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Invited Review Article

Barrier dysfunction in the nasal allergy

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AR, allergic rhinitis; CRS, chronic rhinosinusitis; DEP, diesel exhaust particles; HDM, house dust mite; HNECs, human nasal epithelial cells; JAMs, junction adhesion molecules; PM, particulate matter; ROS, reactive oxygen species; TJ, tight junction; ZO-1, zonula occludens-1

ABSTRACT

Epithelial cells form the first physiological barrier against invasion by pathogens and the infiltration of allergens. Tight junctions (TJ), a cell–cell junctional complex located on the apical side of epithelial cells, have a critical role in the maintenance of epithelial barrier function. Impaired TJ structures are observed in patients with asthma, atopic dermatitis and nasal allergy; therefore, the dysfunction of epithelial barriers might be involved in the initiation or progression of allergic diseases. Protease-containing allergens and environmental pollutants enhance paracellular transport in epithelial cells through disruption of epithelial barrier function. This suggests that the disruption of TJ leads to the promotion of allergen delivery into the subepithelia, resulting in the progression of allergic diseases. Thus, protection of the epithelial barrier function might prevent or inhibit the development or exacerbation of allergic diseases. Recently, we reported that diesel exhaust particles (DEP), the main component of particulate patter 2.5, exacerbated allergic rhinitis (AR) in a mouse model through TJ disruption. In addition, we revealed that the oxidative stress-mediated pathway is involved in the effects caused by DEP and that nasal treatment with a reactive oxygen species (ROS) scavenger suppressed DEP-induced TJ disruption and exacerbation of AR. In this review, we focus on the relationship between TJ disruption and allergic disease. Furthermore, we discuss our recent findings regarding TJ disruption and the exacerbation of AR.

Introduction

Epithelial cells have an important role as a physical barrier to prevent the entry of pathogens, allergens and other foreign particles.[1] Tight junctions (TJ), cell–cell adhesion complexes between epithelial cells, are important for epithelial barrier function.[2] Epithelial TJ disruption has been associated with various human diseases such as inflammatory bowel disease, celiac disease and functional dyspepsia.[3–5] In allergic diseases, TJ disruption is observed in the epithelial cells of patients with asthma, atopic dermatitis, and nasal allergy.[6–9] Thus, TJ disruption, namely epithelial barrier dysfunction, is considered involved in the initiation or progression of allergic diseases.

It was reported that disruption of the TJ barrier is induced by proteases present in pollens or house dust mites (HDM), cytokines or environmental pollutants such as particulate matter (PM) 2.5 and cigarette smoke.[10–14] In addition, SNPs of the *CLDN1* gene, encoding claudin-1 that is essential for TJ function, were present in patients with atopic dermatitis, suggesting genetic factors might influence the weakness of TJ.[7] Paracellular transport is enhanced in epithelial cells with disrupted TJ. Therefore, dysfunction of the TJ barrier might enhance allergen infiltration into the subepithelia and the uptake of allergens by DCs or mast cell degranulation, resulting in the initiation or exacerbation of allergy.

These findings indicate that TJ disruption is linked to various allergic diseases. However, it is unclear whether protection of the TJ barrier can suppress or prevent allergic diseases. In this review, we describe the structure and role of TJ, TJ disruption-inducing factors and relationship between TJ disruption and allergic disease. Finally, we will introduce our recent findings regarding nasal TJ disruption and the exacerbation of AR.

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Structure of tight junctions

TJ, a multiprotein complex located on the apical side of epithelial cells, mediates cell–cell adhesion and tightly regulates the paracellular transport of ions, water and various molecules.[15,16] TJ are essential for paracellular transport and form an epithelial barrier function against foreign invaders such as pathogens, particles, and allergens.[1]

TJ in epithelial cells are composed of three types of transmembrane proteins including occludin, claudin and junction adhesion molecules (JAMs). These transmembrane proteins are important for sealing the paracellular spaces between epithelial cells and the regulation of paracellular transport.[15,17] In addition, TJ structures are supported by adaptor proteins in the cytoplasm such as zonula occludens (ZO) proteins (Fig. 1).[15,17]

Occludin was the first identified integral membrane protein that is ubiquitously expressed in epithelial cells.[15,18,16] Occludin has two extracellular loops and N- and C-terminal cytoplasmic domains.[15,18] The C-terminal of occludin is important for direct interactions with ZO-1, and the N-terminus is involved in the regulation of paracellular permeability.[15,18]

Claudin family members have a short cytoplasmic N-terminal, two extracellular loops and C-terminal cytoplasmic domains. The C-terminal of claudins is required for stability and interactions with ZO-1.[19] The claudin family consists of more than 25 members and the expression pattern of claudin family members varies considerably among tissues.[19] The combination of claudin family members is thought to determine the selectively or strength of TJ.[20,21] It was shown that claudin-1, -4, -7, -8, -12, -13 and -14 are expressed in human nasal mucosa.[1]

JAMs belong to the immunoglobulin superfamily and have a single transmembrane domain and a PDZ domain-binding motif at their C-terminal domain, which interacts with ZO-1.[22,23] JAMs are important for cell–cell adhesion and junctional assembly in epithelial cells.[22]

The adaptor proteins, ZOs, are involved in the connection of transmembrane proteins and the recruitment of other cytoplasmic components such as protein kinase (PKC), GTPase and transcription factors.[16] ZOs regulate junction assembly and selective paracellular permeability by signal transduction.[16]

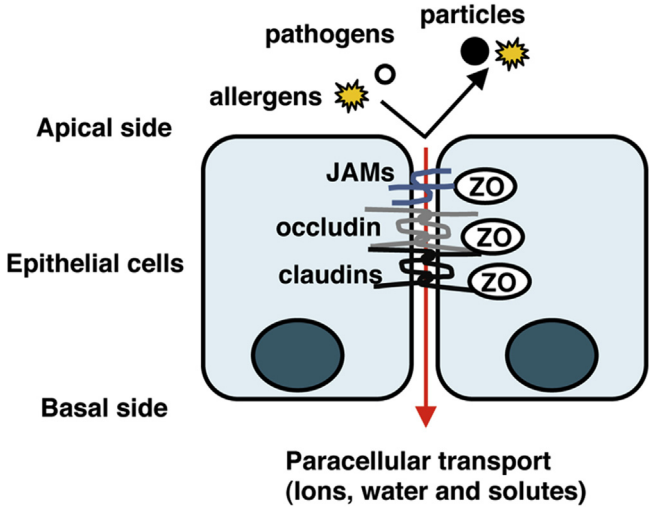

Fig. 1. Structure of tight junctions in epithelial cells. Tight junctions (TJ) contain three types of transmembrane proteins: occludins, claudins, and junction adhesion molecules (JAMs) as well as scaffold proteins such as zonula occludens-1 (ZO). ZO proteins are important for the clustering of occludin and claudin.

TJ and dendritic cells in mucosa

DCs also express TJ proteins. It was reported that DCs in the intestinal mucosa open TJ between epithelial cells, which allows dendrites to move outside the epithelium to directly uptake bacteria.[24] Because intestinal DCs express occludin, claudin-1 and ZO-1, the TJ integrity is preserved when DCs take up bacteria across the epithelial layer.[24] In addition, treatment with thymic stromal lymphopoietin (TSLP), a proallergic cytokine, enhanced the expression of claudin family members in DCs *in vitro*.[25] It is unclear whether DCs in the nasal mucosa directly take up pathogens or allergens via dendrites that move outside the epithelium. However, it was reported that, HLA-DR- and CD11c-postitive DCs express claudin-1 and are increased in human nasal mucosa in patients with AR compared with healthy subjects.[26] This suggests that DCs in nasal mucosa contribute to preserving the TJ barrier while taking up allergens in allergic conditions.

TJ disruption-inducing factors

It is well known that protease-containing allergens such as pollens and HDM, the major allergens for AR, disrupt the TJ barrier. Cysteine proteases such as Der p1 from fecal pellets of HDM, disrupt TJ and increase the permeability of Madin-Darby canine kidney (MDCK) cells and 16HBE14o$^-$ human bronchial epithelial cell lines.[11] The cleaved fragments of occludin and ZO-1 were detected by immunoblotting in Der p1-treated 16HBE14o$^-$ cells *in vitro*, suggesting that these TJ-associated proteins are directly proteolyzed by Der p1.[11] In addition to HDM, previous reports revealed that extracts of various pollens impaired TJ barrier functions.[10,27] Runswick *et al.* showed that Giant Ragweed (*Ambrosia trifida*), White Birch (*Betula pendula*), and Kentucky Blue Grass (*Poa pratensis*) decreased the expression of different TJ proteins in MDCK and Calu-3 cells, human airway epithelial cell lines derived from a patient with lung adenocarcinoma.[10] In addition, these pollen extracts increased the paracellular permeability of Calu-3 cells.[10] The proteolytic activities of Kentucky Blue Grass pollens were inhibited by serine (AEBSF), cysteine (E-64) and trypsin-like protease inhibitors, suggesting that proteases in pollen extracts directly affect TJ proteins.[10] Another group showed that crude extracts of Olive tree (*Olea europaea*), Orchard grass (*Dactylis glomerata*), Italian cypress (*Cupressus sempervirens*) and Scots pine (*Pinus sylvestris*) decreased claudin-1 expression and increased transepithelial permeability in Calu-3 cells.[27] Of note, these pollens had different effects on claudin-1 expression. Scots pine pollens had a greater impact on claudin-1 expression than other pollens. Therefore, although each pollen causes effects on TJ to a different degree, most pollens can disrupt epithelial TJ barriers.

It was reported that Th2 cytokines such as IL-4 and IL-13 disrupt TJ in airway epithelial cells.[28] IL-4 or IL-13-treated Calu-3 cells showed significantly decreased ZO-1 expression and slightly decreased occludin expression.[28] The TJ barrier function of Calu-3 cells was also decreased by IL-4- or IL-13-treatment.[28] In addition, it was shown that IL-4 disrupted TJ structures and increased paracellular transport in 16HBE14o$^-$ cells that was JAK-dependent.[29] IL-13 also impaired epithelial TJ barrier in 16HBE14o$^-$ cells.[29] However, combined IL-4 and IL-13 were not synergistic, suggesting IL-4 and IL-13 disrupt TJ through the same pathway.[29] Similar to 16HBE14o$^-$ cells, IL-4 enhanced paracellular transport in primary human nasal epithelial cells (HNECs).[8] However, the effects of Th2 cytokines on TJ barrier functions in nasal epithelia *in vivo* are unknown.

Environmental pollutants such as PM2.5 and cigarette smoke affect the TJ barrier in pulmonary or nasal epithelial cells.[12–14] PM2.5 (aerodynamic diameter <2.5 μm) is mainly composed of diesel exhaust particles produced by motor vehicles and industrial

plants.[30,31] DEP are comprised of a carbon core and a mixture of absorbed metals and organic chemicals.[30] Epidemiological studies revealed that exposure to PM2.5 increases the risk of allergic diseases, and that the prevalence of AR in urban areas with air pollution is higher than that in rural areas.[32,33] However, it is unclear whether DEP-induced TJ disruption is linked to the exacerbation of AR. We recently revealed that DEP exacerbated AR by disrupting TJ barriers in nasal epithelia *in vivo*.[13] Furthermore, the oxidative stress pathway was involved in the DEP-induced TJ disruption and exacerbation of AR.[13] We will discuss the effect of DEP on AR in more detail in the following section 'Epithelia barrier disruption and allergic diseases'.

Epidemiological studies showed that cigarette smoke is a risk factor for asthma and chronic rhinosinusitis (CRS) with polyps.[14,34] Incubation with cigarette smoke extract decreased ZO-1 and ZO-2 expression, and disrupted TJ barrier function in 16HBE14o$^-$ cells and primary human bronchial epithelial cells (pHBECs).[14] In addition, cigarette smoke extract decreased ZO-1 and JAMAs expressions in primary human sinonasal epithelial cells from healthy subjects.[34] The effect of cigarette smoke extract was inhibited by the pharmacologic activation of nuclear factor erythroid 2-related factor 2 (Nrf2), which is involved in the anti-oxidant pathway.[34] This suggests that cigarette smoke extract disrupts TJ through oxidative stress similar to DEP.

Epithelia barrier disruption and allergic diseases

It was shown that the disruption of TJ is induced in bronchial epithelial cells in patients with asthma.[6] A previous report showed that the epithelial barrier function of primary bronchial epithelial cells from patients with severe asthma was lower than that in healthy subjects or patients with mild asthma.[6] In atopic dermatitis, claudin-1 and -23 expression were decreased in keratinocytes from patients with atopic dermatitis.[7] In addition, the association of atopic dermatitis and four SNPs of the *CLDN1* gene (*rs17501010*, *rs9290927*, *rs9290929* and *rs893051*) in African-American atopic dermatitis patients was previously reported.[7] Furthermore, *rs893051* is associated with the early onset of atopic dermatitis.[35] Claudin-1 deficient mice die within 1 day of birth because of excessive transepidermal water loss, severe dehydration and increased epidermal permeability.[36] Thus, the TJ barrier in keratinocytes is important for maintaining the skin barrier and the dysfunction of TJ might lead to the onset of atopic dermatitis.

In the nose, dysfunction of the nasal epithelial barrier was observed in patients with HDM-induced AR and chronic rhinosinusitis (CRS) with polyps.[8,9] As mentioned above, treatment with pollen extract Der p1 and IL-4 disrupted TJ in primary human nasal epithelial cells (HNECs).[29] However, it is unknown whether TJ disruption is directly linked to the exacerbation of AR and whether protection of the TJ barrier suppresses AR symptoms.

Epithelia barrier disruption and AR

Previous epidemiological studies reported that DEP exacerbate AR. However, the exact role of DEP in the exacerbation of AR is unknown. Recently, we revealed that nasal TJ disruption induced by DEP exacerbated AR using an AR mouse model.[13] We will introduce our recent study regarding TJ disruption and AR symptoms.

First, to investigate whether DEP worsened AR, ragweed-sensitized mice were simultaneously challenged with ragweed pollen alone or ragweed pollen plus DEP (Fig. 2A).[13] We treated mice with ragweed pollen at a low dose that barely induced AR symptoms.[13] At the first challenge, the frequency of sneezing was comparable between ragweed-alone- and ragweed-plus-DEP-challenged mice.[13] However, the frequency of sneezing was

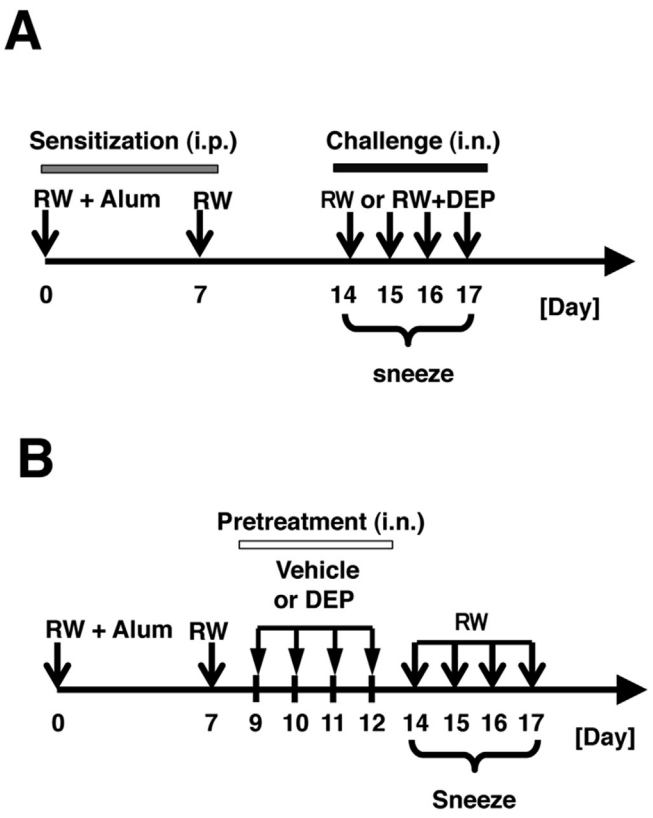

Fig. 2. Experimental schema for the effect of DEP on the exacerbation of AR. (**A**) Mice were sensitized with ragweed pollen (RW) at days 0 and 7. At day 14, mice were challenged intranasally with RW (0.1 mg) or RW plus DEP (10 μg) for 4 days. The number of sneezes was counted for 10 min immediately after each challenge. (**B**) Mice were sensitized as described in (**A**). At day 9, sensitized mice were administrated intranasally with DEP for 4 days, and then challenged with RW.

significantly increased in mice challenged with ragweed pollen plus DEP (40–60 times), but remained low (10–20 times) in ragweed-alone-challenged mice during the challenge.[13] Although it was reported that DEP has an adjuvant effect when used in the sensitization phase,[37,38] the production of Th2 cytokines (IL-4, IL-5 and IL-13) from cervical lymph nodes and serum IgE levels were comparable between the groups.[13] These results reveal that the DEP-mediated exacerbation of AR is not caused by the adjuvant activity of DEP.

DEP might not always be inhaled concurrent with allergen exposure by rhinitis patients. Thus, we next treated sensitized mice with DEP before ragweed pollen-challenge (Fig. 2B). Pretreatment with DEP also increased the frequency of sneezing.[13] Remarkably, although the co-administration of DEP did not increase sneezing frequency at the first ragweed-challenge, mice pretreated with DEP showed a remarkably increased frequency of sneezing at all time points.

DEP impairs nasal epithelial barrier functions

It was shown that DEP disrupt TJ in pulmonary epithelial cells *in vitro*.[12] We analysed the effect of DEP on TJ in nasal epithelial cells. Monolayer RPMI 2650 cells, a human nasal epithelial cell line, were treated with DEP for 24 h, and TJ structure and epithelial barrier function in RPMI 2650 cells were analysed. We used three methods for the analysis of TJ in RPMI 2650 cells: immunostaining for ZO-1, measurement of Transepithelial Electric Resistance (TER) and permeability of FITC-dextran (Fig. 3).[13] At 24 h after DEP-treatment, the expression of ZO-1 was decreased and TER was

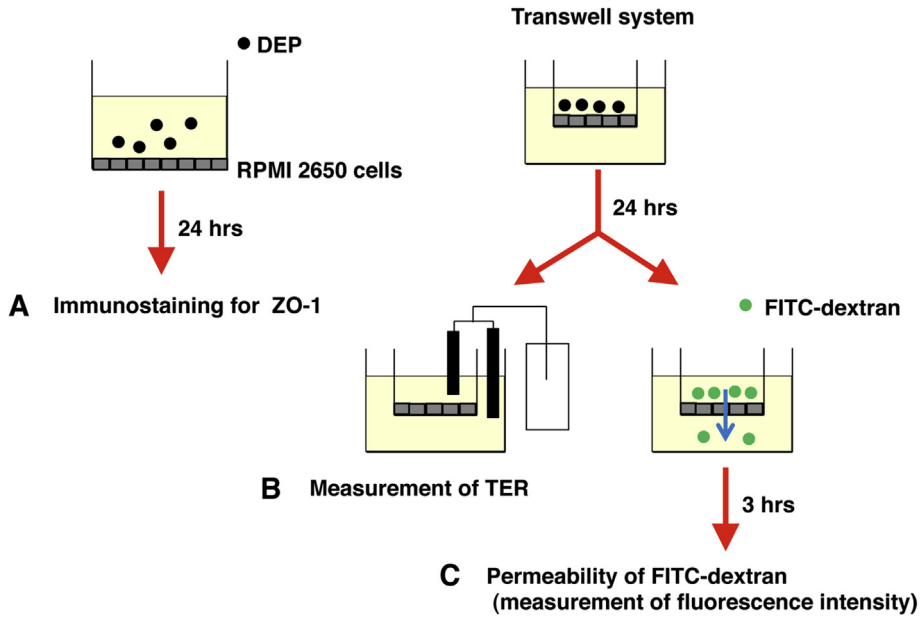

Fig. 3. Analysis of nasal epithelial barrier function. Monolayer RPMI 2650 cells cultured in upper wells were treated with DEP for 24 h. Immunostaining for ZO-1 (**A**). Transepithelial electric resistance (TER) was measured (**B**). After 24 h of DEP treatment, RPMI 2650 cells were incubated with FITC-dextran for 3 h, and then culture supernatants were collected. Fluorescence intensity in culture supernatants was measured (**C**).

reduced in DEP-treated cells. For measure permeability of FITC-dextran, at 24 h after DEP-treatment, FITC-dextran was added into upper wells for 3 h, and then the fluorescence intensity of FITC in bottom wells, which passed through epithelial layer, was measured. DEP-treated RPMI 2650 cells had an increased permeability to FITC-dextran.[13]

To confirm the effect of DEP on nasal TJ *in vivo*, we analysed ZO-1 expression in nasal epithelia from mice treated with DEP. Immunohistochemistry analysis showed that ZO-1 expression in nasal epithelia was decreased by treatment of DEP alone for 4 days, while ragweed pollen-alone-challenge did not influence TJ.[13] Moreover, a single treatment of DEP was sufficient to disrupt nasal TJ. Next, we investigated whether decreased ZO-1 expression correlated with increased sneezing. Ragweed-sensitized mice were treated with a single DEP exposure, and ZO-1 expression in nasal epithelia and the frequency of sneezing at days 2, 4, 6, and 8 after DEP treatment were analysed (Fig. 4). ZO-1 expression was significantly decreased at days 2 and 4; however, ZO-1 expression recovered 6 days after the single exposure of DEP.[13] Furthermore, the frequency of sneezing was increased at days 2 and 4, but an increased frequency was not observed at days 6 and 8.[13] Thus, decreased ZO-1 expression and increased sneezing were inversely correlated, indicating the involvement of TJ disruption in the exacerbation of AR (Fig. 5).

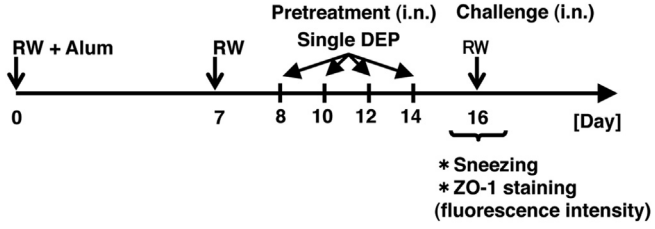

Fig. 4. Experimental schema for determining the relationship between TJ disruption and exacerbation of AR. Sensitized mice as described in Fig. 2A were intranasally administrated with DEP at days 8, 10, 12 or 14. At day 16, mice were challenged with RW, and the frequency of sneezing was counted and ZO-1 expression was analysed.

Nasal TJ disruption by DEP is induced by a reactive oxygen species (ROS)-mediated pathway

Next, to identify the pathway involved in DEP-induced TJ disruption, we focused on ROS production. Previous reports suggested that DEP decreased the expression of TJ proteins in pulmonary epithelial cells *in vitro* through oxidative stress.[39,40] Thus, we treated RPMI 2650 cells with N-acetyl-L-cysteine (NAC), a ROS scavenger, together with DEP. NAC-treated RPMI 2650 cells did not show a DEP-induced decrease of ZO-1 expression or increased permeability to FITC-dextran.[13] Furthermore, *in vivo* intranasal treatment with NAC prevented the DEP-induced increase in sneezing and decrease in ZO-1 expression in nasal epithelia.[13] Therefore, DEP disrupts nasal TJ by a ROS-mediated pathway, and nasal treatment with NAC might be a novel therapeutic strategy for the DEP-induced exacerbation of AR.

Conclusions

In this review, we described the importance of epithelial barrier and the relationship between TJ disruption and allergic diseases. In addition, we discussed our new findings regarding the association of TJ disruption and exacerbation of AR induced by DEP. Our study revealed that ROS production is involved in DEP-induced TJ disruption and the exacerbation of AR. In addition, a ROS scavenger, NAC, was effective against the DEP-induced exacerbation of AR, suggesting that protection of the TJ barrier might suppress or prevent AR.

Dysfunction of the TJ barrier was observed in patients with various allergic diseases. Our study showed that the intranasal administration of NAC prevented DEP-induced TJ disruption, resulting in suppression of the exacerbation of AR. However, the TJ barrier is impaired by ROS production as well as direct proteolysis by proteases or Th2 cytokine-mediated pathways. Thus, a combination of protease inhibitor and the neutralization of Th2 cytokines might be effective for AR induced by protease-containing allergens. Although further studies are needed, protection of the nasal TJ barrier might be a promising approach for the development of therapeutic or preventive strategies for AR.

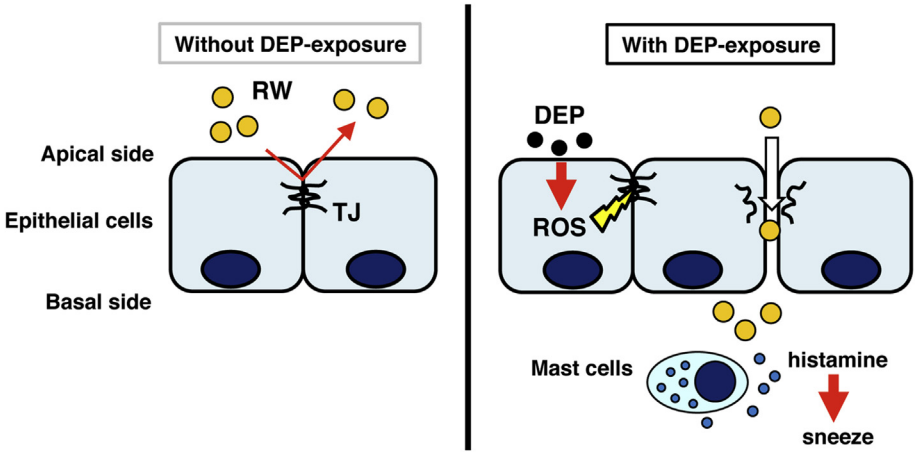

Fig. 5. Schematic diagram of DEP-induced TJ disruption and exacerbation of AR. Without DEP exposure, the penetration of allergens is prevented by the TJ barrier. Nasal TJ are disrupted by ROS production when nasal mucosa are exposed to DEP. The disruption allows allergens to penetrate into the subepithelial tissue, resulting in increased sneezing.

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Conflict of interest

The authors have no conflict of interest to declare.

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