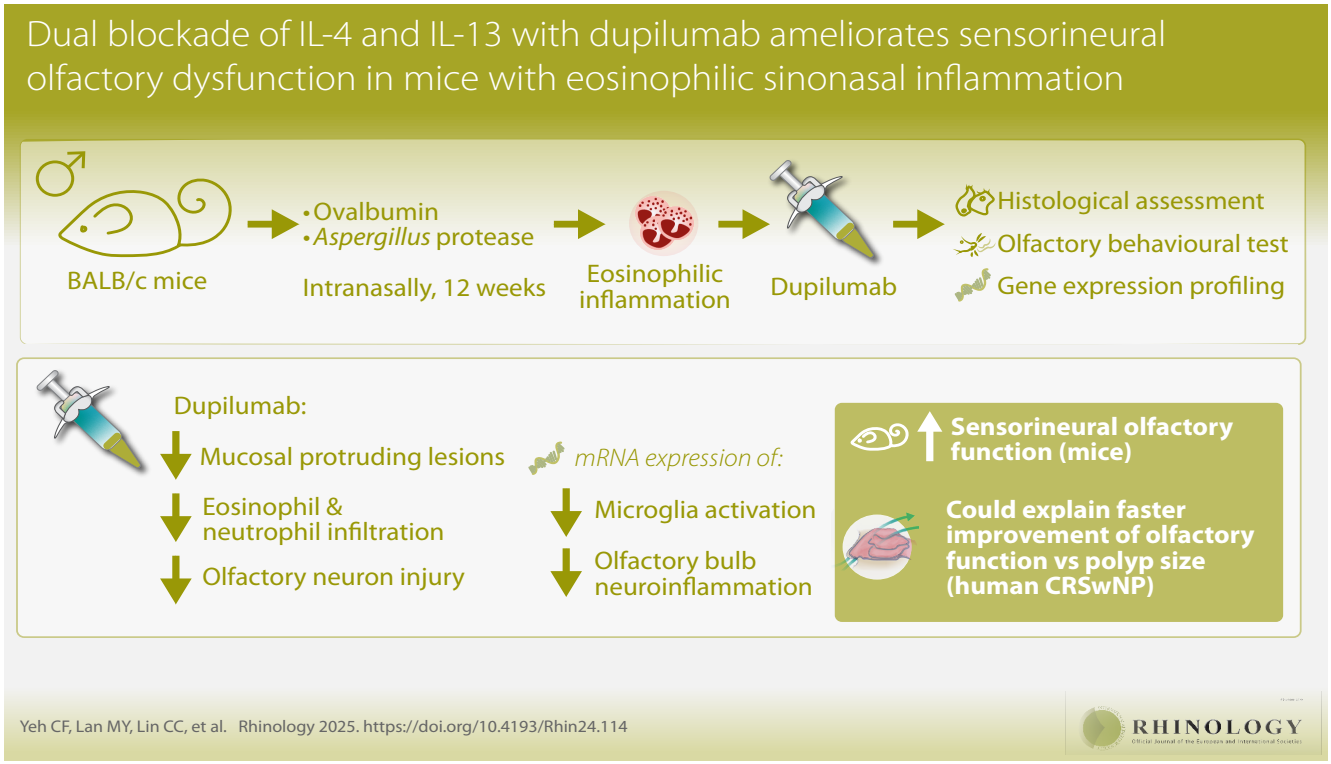
Dual blockade of IL-4 and IL-13 with dupilumab ameliorates sensorineural olfactory dysfunction in mice with eosinophilic sinonasal inflammation

Chien-Fu Yeh[1,2], Ming-Ying Lan[1,2], Ching-Chia Lin[1], Yu-Wen Hung[3], Wei-Hao Huang[1], Yi-Ling Lai[2]

Rhinology 63: 1, 32 - 42, 2025
https://doi.org/10.4193/Rhin24.114



Abstract

Background: Dupilumab, an antibody that binds IL-4Rα and inhibits IL-4 and IL-13 signals, has demonstrated efficacy in chronic rhinosinusitis with nasal polyps (CRSwNP) primarily characterized by type 2 inflammation. Current evidence suggests that the rate of improvement in olfactory dysfunction with dupilumab exceeds that of nasal polyp reduction, yet the underlying mechanism remains undisclosed. We hypothesize that dupilumab may initially ameliorate sensorineural olfactory dysfunction. **Methodology**: Male BALB/c mice were intranasally administered ovalbumin and *Aspergillus* protease for 12 weeks to induce eosinophilic sinonasal inflammation. Dupilumab treatment was also administered. The mice underwent histological assessment, olfactory behavioural test, and gene expression profiling to identify neuroinflammatory markers within the olfactory bulb. **Results**: Dupilumab treatment resulted in a reduction in the number of mucosal protruding lesions, as well as decreased infiltration of eosinophils and neutrophils, along with a decrease in olfactory sensory neuron injury. Furthermore, there was a downregulation in the mRNA expression related to microglia activation and neuroinflammation in the olfactory bulb. **Conclusions**: Dupilumab improves the sensorineural pattern of olfactory dysfunction in mice, potentially explaining why olfaction improves more rapidly than polyp reduction in patients with CRSwNP.

Key words: chronic rhinosinusitis, dupilumab, eosinophil, neuroinflammation, olfactory dysfunction

Introduction

Chronic rhinosinusitis (CRS) is characterized by inflammation of the nasal cavity and sinuses lasting more than 12 weeks. Historically, the classification of CRS was primarily based on phenotype. However, in recent years, there has been a shift towards a classification primarily centred on the presence or absence of type 2 inflammatory responses [1]. Conventional treatments involve medications and surgical intervention, with biologic agents emerging as potential options for treating patients with severe CRS exhibiting type 2 inflammation [2].

Typically, odour molecules enter the olfactory epithelium, and the signals are transmitted by olfactory sensory neurons to the olfactory bulb, eventually reaching the olfactory cortex. Olfactory dysfunction, including both conductive and sensorineural patterns, occurs in up to 84% of individuals diagnosed with CRS [3]. According to the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020 guidelines, a decreased sense of smell and taste is associated with type 2 disease more than type 1 disease [1]. Any obstruction in the sinonasal cavity, such as a large nasal polyp, can impede the passage of odour molecules. Additionally, any impairment along this neural pathway can result in sensorineural olfactory dysfunction. Olfactory receptor neurons (ORNs), also known as olfactory sensory neurons (OSNs), can be identified by olfactory marker protein (OMP) [4]. Previous in vivo studies have demonstrated a positive correlation between sensorineural olfactory dysfunction and reduced expression of OMP [5].

Currently, there are several biologic agents, including dupilumab, an interleukin (IL)-4Rα antibody that can block both IL-4 and IL-13 signals [6]. In recent years, the SINUS 24 and SINUS 52 trials have demonstrated the efficacy of dupilumab in treating chronic rhinosinusitis with nasal polyps (CRSwNP) [7]. Nasal polyp size reduction and olfactory improvement after dupilumab treatment have been demonstrated in several studies using real-world clinical data [6,8-10]. After two weeks of initiating dupilumab, some patients showed a reduction in nasal polyps, while improvements in olfactory performance could be noticeable as early as three days into the treatment for some patients [11-13]. Currently, there is no exact mechanism supporting the inconsistency in the timeline of improvement between nasal polyps and olfactory dysfunction. Our hypothesis suggests that the use of dupilumab may initially improve damage to the olfactory nervous system, thereby improving olfactory dysfunction.

Materials and methods

Animals

Male BALB/c mice aged four weeks and weighing between 22-25g were procured from BioLASCO (Taipei, Taiwan). Female mice were deliberately omitted to minimize the potential influence of sex hormones. These mice were housed in sterile cages under a 12-hour light/dark cycle at a consistent temperature of 25 ± 2 °C, with unrestricted access to water and food. All animal experiments adhered to the National Institute of Health guidelines and received approval from the Institutional Animal Care and Utilization Committee of Taipei Veterans General Hospital.

Induction of eosinophilic sinonasal inflammation with olfactory dysfunction and administration of dupilumab

The intranasal administration in mice was carried out according to a protocol previously outlined by Kim et al., with some adjustments [14,15]. After a one-week acclimatization period, a solution containing 75 µg of ovalbumin (OVA) (grade V; Sigma-Aldrich, St. Louis, MO, USA) and 2U of *Aspergillus* protease (AP) (Sigma-Aldrich) was diluted in phosphate-buffered saline (PBS) to a total volume of 20 µL. This mixture was then intranasally administered into each naris (10µL/naris) three times a week for a duration of 12 weeks. A separate control group received intranasal administration of 20 µL of PBS. Following nasal instillation, the mouse's head was maintained in a downward position to prevent the reagent from entering the lungs. To minimize potential anaesthesia-induced mortality, morbidity and inefficiency, the intranasal administration was conducted without anaesthesia. In mice receiving dupilumab treatment, the drug was administered at 10mg/kg intraperitoneally either every other week or once a week for a period of 4 weeks after the induction of OVA/AP for 8 weeks. The details of the four groups mentioned above are also presented in Figure 1A.

Histological assessment

Following the 12-week experimental period, mice were anesthetized through intraperitoneal injection of Zoletil (30 mg/kg) and Xylazine (8 mg/kg). Post-anaesthesia, mice were decapitated, and the head and mandible's skin was removed. The collected samples underwent fixation in 4% paraformaldehyde and subsequent decalcification in a 10% ethylenediaminetetraacetic acid solution in PBS over 14 days at 4°C. After decalcification, tissues were dehydrated, embedded in paraffin, and sectioned into 4-µm-thick coronal slices. Selected sections, located just anterior to the eyes' anterior border, underwent haematoxylin and eosin (H&E) staining for eosinophil count and respiratory mucosal thickness measurement. Immunohistochemistry analysis employed a fully automated Bond-Max staining system (Leica Microsystems, Vista, CA, USA) with antibodies against eosinophil major basic protein (MBP) antibody (BMK-13, 1:100, #NBP1-42140, Novus Biologicals, USA), Myeloperoxidase (MPO, 1:6000; #E-AB-70091, Elabscience, USA), and olfactory marker protein (OMP, 1:6000; #ab183947, Abcam Inc., Cambridge, MA, USA). Eosinophil count and the presence of mucosal protruding lesions were also confirmed through sections stained with anti-eosinophil MBP. Eosinophil infiltration, and microcavity

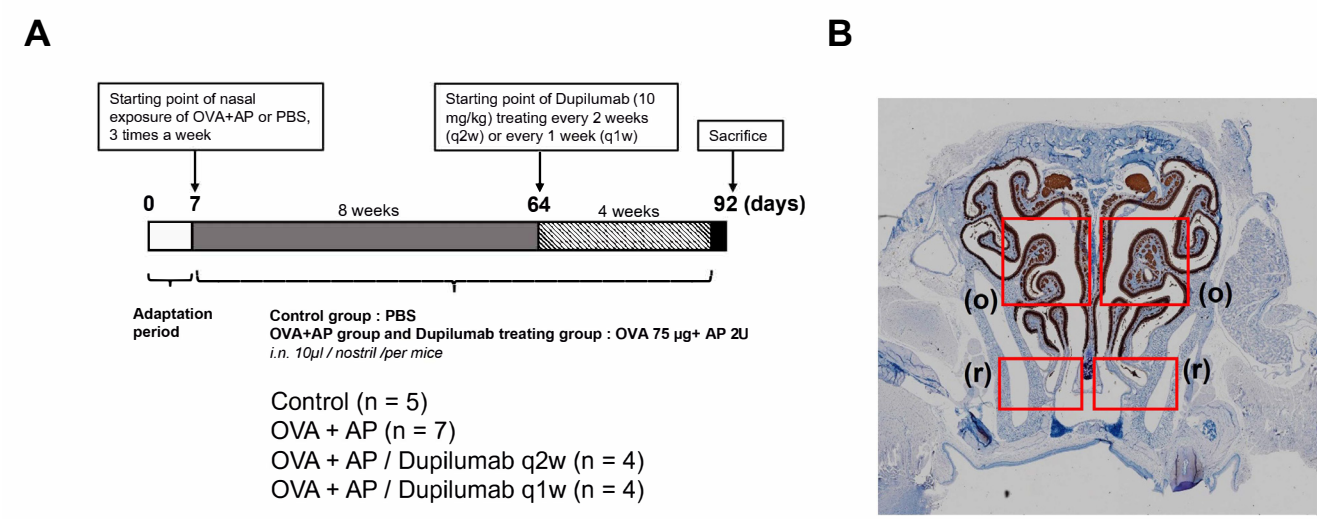


Figure 1. (A) Experimental protocol for intranasal administration of OVA/AP. Four groups of mice were subjected to treatment: OVA/AP (n = 7), PBS (control group, n = 5), OVA/AP + dupilumab treatment every 2 weeks (n = 4), and OVA/AP + dupilumab treatment every week (n = 4). Following a 1-week adaptation period, the mice received intranasal administration of OVA/AP or PBS three times per week. The dupilumab treatment groups were administered dupilumab starting 8 weeks after the initial OVA/AP administration. (B) The section selected for neutrophil and eosinophil infiltration evaluation is positioned anterior to the anterior border of the eyes in the mice. Two olfactory areas (o) and two respiratory areas (r) for measurement are chosen in each mouse (red box). OVA, ovalbumin; AP, *Aspergillus* protease; q2w, every 2 weeks; q1w, every 1 week; i.n., intranasal.

formation were also recorded [16]. Microcavities located near the surface of the sinus lumen are about to rupture and connect to the surrounding sinus cavity. Eventually, the stalk of a polypoid lesion forms [16]. Neutrophil infiltration was quantified using anti-MPO sections. The average eosinophil and neutrophil count were determined in a high-power field (HPF, x400) in two respiratory and two olfactory areas (Figure 1B). Additionally, the OMP signal in the olfactory epithelium was measured, including the sublayer containing OMP-positive (OMP+) axon bundles. The OMP+ sections were utilized to measure the thickness of the olfactory epithelium from the nasal septum. The brown-coloured OMP+ area and length were quantified using the thresholding method with ImageJ software. All sections underwent examination under a light microscope (Ni-E system) by two independent examiners blinded to the experimental groups, and three consecutive slides were reviewed to exclude processing errors.

Olfactory behaviour test - buried food test

The buried food test involves burying food 1 cm beneath fresh bedding to assess each mouse's ability to detect odours [17]. Mice underwent a 20-hour fasting period before and after 12 weeks of OVA/AP intranasal administration. For habituation, each mouse spent 10 minutes in an individual cage with fresh bedding. Concurrently, a piece of standard chow (Laboratory Autoclavable Rodent Diet 5010; PMI Nutritional International) was buried 1 cm under the fresh bedding in the middle of each cage, with the mouse placed in the corner. The latency time, representing the duration it took for a mouse to locate and begin consuming the buried food after it started moving, was recorded. To eliminate considerations related to motor disorders or changes in food motivation, an exposed food test, where food was placed on the surface of the fresh bedding, was subsequently conducted for each mouse following the buried food test.

Measurement of the mRNA of neuronal and microglia markers and inflammatory cytokine expressions in olfactory bulb

After 12 weeks of intranasal administration of OVA/AP/PBS, the mice were euthanized, and their olfactory bulb tissues were collected for total RNA extraction. Subsequently, cDNA was synthesized using a reverse transcription kit. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed on an ABI QuantStudio 3 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with SYBR green PCR Master Mix (Vazyme Biotech, Nanjing, China). The $2^{-\Delta\Delta CT}$ method was employed to determine the relative expression levels of the target mRNAs as fold changes compared to the control, with olfactory bulb samples normalized to GAPDH expression. The primer sequences used for PCR are provided in Supplementary Table 1.

Statistical analysis

Statistical analyses were performed using SPSS 17.0 software (SPSS, Inc). Data were presented as mean ± standard deviation. Multiple group comparisons were conducted using one-way ANOVA with LSD post hoc test. Pearson's correlation was em-

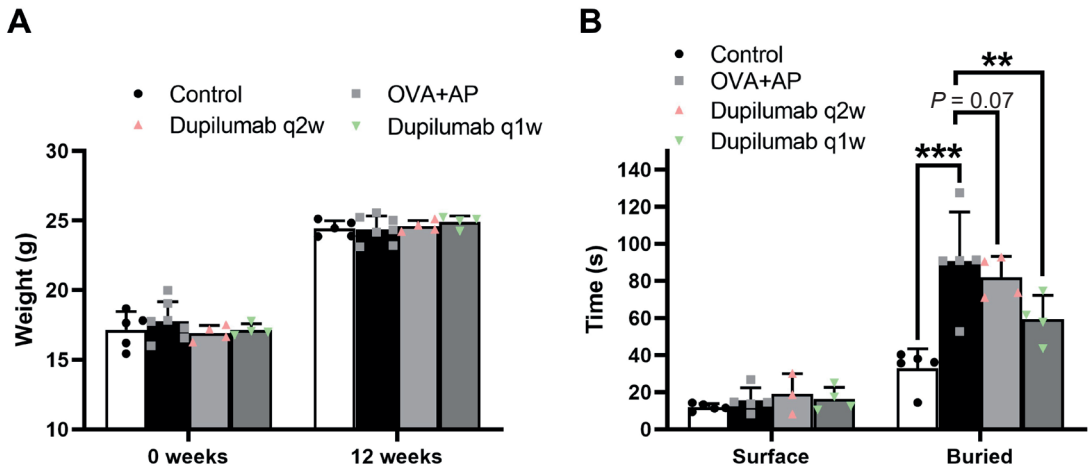


Figure 2. Weight changes and olfactory behaviour test results after OVA/AP induction and dupilumab treatment. (A) There were no significant differences in the body weight of the four mouse groups before the experiment. After 12 weeks of experimentation, all four groups showed weight growth, with no noticeable differences between the groups. (B) In the buried food test, the OVA/AP-treated group (n=5) showed a significantly longer time spent finding the hidden food compared to the control group (n=5). Following dupilumab treatment (n=4), the time spent locating the concealed food decreased, particularly in mice receiving weekly dupilumab. No differences were observed between the groups in finding the visible food (n=3-5 in each group). Data are presented as mean ± SD, with **P < 0.01 and ***P < 0.001 indicating statistical significance. OVA, ovalbumin; AP, *Aspergillus* protease; q2w, every 2 weeks; q1w, every 1 week.

ployed to examine the correlation between the OMP positive area and length and the latency of the buried food test. Statistical significance was considered at P < 0.05.

Results

Improvement of olfactory function after dupilumab treatment

We induced eosinophilic sinonasal inflammation in mice by intranasal injection of OVA/AP and treated them with dupilumab at different frequencies. There were no significant differences in body weight among the control group (n=5), OVA/AP group (n=7), and two Dupilumab treatment groups (n=4) before and after the experiment (Figure 2A), indicating that both OVA/AP induction and Dupilumab treatment did not affect the mice's growth. The buried food test (n=4-5 in each group) was used to assess olfactory function. Following intranasal injection of OVA/AP, the mice exhibited a significant increase in the time spent searching for food, indicating a deterioration in olfactory function (P < 0.001). Treatment with dupilumab every other week did not improve olfactory function. However, treatment with dupilumab once a week significantly reduced the time spent searching for food, suggesting an improvement in olfactory function (P < 0.01). When food was placed on the surface, there were no statistical differences between groups, indicating that mice in all groups (n=3-5) had the motivation and motor ability to search for food (Figure 2B).

Reduction of sinonasal mucosal thickness, mucosal protruding lesions and inflammatory cell infiltration by dupilumab

We measured respiratory area mucosal thickness, protruding lesions, eosinophils, and neutrophils in the sinonasal mucosa of the control group mice (n=5), mice exposed to OVA/AP administration (n=6-7), and mice subjected to OVA/AP administration treated with dupilumab every 2 weeks (n=3-4) or every week (n=4). After OVA/AP induction, mucosal thickness (Figure 3A), mucosal protruding lesions (Figure 3B), eosinophils (Figure 4 A,B), and neutrophils (Figure 4C) were significantly higher than in the control group (all P < 0.001). Following dupilumab treatment, mucosal thickness (Figure 3A) and mucosal protruding lesions (Figure 3B) were significantly lower than in the OVA/AP-induced group (all P < 0.01). Similarly, eosinophil (Figure 4 A,B) and neutrophil (Figure 4C) infiltration were significantly lower than in the OVA/AP-induced group after once-weekly dupilumab treatment (all P < 0.05).

Reduction of damage to olfactory sensory neurons by dupilumab

We quantified olfactory sensory neurons in the nasal cavity through OMP staining in the control group mice (n=5), mice exposed to OVA/AP administration (n=4), and mice subjected to OVA/AP administration treated with dupilumab every 2 weeks (n=4) or every week (n=3) (Figure 5A,B). Following OVA/AP induction, the distribution area and length of olfactory sensory neurons in the nasal septum decreased significantly compared to the control group (all P < 0.001), indicating nerve damage. Dupilumab treatment mitigated this condition (all P < 0.05).

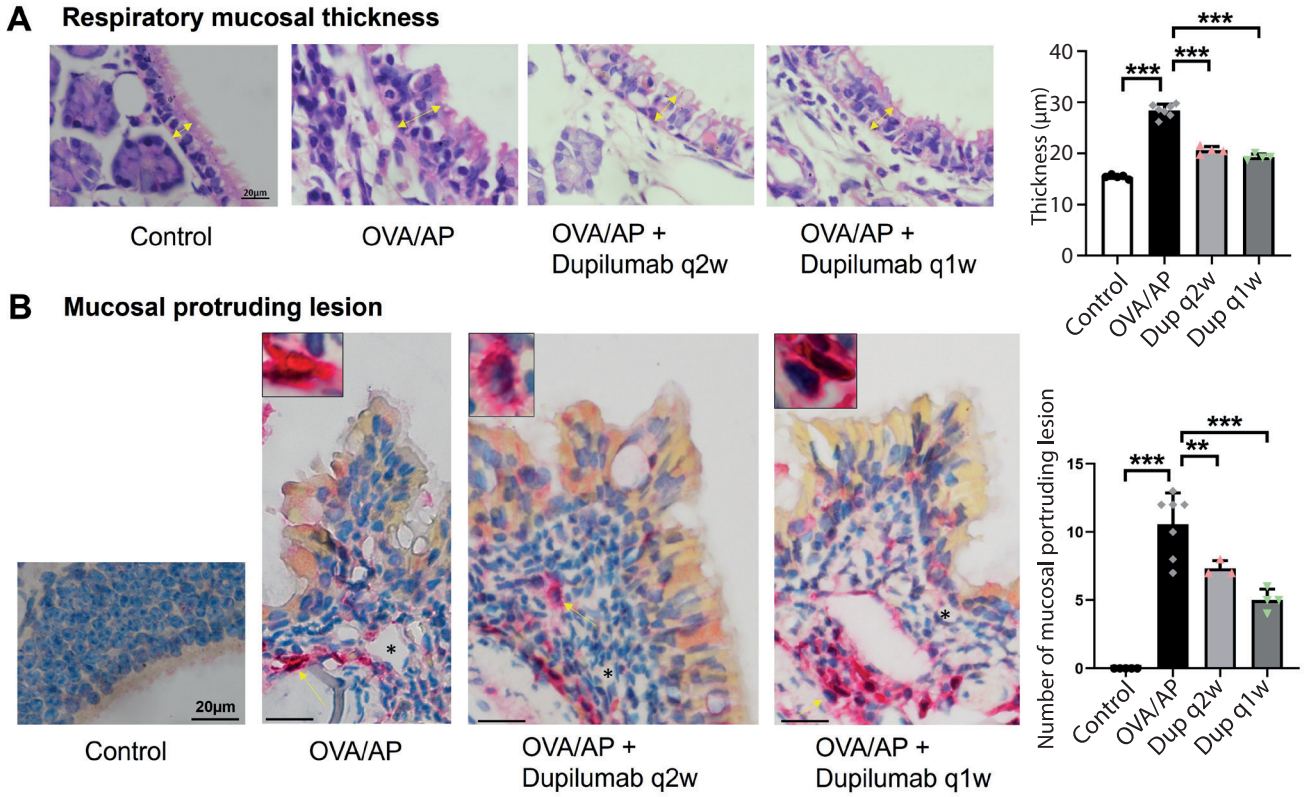


Figure 3. Dupilumab treatment reduces both respiratory mucosal thickness and the occurrence of mucosal protruding lesions. The evaluated section, located just anterior to the anterior border of the eyes, includes control group mice (n=5), mice exposed to OVA/AP administration (n=7), and mice subjected to OVA/AP administration treated with dupilumab every 2 weeks (n=3-4) or every week (n=4). (A) Representative photomicrographs of H&E staining capture mucosal thickness in the respiratory area, located in the lower part of the sinonasal cavity without olfactory nerve (scale bar =20 μm). Mice subjected to OVA/AP administration also exhibited irregular mucosal surface. The right panel quantifies respiratory mucosal thickness in the mentioned area. (B) Representative photomicrographs of eosinophil major basic protein IHC staining illustrate nasal mucosal protruding lesions with eosinophil infiltration (arrow) and microcavity formation (asterisk) (scale bar =20 μm). Enlarged view of eosinophil is shown in left upper corner of the images. The right panel provides quantitative data on the number of mucosal protruding lesions. Data are expressed as mean ± SD, with **P < 0.01 and ***P < 0.001 indicating statistical significance. OVA, ovalbumin; AP, *Aspergillus* protease; Dup, dupilumab; q2w, every 2 weeks; q1w, every 1 week.

However, we did not observe changes in olfactory mucosa thickness after OVA/AP induction or Dupilumab treatment (Figure 5C). Additionally, we identified a positive correlation between the OMP+ area in the sinonasal section and the length of OMP+ epithelium on the nasal septum (r=0.774, P=0.001) (Figure 5D). Both factors were negatively correlated with latency in the buried food test (r=-0.833 and -0.874, both P < 0.001) (Figure 5D). The above results show that dupilumab reduces olfactory sensory neuron injury, and this reduction is correlated with improved olfactory performance.

Reduction of gene expression of M1 microglia and inflammatory substances in the olfactory bulb by dupilumab

The olfactory bulb plays a vital role in the olfactory system. We examined the mRNA expression of key markers in this region among the control group mice, mice exposed to OVA/AP administration, and mice subjected to OVA/AP administration treated with dupilumab every 2 weeks or every week (n=3-4 in each group). Neuronal nuclear protein (NeuN) is a neuronal marker, and we observed no change in mRNA expression following OVA/AP induction and dupilumab treatment. Our attention then turned to olfaction-specific genes. OMP functions as an indicator of mature olfactory sensory neurons, while growth-associated protein-43 (GAP43) signifies immature olfactory sensory neurons. Following OVA/AP induction, there was a decline in OMP mRNA expression (P <0.01) and an increase in GAP43 mRNA expression (P <0.001). Subsequent dupilumab treatment showed no alteration in OMP mRNA expression but was accompanied by a significant decrease in GAP43 mRNA expression (P <0.01) (Figure 6A).

Ionized calcium binding adaptor molecule 1 (Iba1) and CD68 are markers for microglia/macrophages, and CD86 is a marker for M1 type. OVA/AP induction of CRS increased the expression of these genes (all P < 0.01), while dupilumab treatment

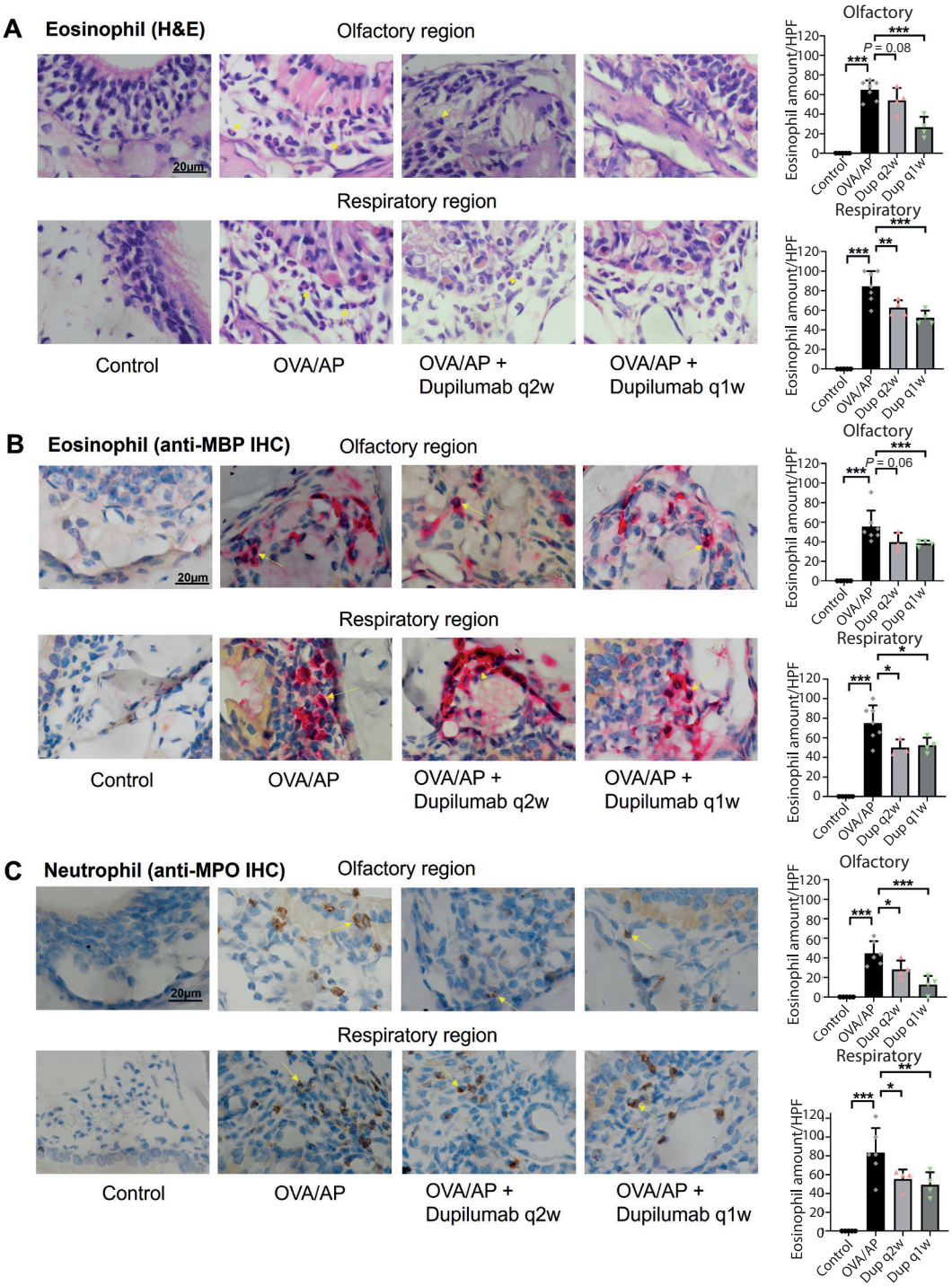


Figure 4. Dupilumab treatment demonstrates a reduction in both eosinophil and neutrophil infiltration within the olfactory and respiratory regions of sinonasal tissue. The designated region for assessing neutrophil and eosinophil infiltration lies anterior to the anterior border of the eyes in control group mice (n=5), mice exposed to OVA/AP administration (n=6-7), and mice subjected to OVA/AP administration treated with dupilumab every 2 weeks (n=3-4) or every week (n=4). (A) Representative photomicrographs of H&E staining capture eosinophil infiltration (yellow arrow) in the olfactory and respiratory area of the mice (scale bar =20 μm). The right panel quantifies the number of eosinophils in the aforementioned area. (B) Representative photomicrographs of eosinophil major basic protein IHC staining also reveal eosinophil infiltration (yellow arrow) in the olfactory and respiratory area of the mice (scale bar =20 μm). The right panel quantifies the number of eosinophils in the aforementioned area. (C) Representative photomicrographs of MPO IHC staining capture neutrophil infiltration (yellow arrow) in the olfactory and respiratory area of the mice (scale bar =20 μm). The right panel quantifies the number of neutrophils in the aforementioned area. Data are expressed as mean ± SD, with *P < 0.05, **P < 0.01 and ***P < 0.001 indicating statistical significance. HPF: high-power field (x400); OVA, ovalbumin; AP, *Aspergillus* protease; Dup, dupilumab; q2w, every 2 weeks; q1w, every 1 week.

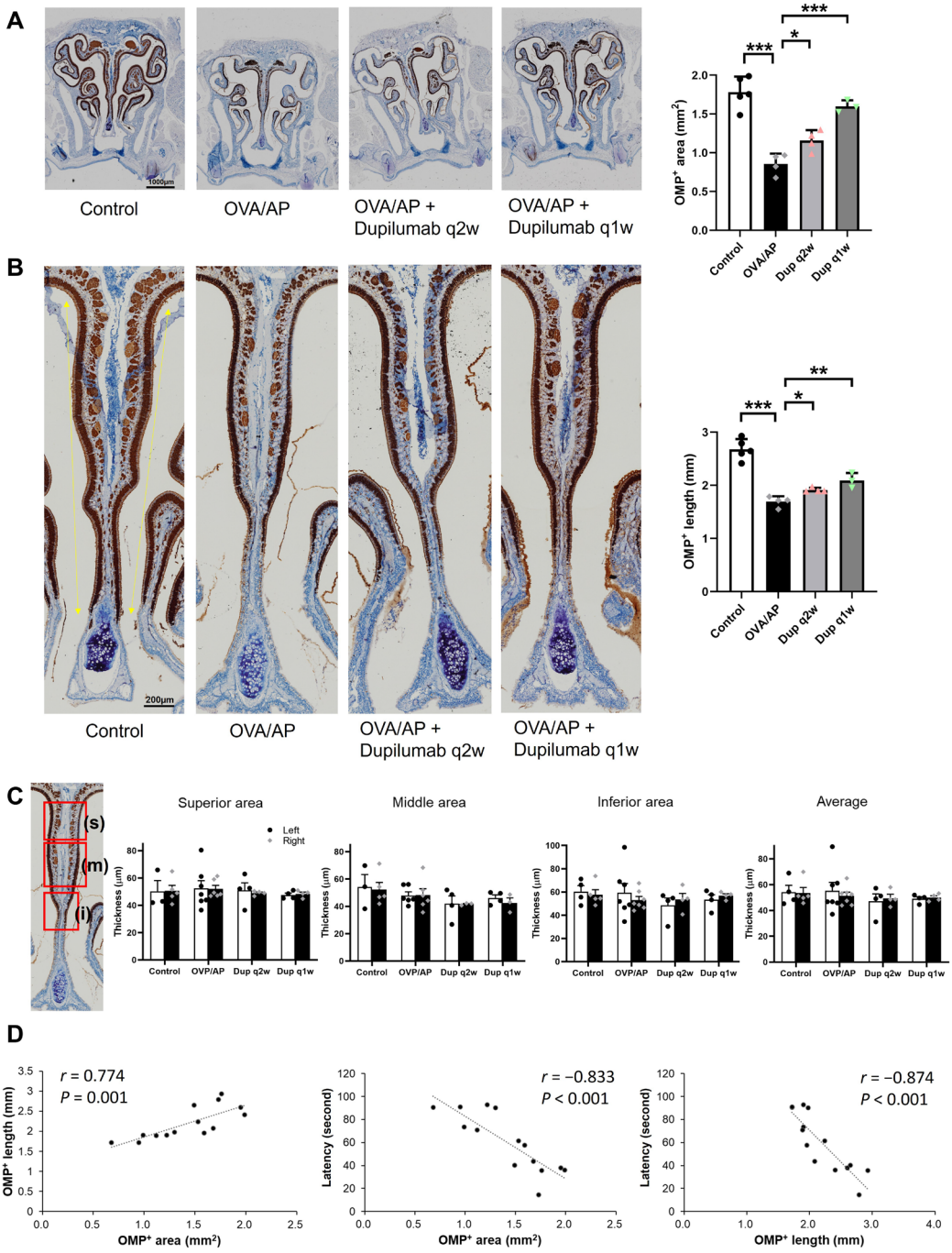


Figure 5. Dupilumab treatment demonstrates an improvement of olfactory sensory neuron injury induced by intranasal OVA/AP administration in the olfactory epithelium. (A) Representative photomicrographs of immunohistochemistry with anti-OMP antibody of olfactory mucosa illustrate mature olfactory sensory neurons in control group mice (n=5), mice exposed to OVA/AP administration (n=4), and mice subjected to OVA/AP administration treated with dupilumab every 2 weeks (n=4) or every week (n=3) (scale bar =1000 μm). The right panel quantifies OMP+ area of olfactory epithelium. (B) Representative photomicrographs of immunohistochemistry with anti-OMP antibody detect olfactory mucosa in the nasal septum (yellow double arrow) (scale bar =200 μm). The right panel quantifies OMP+ length of olfactory epithelium in the nasal septum. (C) Representative photomicrographs of immunohistochemistry with anti-OMP antibody display olfactory epithelium thickness in the upper part of the nasal septum, divided into (s) superior, (m) middle, and (i) inferior sections. The right panel quantifies olfactory mucosal thickness in the aforementioned area. (D) The correlation between OMP+ area in the sinonasal cavity, OMP+ length along the nasal septum and the latency revealed by the buried food test is depicted. Data are expressed as mean ± SD, with *P < 0.05, **P < 0.01 and ***P < 0.001 indicating statistical significance. OMP+: olfactory marker protein positive; OVA, ovalbumin; AP, *Aspergillus* protease; Dup, dupilumab; q2w, every 2 weeks; q1w, every 1 week.

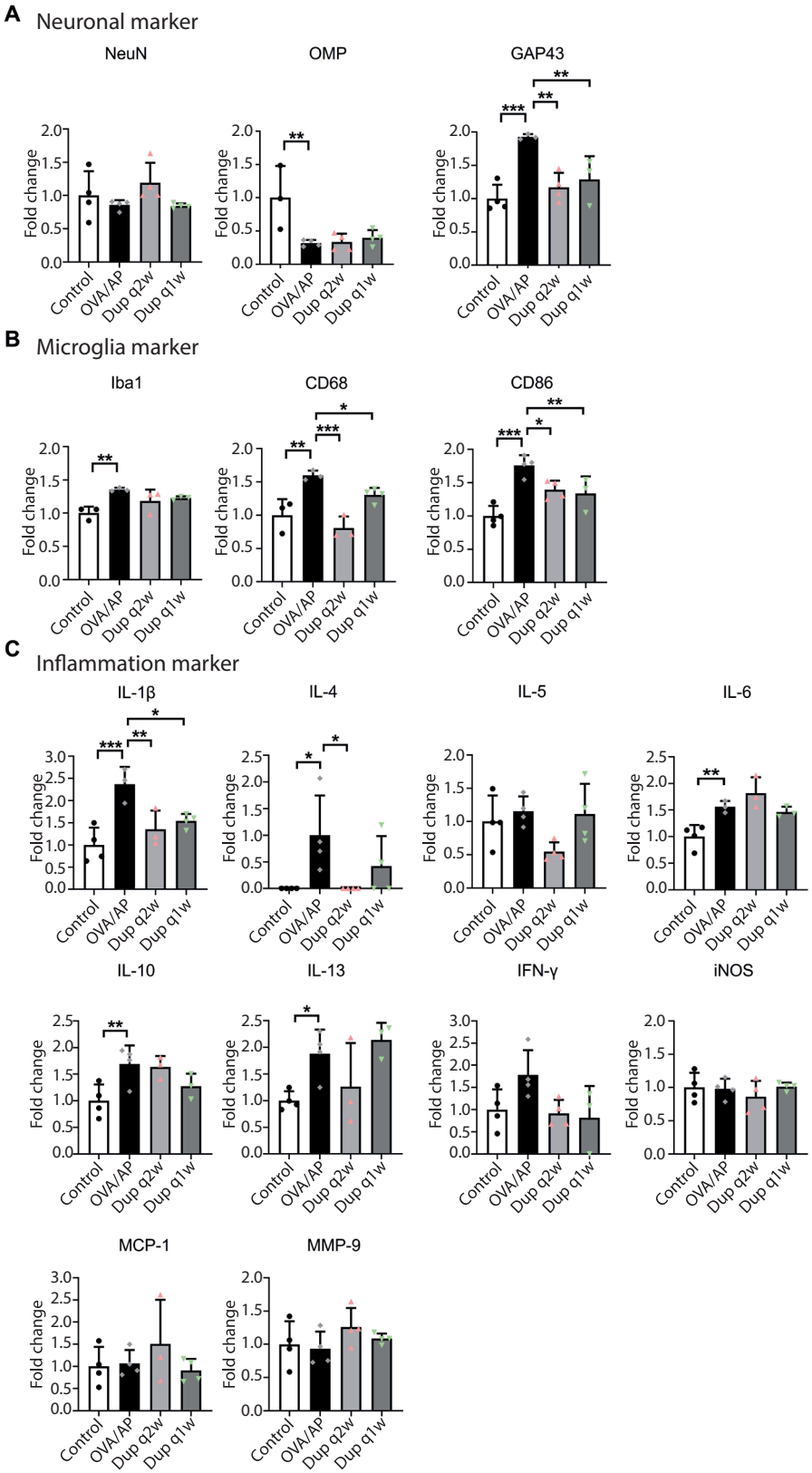


Figure 6. The mRNA levels of (A) neuronal markers, (B) microglia markers, and (C) inflammation markers in the olfactory bulb are analysed in control group mice, mice exposed to OVA/AP administration, and mice subjected to OVA/AP administration treated with dupilumab every 2 weeks or every week (n=3-4 in each group). The vertical axis represents the relative mRNA expression of each marker compared to the control group. In the IL-4 panel, the vertical axis is the relative mRNA expression compared to the OVA/AP group. Data are presented as mean ± SD, with *P < 0.05, **P < 0.01 and ***P < 0.001 indicating statistical significance. OVA, ovalbumin; AP, *Aspergillus* protease; Dup, dupilumab; q2w, every 2 weeks; q1w, every 1 week.

decreased the mRNA expression of CD68 and CD86 (all $P < 0.05$) (Figure 6B). Various mRNA expression of inflammatory markers, including IL-1β, IL-6, IL-4, IL-5, IL-10, IL-13, interferon-γ (IFN-γ), inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP-1), and matrix metalloproteinase-9 (MMP-9) were checked. The inflammatory substance IL-1β, IL-4, IL-6, IL-10 and IL-13 showed increased gene expression after OVA/AP-induced sinusitis (all $P < 0.05$). Dupilumab treatment, administered both weekly and every other week, decreased IL-1β expression (both $P < 0.05$) (Figure 6C).

Discussion

The reason why olfaction improves more rapidly than polyp reduction in patients with CRSwNP after dupilumab treatment remains unclear. Our study is the first to provide evidence that dupilumab not only reduces the infiltration of inflammatory cells in sinonasal tissues but also improves olfactory neuronal injury and inflammation. This may explain why dupilumab improves olfactory dysfunction before polyp reduction.

Ideal substances that induce eosinophilic sinonasal inflammation or therapeutic drugs should have minimal systemic impact, including weight reduction. In this study, referencing Kim et al., the eosinophilic sinonasal inflammation model was induced using OVA/AP, and the body weight showed growth after 12 weeks, with no significant difference compared to the control group [14]. Additionally, the treatment group receiving the drug dupilumab also exhibited weight gain after 12 weeks, with no significant difference compared to the control group.

The buried food test is a straightforward assessment to conduct in mice, as they possess a highly sensitive sense of smell [17]. If mice take an extended period to locate buried food, it indicates impaired olfaction. Dupilumab has the potential to ameliorate olfactory dysfunction in patients with CRSwNP. In our present study, mice with eosinophilic sinonasal inflammation exhibited diminished olfaction. While those treated with dupilumab every other week showed no improvement in olfactory function, mice receiving more frequent dupilumab injections every week demonstrated significant enhancement in olfactory function. Although it cannot be determined whether this represents a recovery of smell function or a prevention of further olfactory loss, this finding is reasonable and can be considered analogous to the human condition.

In this study, dupilumab was found to decrease respiratory mucosal thickness and sinonasal mucosal protruding lesions, aligning with observed polyp reduction in humans. Notably, weekly dupilumab demonstrated the ability to reduce eosinophil and neutrophil infiltration in the sinonasal region, a novel finding not previously reported. Although dupilumab administered every other week failed to reduce eosinophil infiltration in the olfactory region, this might be attributed to the necessity for more frequent dupilumab treatment in the deeper olfactory regions. In rodent studies, Le Floc'h et al. reported a reduced eosinophil count in lung tissue following dupilumab treatment; however, there is a lack of eosinophil count data in rodent models specifically focused on sinonasal tissue after dupilumab administration [18]. Investigations into blood eosinophil levels following dupilumab treatment in humans have been conducted, revealing instances of transient eosinophilia [19,20]. There is limited data on eosinophil counts in human sinonasal deep tissues. This scarcity may be attributed to the challenges associated with obtaining sinonasal tissue biopsies, a procedure that can be burdensome for patients. While biopsies conducted before dupilumab treatment could offer detailed information on the degree of type 2 inflammation, patient reluctance to undergo repeated biopsies post-treatment due to potential complications such as pain, bleeding, and poor wound healing may limit the availability of such data. Instead, blood samples are more accessible for evaluating eosinophil levels after dupilumab treatment. However, it is essential to acknowledge that systemic eosinophil conditions assessed through blood tests may not necessarily correlate with eosinophilic infiltration in sinonasal tissue.

In patients with CRSwNP, the presence of large polyps may lead to conductive olfactory dysfunction. However, our mouse model did not exhibit nasal polyps large enough to induce conductive olfactory dysfunction. Instead, the mice in our model developed sensorineural olfactory dysfunction. Dupilumab was found to improve olfactory dysfunction in our model, indicating an improvement in the sensorineural pattern. OMP serves as a marker for olfactory sensory neurons [4]. In this study, there was a significant loss of OMP in the sinonasal region following induction with OVA/AP, and OMP demonstrated recovery after dupilumab treatment. Consistent with our previous studies highlighting the strong association between OMP and olfaction, the current study reinforced this relationship [5,21]. Olfactory performance, assessed through the buried food test, was found to be correlated with both the OMP+ area in the sinonasal section and the length of OMP+ epithelium on the nasal septum.

The olfactory bulb plays a crucial role in the olfactory system, and our study investigated the gene expression of neuronal markers in this region. OMP serves as a marker for mature olfactory sensory neurons, while GAP43 is indicative of immature olfactory sensory neurons [22]. Their expression can be reciprocal; when OMP expression is low, GAP43 tends to be upregulated, facilitating the preparation for new neuron growth to aid in the repair of the injury [23]. In our study, following OVA/AP induction, OMP mRNA expression decreased while GAP43 mRNA expression increased. After treatment with dupilumab, there was no

change in OMP mRNA expression, while GAP43 mRNA expression significantly decreased.

Microglia in the olfactory bulb can be activated into pro-inflammatory M1 type or anti-inflammatory M2 type in response to external stimuli [24,25]. An increased presence of M1 type results in heightened inflammation and subsequent olfactory dysfunction [24]. Iba1/CD68 serves as a marker for microglia/macrophages, while CD86 is indicative of M1 microglia [26]. Consistent with our prior reports, these markers increased following OVA/AP injection [21]. Our study utilized dupilumab and demonstrated a decrease in CD68 and CD86, suggesting the potential of this drug to reduce M1 microglia in the olfactory bulb. Microglial activation is a pivotal factor in neuroinflammation, with M1 microglia capable of releasing proinflammatory cytokines such as IL-1β and IL-6, leading to neuronal injury [5,21,27]. Suppression of M1 microglial activation represents a strategy to inhibit neuroinflammation [28].

Inflammation in the olfactory bulb can lead to olfactory dysfunction [29]. We investigated the relative gene expression in the olfactory bulb, examining various inflammatory markers, including IL-1β, IL-6, IL-4, IL-5, IL-10, IL-13, IFN-γ, iNOS, MCP-1, and MMP-9. Following OVA/AP induction, only IL-1β, IL-4, IL-6, IL-10, and IL-13 exhibited increased expression. These inflammatory cytokines represent a mix, not solely related to type 2 inflammation, which is characteristic of cytokines in sinonasal tissue. Even though only IL-1β decreased after dupilumab treatment, administered both weekly and every other week, the data suggests that dupilumab has the potential to reduce inflammation in the olfactory bulb.

A limitation of this study is its preclinical design. However, obtaining specimens from the olfactory epithelium and olfactory bulb in humans poses significant challenges due to the potential for causing olfactory neuronal injury through biopsies in these regions. In the absence of readily available human specimens, animal studies can offer valuable insights.

Conclusion

This study demonstrates that dupilumab, an antibody that binds IL-4Rα and inhibits IL-4 and IL-13 signals, can improve the sensorineural pattern of olfactory dysfunction. Dupilumab not only restores olfactory sensory neuron injury in the sinonasal region but also reduces neuroinflammation in the olfactory bulb. This finding may elucidate why, in humans, olfactory dysfunction improves more rapidly than the reduction in polyp size in patients with CRSwNP after dupilumab treatment.

Acknowledgements

The authors thank the Clinical Research Core Laboratory at Taipei Veterans General Hospital for facility support and Professor Shang-Liang Wu for statistical analysis. This work was assisted in part by the Division of Experimental Surgery of the Department of Surgery, Taipei Veterans General Hospital. This research was supported by Ministry of Science and Technology of Taiwan (Grant number MOST 110-2314-B-075-055), National Science and Technology Council of Taiwan (Grant number NSTC 112-2314-B-075-010), and Taipei Veterans General Hospital (Grant numbers V109B-008, V110B-025, V111B-006, V112C-148, and V113C-198).

Authorship contribution

CFY: conception, analysis of data, drafting and revising the article; MYL, revising the article; CCL: conception, drafting the article; YWH: analysis of data; WHH: acquisition and analysis of data; YLL: conception, analysis of data.

Conflict of interest

None.

Funding

This research was supported by Ministry of Science and Technology of Taiwan (Grant number MOST 110-2314-B-075-055), National Science and Technology Council of Taiwan (Grant number NSTC 112-2314-B-075-010), and Taipei Veterans General Hospital (Grant numbers V109B-008, V110B-025, V111B-006, V112C-148, and V113C-198).

References

1. Fokkens WJ, Lund VJ, Hopkins C, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. Rhinology. 2020;58(Suppl S29):1-464.
2. Bachert C, Han JK, Wagenmann M, et al. EUFOREA expert board meeting on uncontrolled severe chronic rhinosinusitis with nasal polyps (CRSwNP) and biologics: definitions and management. J Allergy Clin Immunol. 2021;147(1):29-36.
3. Katotomichelakis M, Gouveris H, Tripsianis G, Simopoulou M, Papathanassiou J, Danielides V. Biometric predictive models for the evaluation of olfactory recovery after endoscopic sinus surgery in patients with nasal polyposis. Am J Rhinol Allergy. 2010;24(4):276-280.
4. Albeanu DF, Provost AC, Agarwal P, Soucy ER, Zak JD, Murthy VN. Olfactory marker protein (OMP) regulates formation and refinement of the olfactory glomerular map. Nat Commun. 2018;9(1):5073.
5. Yeh CF, Chuang TY, Lan MY, Chin YC, Wang WH, Lin YY. Excessive expression of microglia/macrophage and proinflammatory mediators in olfactory bulb and olfactory dysfunction after stroke. In Vivo. 2019;33(6):1893-1899.
6. De Corso E, Pasquini E, Trimarchi M, et al. Dupilumab in the treatment of severe uncontrolled chronic rhinosinusitis with nasal polyps (CRSwNP): a multicentric observational phase IV real-life study (DUPIREAL). Allergy. 2023;78(10):2669-2683.
7. Bachert C, Han JK, Desrosiers M, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY

NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. Lancet. 2019;394(10209):1638-1650.
8. van der Lans RJL, Otten JJ, Adriaensen GFJPM, et al. Two-year results of tapered dupilumab for CRSwNP demonstrates enduring efficacy established in the first 6 months. Allergy. 2023;78(10):2684-2697.
9. Haxel BR, Hummel T, Fruth K, et al. Real-world-effectiveness of biological treatment for severe chronic rhinosinusitis with nasal polyps. Rhinology. 2022;60(6):435-443.
10. Boscke R, Heidemann M, Bruchhage KL. Dupilumab for chronic rhinosinusitis with nasal polyps: real-life retrospective 12-month effectiveness data. Rhinology. 2023;61(3):203-213.
11. Mullol J, Bachert C, Amin N, et al. Olfactory outcomes with dupilumab in chronic rhinosinusitis with nasal polyps. J Allergy Clin Immunol Pract. 2022;10(4):1086-1095 e5.
12. Cantone E, De Corso E, Ricciardiello F, et al. Olfaction recovery following dupilumab is independent of nasal polyp reduction in CRSwNP. J Pers Med. 2022;12(8):1215.
13. La Mantia I, Grigaliute E, Ragusa M, et al. Effectiveness and rapidity on olfatory fuction recovery in CRS patients treated with dupilumab: a real life prospective controlled study. Eur Arch Otorhinolaryngol. 2024;281(1):219-226.
14. Kim HC, Lim JY, Kim S, Kim JH, Jang YJ. Development of a mouse model of eosinophilic chronic rhinosinusitis with nasal polyp by nasal instillation of an Aspergillus protease and ovalbumin. Eur Arch Otorhinolaryngol. 2017;274(11):3899-3906.
15. Huang WH, Hung YW, Hung W, Lan MY, Yeh CF. Murine model of eosinophilic chronic rhinosinusitis with nasal polyposis inducing neuroinflammation and olfactory dysfunction. J Allergy Clin Immunol. 2024;154(2):325-339.e3.
16. Kim DW, Khalmuratova R, Hur DG, et al. Staphylococcus aureus enterotoxin B contributes to induction of nasal polypoid lesions in an allergic rhinosinusitis murine model. Am J Rhinol Allergy. 2011;25(6):e255-261.
17. Yang M, Crawley JN. Simple behavioral assessment of mouse olfaction. Curr Protoc Neurosci. 2009;Chapter 8:Unit 8 24.
18. Le Floc'h A, Allinne J, Nagashima K, et al. Dual blockade of IL-4 and IL-13 with dupilumab, an IL-4Ralpha antibody, is required to broadly inhibit type 2 inflammation. Allergy. 2020;75(5):1188-1204.
19. Wechsler ME, Klion AD, Paggiaro P, et al. Effect of dupilumab on blood eosinophil counts in patients with asthma, chronic rhinosinusitis with nasal polyps, atopic dermatitis, or eosinophilic esophagitis. J Allergy Clin Immunol Pract. 2022;10(10):2695-2709.
20. Ryser FS, Yalamanoglu A, Valaperti A, et al. Dupilumab-induced eosinophilia in patients with diffuse type 2 chronic rhinosinusitis. Allergy. 2023;78(10):2712-2723.
21. Yeh CF, Huang WH, Lan MY, Hung W. Lipopolysaccharide-initiated rhinosinusitis causes neuroinflammation and olfactory dysfunction in mice. Am J Rhinol Allergy. 2023;37(3):298-306.
22. Liberia T, Martin-Lopez E, Meller SJ, Greer CA. Sequential maturation of olfactory sensory neurons in the mature olfactory epithelium. eNeuro. 2019;6(5):ENEURO.0266-19.2019.
23. Nakashima N, Nakashima K, Takaku-Nakashima A, Takano M. Olfactory receptor neurons express olfactory marker protein but not calpain 5 from the same genomic locus. Mol Brain. 2019;12(1):54.
24. Seo Y, Kim HS, Kang KS. Microglial involvement in the development of olfactory dysfunction. J Vet Sci. 2018;19(3):319-330.
25. Reshef R, Kreisel T, Beroukhim Kay D, Yirmiya R. Microglia and their CX3CR1 signaling are involved in hippocampal- but not olfactory bulb-related memory and neurogenesis. Brain Behav Immun. 2014;41:239-250.
26. Jurga AM, Paleczna M, Kuter KZ. Overview of general and discriminating markers of differential microglia phenotypes. Front Cell Neurosci. 2020;14:198.
27. Yeh CF, Chuang TY, Hung YW, et al. Inhibition of soluble epoxide hydrolase regulates monocyte/macrophage polarization and improves neurological outcome in a rat model of ischemic stroke. Neuroreport. 2019;30(8):567-572.
28. Hu X, Leak RK, Shi Y, et al. Microglial and macrophage polarization-new prospects for brain repair. Nat Rev Neurol. 2015;11(1):56-64.
29. Kagoya R, Toma-Hirano M, Yamagishi J, Matsumoto N, Kondo K, Ito K. Immunological status of the olfactory bulb in a murine model of Toll-like receptor 3-mediated upper respiratory tract inflammation. J Neuroinflammation. 2022;19(1):13.

Chien-Fu Yeh
Department of Otorhinolaryngology
Head and Neck Surgery
Taipei Veterans General Hospital
No. 201, Sec. 2, Shipai Rd.
Taipei 11217
Taiwan

Tel: +886-2-2875-7337
Fax: +886-2-2875-7338
E-mail: rihney@hotmail.com

Chien-Fu Yeh[1,2], Ming-Ying Lan[1,2], Ching-Chia Lin[1], Yu-Wen Hung[3], Wei-Hao Huang[1], Yi-Ling Lai[2]

[1] Department of Otolaryngology-Head and Neck Surgery, Taipei Veterans General Hospital, Taipei, Taiwan
[2] School of Medicine, National Yang-Ming Chiao Tung University, Taipei, Taiwan
[3] Department of Nursing, College of Medical Technology and Nursing, Yuanpei University of Medical Technology, Hsinchu, Taiwan

Rhinology 63: 1, 32 - 42, 2025
https://doi.org/10.4193/Rhin24.114

Received for publication:
March 14, 2024
Accepted: July 24, 2024

Associate Editor:
Sietze Reitsma

This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Supplementary Table 1. The detected target gene primers.

Gene name	Forward primer	Reverse primer
Rbfox3 (NeuN)	CACCACTCTCTTGTCCGTTTGC	GGCTGAGCATATCTGTAAGCAGC
Omp	AGCAGGATGGTGAGAAGCTGAT	TTGTCCAGAACCACGTTCCA
Gap43	CTCCAACGGAGACTGCAGAAA	GGCATGTTCTTGGTCAGCCT
Iba1	AGGAGACGTTCAGCTACTCTGACTT	ACCAGTTGGCCTCTTGTGTTCT
CD68	AGCTGTTCACCTTGACCTGCTC	CCAATGATGAGAGGCAGCAAG
CD86	TGGGCTTGGCAATCCTTATC	TGTAAATGGGCACGGCAGAT
Il1β	GCACTACAGGCTCCGAGACTAAC	TTGTCGTTGCTTGGTTCTCCTTGT
Il4	CACGGAGATGGATGTGCCA	GTTCTTCTTCAAGCATGGAGTTTTC
Il5	GTTCTGACTCTCAGCTGTGTCTGG	CAACAGAGCTCGGTGAGCG
Il6	CCACTCCCAACAGACCTGTCTAT	GCATCATCGTTGTTCATACAATCA
Il10	TGAAAATAAGAGCAAGGCAGTGG	TGATCATCATGTATGCTTCTATGCA
Il13	TCTGTGTAGCCCTGGATTCCC	GGCCTTGCGGTTACAGAGG
Ifnr	CAGCAACAGCAAGGCGAAAAAGG	TTTCCGCTTCCTGAGGCTGGAT
Nos2 (iNOS)	AAGATGGCCTGGAGGAATGC	TGCTGTGCTACAGTTCCGAG
Mcp1	GCTACAAGAGGATCACCAGCAG	GTCTGGACCCATTCCTTCTTGG
Mmp9	TCGCGTGGATAAGGAGTTCTC	GGAAACTCACACGCCAGAAGA
Gapdh	AATGTGTCCGTCGTGGATCTG	GCCCAAGATGCCCTTCAGT