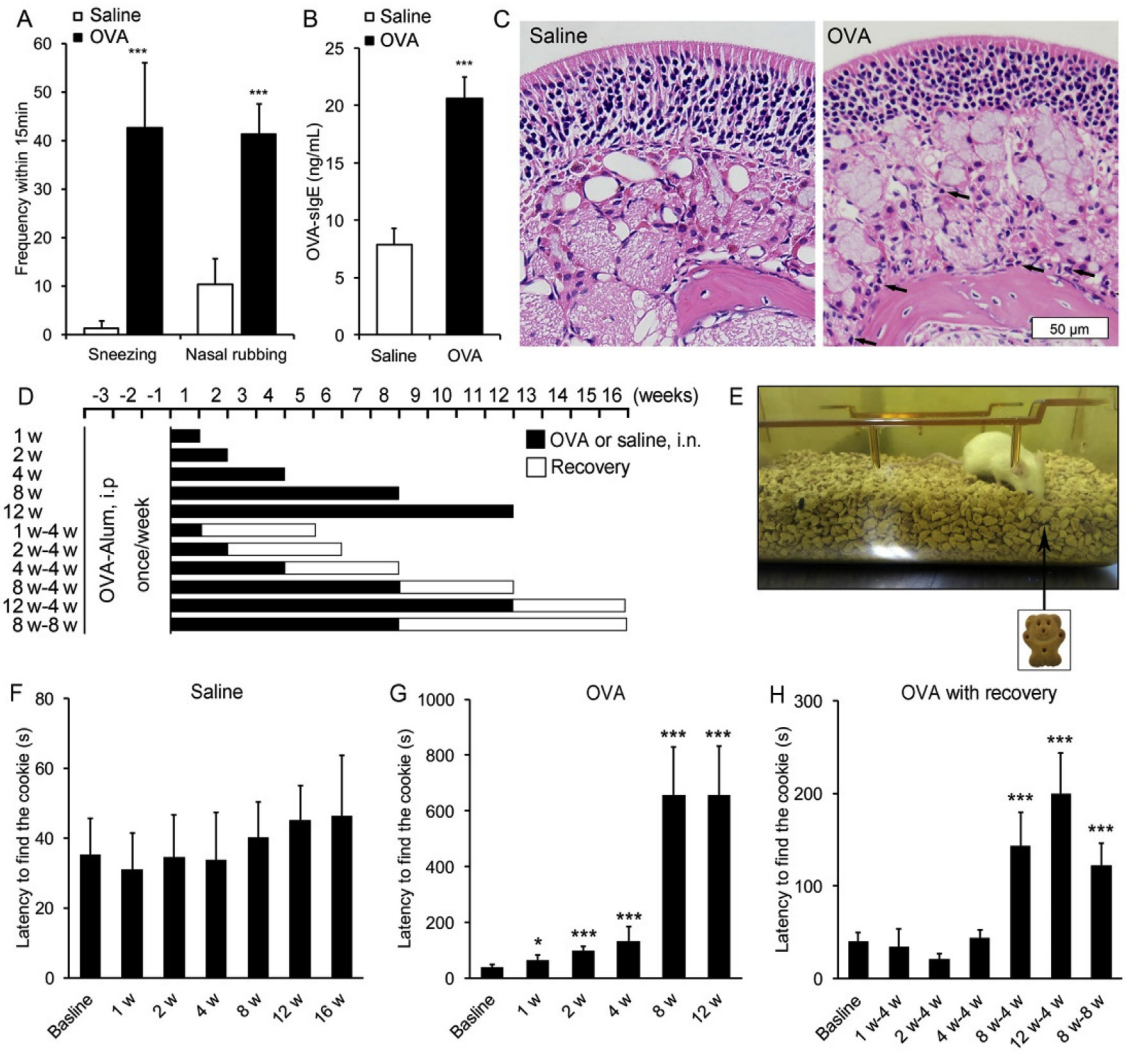


Fig. 1. Establishment of ovalbumin (OVA) induced mouse sinonasal inflammation and evaluation of olfaction. (A) Frequency of sneezing and nasal rubbing are significantly increased in OVA group as compared with saline group. (B) OVA specific IgE in serum is markedly elevated after OVA intranasal challenge. (C) Representative images show a robust infiltration of eosinophil into the olfactory mucosa after OVA challenge (black arrows). (D) Schematic illustration of experimental design. The black bars indicate the duration of treatments (OVA or saline intranasal challenge, i.n.), and the white bars indicate the duration of recovery interval. (E) A picture showing the mouse searching for a buried cookie. (F–H) Statistics of mouse olfaction. Data are shown as mean ± SD (N = 6), *p < 0.05, ***p < 0.001.

sections were rinsed with PBS 5 min for 3 times, incubated with secondary antibody for 50 min at room temperature and then staining was performed with DAB Peroxidase Substrate Kit (DAKO). Subsequently, sections were counterstained with hematoxylin (G1004, Servicebio). Photographs were acquired with Olympus BX53 microscope.

2.9. Statistics

The results are presented as mean ± standard deviation (SD). Statistics were analyzed by the IBM SPSS Ver. 21.0 (IBM, Shanghai, China), and a two-tailed $p < 0.05$ was taken to indicate statistical significance. Mann-Whitney *U* test were used for in-between-group comparisons. Pearson Correlation Coefficient was applied to analyze the correlation between olfactory function and ORNs density, $CD45^+$ cells and ORN density, and $TUNEL^+$ cells and ORN density. All data from histology were measured in the same anatomic location of different mice to control the inter-animal heterogeneity.

3. Results

3.1. Induction of AR mouse model

In order to construct a mouse model with AR, mice were sensitized with OVA and then received intranasal challenge by the same allergen. As described before, mice exhibited typical characteristics of AR, including increased frequency of sneezing and nasal rubbing, elevated level of OVA-specific IgE in serum, and massive infiltration of eosinophils into nasal mucosa when compared with saline group (Fig. 1A–C), demonstrating a successful induction of AR mouse model.

3.2. Olfactory loss continued as AR sustained

To know the olfactory loss in terms of disease duration of AR, mice were continuously intranasal challenged with saline or OVA for 1–12 weeks. Before intranasal challenge, mice showed no abnormality in nasal symptoms of sneezing and nasal rubbing, which were burst out and persisted to the end of experiments after

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intranasal challenge. In the buried food test, mice of OVA groups spent 2–3 times as much the time than saline groups to found the buried cookies during the first four weeks (Fig. 1G). This latency increased to a surprisingly high level at the 8 t h week and thereafter, indicating a mild to severe olfactory loss in acute or early phase sinonasal inflammation, which was deteriorated to anosmia as the inflammation sustained (Fig. 1G).

To further know the recovery potential of olfactory loss after sinonasal inflammation, a 4-week recovery interval was conducted following the corresponding time points [19], and olfaction were evaluated at the end of the interval. Results showed the olfaction recovery potential depends on the duration of AR. Olfaction recovered almost to baseline before four weeks of OVA challenge but not after, even a prolonged recovery interval (as long as eight weeks) has few effects to olfaction recovery as compared to that of four weeks recovery interval (Fig. 1H). Thus, the irreversible AR-induced olfactory loss model was constructed, of which the olfactory loss is not recovered after 8-week recovery interval.

3.3. ORNs loss contributes to olfactory loss after 8-week intranasal challenge

ORNs are the first line of olfaction and injuries to the neurons impair olfactory function directly. IF staining of olfactory marker protein (OMP), a marker of mature ORNs, showed the intensive OMP^+ significantly decreased after 2-week intranasal challenge and the neuronal loss continued thereafter (Fig. 2A and B). Olfactory loss can be measured after 1-week intranasal challenge while the OE seems still intact. This may be explained by the physical congestion of airway by nasal secretion, and when allergen stimulation stopped, the olfaction was easy to recover (Fig. 1H). From 2-week and on, both nasal congestion and ORNs loss may count for the olfactory loss. Before the time point of four weeks, those lost neurons still can be regenerated after a 4-week recovery interval but not when OVA challenge continued. Accordingly, the olfactory loss is irreversible after the time point of eight weeks, even with a prolonged recovery interval (Fig. 1H). Taken together, these data showed a highly correlation of ORNs loss and olfactory loss (Fig. 2C and D), with disease duration around eight weeks as the divide, after which, the neuronal loss along with olfactory loss became irreversible.

3.4. $CD45^+$ leukocytes accumulation may contribute to ORNs loss

Leukocytes recruitment to nasal mucosa is one of the key events in sinonasal inflammation which have been reported by numbers of literatures [20–22]. In this study, IF staining of CD45 showed a massive infiltration of leukocytes into olfactory mucosa immediately after 1-week OVA challenge (Fig. 3A and B). And statistics showed the number of $CD45^+$ cells reached to a peak at the 2-week time point and stayed a high level during OVA challenge continued (Fig. 3B). Of note, most of the leukocytes located beneath or around the basement membrane at early phase (within one week) and gradually migrated toward the surface of mucosa at latter phase (after two weeks) (Fig. 3A). Furthermore, $CD45^+$ cells number was reverse correlated with OMP^+ cells number. This correlation at individual level was in line with that of histological level within an identical mouse, indicating the enhanced of mucosa immunity or a hyper immunological response may contribute to the ORNs loss and subsequently olfactory loss.

3.5. Neutrophils dominated among other cell types

To further investigate the specific subtypes of those infiltrated leukocytes, the eosinophils, neutrophils, T-lymphocytes and macrophages, four main cell types that play important immunological

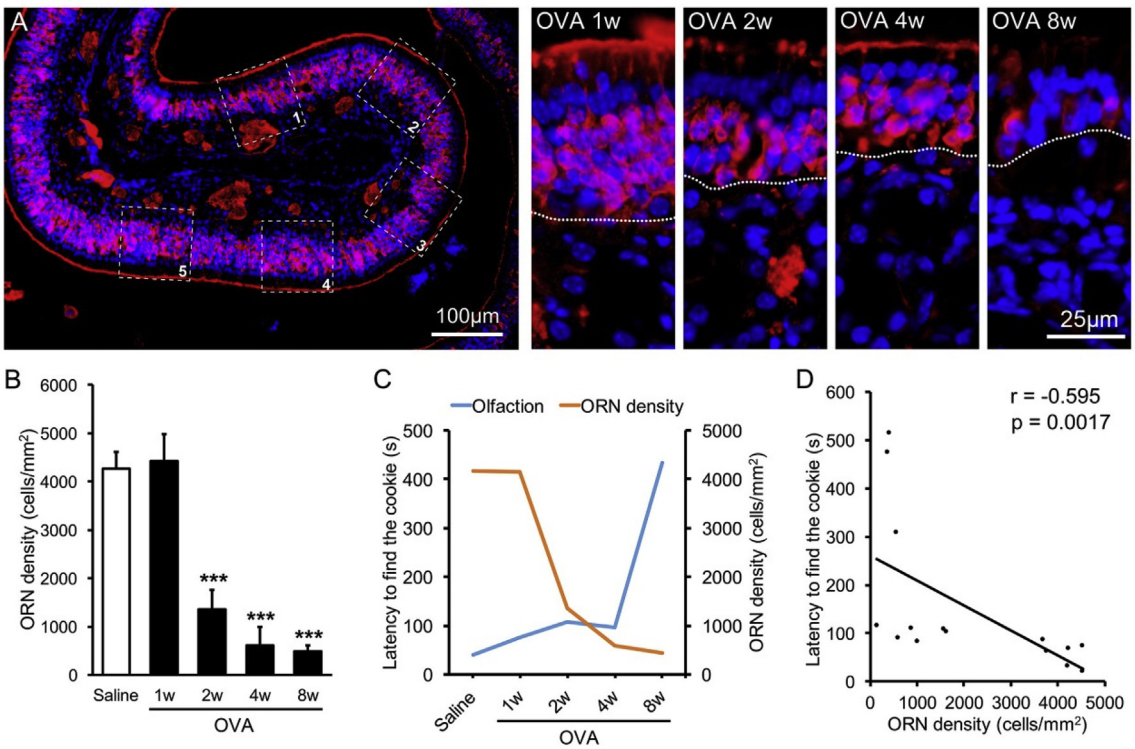


Fig. 2. The neuronal loss of OE in a time dependent manner in terms of sinonasal inflammation. (A) Olfactory marker protein (OMP) IF staining. Mouse challenged with OVA exhibited significant pathological changes to OE with massive neuronal loss when compared with saline group, which the mouse shows healthy turbinate with robust olfactory receptor neurons (ORNs) layers. (B) Statistics of ORNs density. OMP^+ cells are counted within a 0.1 × 0.1 mm area of the epithelial layer and the density is expressed by cells per mm^2. (C and D) The ORNs density is reverse correlated to olfactory loss. Results are expressed as means ± SD (N = 3). All data are at least from 3 pictures/mouse, $^{***}p < 0.001$.

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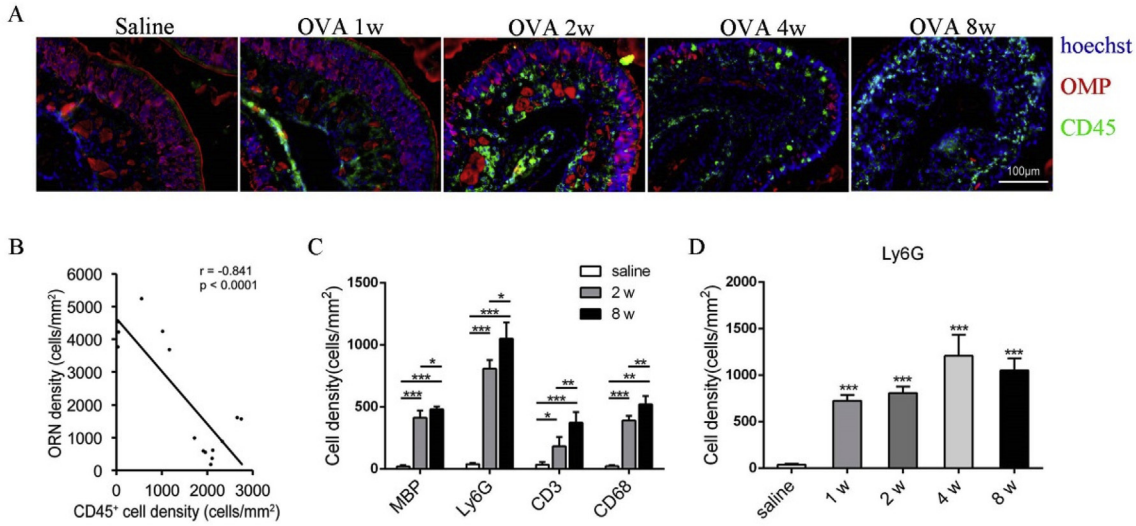


Fig. 3. Leukocytes infiltration in a time dependent manner that positively correlated to ORNs loss. (A) OMP and CD45 double staining demonstrating a continuous leukocytes infiltration corresponding to the ORNs loss when challenged with OVA. (B) The leukocytes infiltration is strictly reverse correlated to ORNs density. (C) Statistics of four immuno-cell subtypes within OE. (D) The number of infiltrated leukocytes increases immediately after OVA challenge. Results are expressed as means ± SD (N = 3). All data are at least from 3 pictures/mouse, $^{**}p < 0.01$, $^{***}p < 0.001$.

roles in AR, were labeled with antibodies to MBP, Ly6G, CD3 and CD68 respectively. Results showed that all of the four subtypes had a marked increase after OVA challenge, among which neutrophils dominated in number during the whole experimental scheme (Fig. 3C and D).

3.6. Apoptotic neutrophils is positively correlated with olfactory loss

It is considered that the apoptotic death of ORNs and supporting cells result in olfactory dysfunction [23,24]. In this study TUNEL assay was used to determine apoptotic cells. Interestingly, a significant increase of TUNEL signals was observed after challenge, but the results of OMP/TUNEL double staining showed the TUNEL signals barely overlaid with OMP throughout the whole experiments (Fig. 4A). The increasing TUNEL cells were accompanied by a looser structure of OM (decreased cell density of Hoechst) and less apical OMP signals (Fig. 4B). Further research found that Ly6G^{+} neutrophils and TUNEL^{+} cells are overlaid, suggesting that the detected

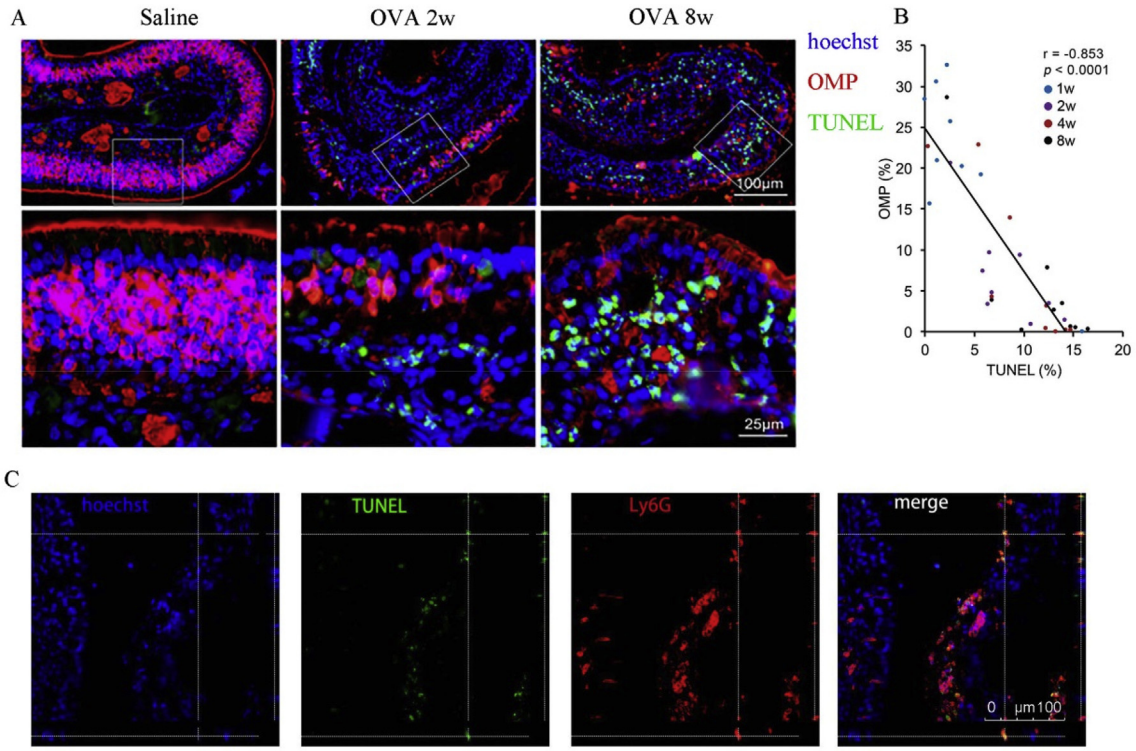


Fig. 4. Apoptotic neutrophils are positively correlated with olfactory Loss. (A) Representative images of OMP IF staining and TUNEL staining. (B) A reverse correlation of cell apoptotic ratio and ORNs cell density. (C) Representative images of Ly6G IF staining and TUNEL staining. Results are expressed as means ± SD (N = 3). All data are at least from 3 pictures/mouse, $^{*}p < 0.05$, $^{**}p < 0.01$.

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apoptotic cells are neutrophils during olfactory loss progression (Fig. 4C), which may contribute to AR-induced olfactory loss.

4. Discussion

In this study, our data showed that inflammation in AR mice model results in significant ORNs loss in a time dependent manner, causing subsequent olfactory loss. Chronic inflammation that lasted longer than eight weeks impaired the regenerative ability of ORNs, leading to irreversible recovery of olfactory loss. The dynamic changes of infiltrated leukocytes in terms of disease duration provided important evidence for the pathology of sinonasal inflammation associated olfactory dysfunction. Domination of neutrophils in number among all immunological cell types during the process of disease implying their pivotal roles in sinonasal inflammation, and may be the core contributors to ORNs loss and olfactory dysfunction. Meanwhile, this mouse model provides an easy and reliable system to clarify the mechanisms of AR-induced irreversible olfactory dysfunction.

To our knowledge, impaired olfaction has been observed in a mouse model of AR [25], while the dynamic changes of olfaction as well as the pathological changes of OE have not been reported yet. ORNs are normally updated every 30–60 days and replaced through the proliferation and differentiation of a series of immature precursors and multipotent progenitor cells [19,26]. In this study, a significant reduction of ORNs was observed within the mice OE after 2-week intranasal challenge. A 4-week interval of stopping intranasal challenge endows the OE recover from injuries, and we found the mice lost the ability to recover to normal level if the disease duration more than eight weeks. It may explain the severity of olfactory dysfunction and its recovery capacity varies with the severity or duration of patients suffered from AR or CRS. It is strongly suggested that early diagnose and treatment are necessary for better recovery of olfactory function.

There is growing evidence that inflammation induced pathological changes of olfactory epithelium may be the main reason for olfactory loss in patients with AR or CRS. It is reported that a reduction of normal OMP^+ ORNs in biopsy specimens from CRS patients [27] and morphological abnormal OMP^+ cells in those patients [28]. A clinical study showed topical steroids are effective in olfactory improvement in AR patients when applied in combination with an antihistamine [29]. In this study, the general cell density of OM is statistically different between OVA and saline groups at 8 t h week. One explanation for this is that ORNs homeostasis is broken by infiltrated leukocytes. By inducing cytotoxicity and neurotoxicity, leukocytes play important roles in AR-induced olfactory loss [30]. It is remarkable that few apoptosis was found in OMP^+ cells, but most of the TUNEL signals were $Ly6G^+$ neutrophils. The reverse correlation of infiltrated leukocytes to ORNs or olfactory loss further suggests that sinonasal inflammation should be treated timely to prevent the olfactory loss exacerbation. Meanwhile, the olfactory loss may also be indicative for the disease severity like AR or CRS.

In conclusion, the present study describes the olfaction changes and recovery capacities in terms of disease durations in AR mice model of OVA induced sinonasal inflammation, and finds that the duration of 8-week as the divide between reversible and irreversible olfactory loss. Our research suggests that a mild olfactory loss induced by acute or short term sinonasal inflammation is favorable to recover once the inflammation is cured; a moderate olfactory loss in the early phase of chronic inflammation still have the potential to recover for the robust regeneration capability of OE; when the stem cells or progenitors of OE lost its regenerative ability or died when uncontrolled chronic inflammation sustained for enough long, anosmia as the consequence may be irreversible.

Disclosure statement

The authors have no conflicts of interest to declare.

Author contributions

Caiquan Liang and Zixuan Yang performed the experiments, collected the data, and contributed equally to the work as first authors. Qingyun Zou and Mengxia Zhou contributed to the data analysis and revision of the article. Jingping Fan and Huanhai Liu supervised the study, wrote the article and contributed equally as senior authors.

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References

[1] R.M. Patel, J.M. Pinto, Olfaction: anatomy, physiology, and disease, Clin. Anat. 27 (2013) 54–60.
[2] I. Croy, S. Nordin, T. Hummel, Olfactory disorders and quality of life–an updated review, Chem. Senses 39 (2014) 185–194.
[3] J.M. Pinto, Olfaction, Proc. Am. Thorac. Soc. 8 (2011) 46–52.
[4] P. Rombaux, C. Huart, P. Levie, C. Cingi, T. Hummel, Olfaction in chronic rhinosinusitis, Curr. Allergy Asthma Rep. 16 (2016) 41.
[5] S. Boesveldt, E.M. Postma, D. Boak, A. Welgeluessen, V. Schöpf, J.D. Mainland, J. Martens, J. Ngai, V.B. Duffy, Anosmia-a clinical review, Chem. Senses 42 (2017) 513.
[6] K. Enriquez, E. Lehrer, J. Mullol, The optimal evaluation and management of patients with a gradual onset of olfactory loss, Curr. Opin. Otolaryngol. Head Neck Surg. 22 (2014) 34–41.
[7] B.A. Stuck, T. Hummel, Olfaction in allergic rhinitis: a systematic review, J. Allergy Clin. Immunol. 136 (2015) 1460–1470.
[8] C.Y. Su, K. Menuz, J.R. Carlson, Olfactory perception: receptors, cells, and circuits, Cell 139 (2009) 45–59.
[9] N. El-Habashi, E.N. El-S, H. Fukushi, D. Hibi, H. Sakai, V. Sasseville, T. Yanai, Experimental intranasal infection of equine herpesvirus 9 (EHV-9) in suckling hamsters: kinetics of viral transmission and inflammation in the nasal cavity and brain, J. Neurovirol. 16 (2010) 242–248.
[10] R. Ueha, S. Ueha, K. Kondo, T. Sakamoto, S. Kikuta, K. Kanaya, H. Nishijima, K. Matsushima, T. Yamasoba, Damage to olfactory progenitor cells is involved in cigarette smoke-induced olfactory dysfunction in mice, Am. J. Pathol. 186 (2016) 579–586.
[11] Z.F. Sun, X. Gao, J.M. Pinto, Y. He, Q.X. Yang, J. Tian, Q.W. Lv, Y.X. Wei, Morphological evaluation using MRI of the olfactory filaments (fila) in a post-traumatic olfactory rat model, World J. Otorhinolaryngol. d Neck Surg. 4 (2018) 50–56.
[12] J. Li, T. Ishii, P. Feinstein, P. Mombaerts, Odorant receptor gene choice is reset by nuclear transfer from mouse olfactory sensory neurons, Nature 428 (2004) 393.
[13] R.C. Kern, Candidate's thesis: chronic sinusitis and anosmia: pathologic changes in the olfactory mucosa, Laryngoscope 110 (2010) 1071–1077.
[14] H. Saito, K. Matsumoto, A.E. Denburg, L. Crawford, R. Ellis, M.D. Inman, R. Sehmi, K. Takatsu, K.I. Matthaei, J.A. Denburg, Pathogenesis of murine experimental allergic rhinitis: a study of local and systemic consequences of IL-5 deficiency, J. Immunol. 168 (2002) 3017–3023.
[15] J.H. Mo, S.E. Lee, J.H. Wee, J.E. Lee, C.S. Rhee, C.H. Lee, D.Y. Kim, Anti-allergic effects of So-Cheong-Ryong-Tang, a traditional Korean herbal medicine, in an allergic rhinitis mouse model, Eur. Arch. Oto-Rhino-Laryngol. 270 (2013) 923–930.
[16] A.P. Lane, J. Turner, L. May, R. Reed, A genetic model of chronic rhinosinusitis-associated olfactory inflammation reveals reversible functional impairment and dramatic neuroepithelial reorganization, J. Neurosci. Off. J. Soc. Neurosci. 30 (2010) 2324–2329.
[17] M. Yang, J.N. Crawley, Simple Behavioral Assessment of Mouse Olfaction, 2009 (Chapter 8):Unit 8.24.
[18] J. Hou, Y. Zhou, Y. Zheng, J. Fan, W. Zhou, I.L. Ng, H. Sun, L. Qin, S. Qiu, J.F. Lee, Hepatic RIG-I predicts survival and interferon-α therapeutic response in hepatocellular carcinoma, Cancer Cell 25 (2014) 49–63.
[19] M. Takanosawa, H. Nishino, Y. Ohta, K. Ichimura, Glucocorticoids enhance regeneration of murine olfactory epithelium, Acta Otolaryngol. 129 (2009) 1002.
[20] P.P. Cao, H.B. Li, B.F. Wang, S.B. Wang, X.J. You, Y.H. Cui, D.Y. Wang,

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M. Desrosiers, Z. Liu, Distinct immunopathologic characteristics of various types of chronic rhinosinusitis in adult Chinese, J. Allergy Clin. Immunol. 124 (2009) 478–484, e472.
[21] H. Wang, Z.Y. Li, W.X. Jiang, B. Liao, G.T. Zhai, N. Wang, Z. Zhen, J.W. Ruan, X.B. Long, H. Wang, The activation and function of IL-36γ in neutrophilic inflammation in chronic rhinosinusitis, J. Allergy Clin. Immunol. (2017) 141.
[22] Y. Haenuki, K. Matsushita, S. Futatsugiyumikura, K.J. Ishii, T. Kawagoe, Y. Imoto, S. Fujieda, M. Yasuda, Y. Hisa, S. Akira, A critical role of IL-33 in experimental allergic rhinitis, J. Allergy Clin. Immunol. 130 (2012) 184–194, e111.
[23] P. Assanasen, P. Choochurn, W. Banhiran, C. Bunnag, Radiofrequency inferior turbinate reduction improves smell ability of patients with chronic rhinitis and inferior turbinate hypertrophy, Allergy Rhinol. 5 (2014) 12–16.
[24] R.S. Jiang, F.J. Lu, K.L. Liang, J.Y. Shiao, M.C. Su, C.H. Hsin, W.K. Chen, Olfactory function in patients with chronic rhinosinusitis before and after functional endoscopic sinus surgery, Am. J. Rhinol. 22 (2008) 445.
[25] S. Ozaki, K. Toida, M. Suzuki, Y. Nakamura, N. Ohno, T. Ohashi, M. Nakayama, Y. Hamajima, A. Inagaki, K. Kitaoka, Impaired olfactory function in mice with allergic rhinitis, Auris Nasus Larynx 37 (2010) 575–583.
[26] P.P. Graziadei, G.A. Graziadei, Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons, J. Neurocytol. 1 (1979) 1–18.
[27] K.K. Yee, E.A. Pribitkin, B.J. Cowart, D. Rosen, P. Feng, N.E. Rawson, Analysis of the olfactory mucosa in chronic rhinosinusitis, Ann. N. Y. Acad. Sci. 1170 (2009) 590–595.
[28] E.H. Holbrook, D.A. Leopold, J.E. Schwob, Abnormalities of axon growth in human olfactory mucosa, Laryngoscope 115 (2010) 2144–2154.
[29] L. Klimek, S.C. Poletti, A. Sperl, M. Spielhaupter, C. Bardenhewer, J. Mullol, K. Hā¶Rmann, T. Hummel, Olfaction in patients with allergic rhinitis: an indicator of successful MP-AzeFlu therapy, Int. Forum Allergy Rhinol. 7 (2017) 287–292.
[30] H. Lou, Y. Meng, Y. Piao, N. Zhang, C. Bachert, C. Wang, L. Zhang, Cellular phenotyping of chronic rhinosinusitis with nasal polyps, Rhinology 54 (2016) 150.

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