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Mechanisms and clinical implications of glucocorticosteroids in the treatment of allergic rhinitis

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Summary

Allergic rhinitis is a common airway disease characterized by hypersensitivity, exudation, hypersecretion, inflammatory cell infiltration and remodelling. Intranasal glucocorticosteroids are the most effective drugs for controlling the inflammation caused by allergic rhinitis. Glucocorticosteroids exert anti-inflammatory effects through at least two pathways: the transactivation pathway and the transrepression pathway. Glucocorticosteroids also exert regulatory functions by inducing regulatory cytokines and forkhead box P3 (FoxP3$^+$) regulatory T cells. Evidence suggests that intranasal glucocorticosteroids control not only nasal symptoms but also ocular symptoms. In contrast to sedating H1 receptor antagonists, intranasal glucocorticosteroids can improve impaired performance symptoms, such as daytime sleepiness, associated with allergic rhinitis. Recent studies suggest that intranasal glucocorticosteroids might also be useful for the prophylactic treatment of pollinosis; this possibility is supported by the molecular mechanism of the anti-inflammatory action of glucocorticosteroids. These findings suggest that intranasal glucocorticosteroids might be positioned as first-line drugs for the treatment of both perennial and seasonal allergic rhinitis.

Keywords: impaired performance, intranasal glucocorticosteroids, ocular symptoms, regulatory T cells

Introduction

Allergic rhinitis (AR) is a common manifestation of allergic diseases, affecting approximately 500 million people worldwide [1]. AR is increasing in prevalence. For example, the prevalence of AR in Japan increased from 29·8% in 1998 to 39·4% in 2008. The prevalence of pollinosis, the typical seasonal AR, has been increased from 19·6% in 1998 to 29·8% in 2008 [2].

AR is a major chronic inflammatory condition in the upper airway characterized by hypersensitivity, exudation, hypersecretion, inflammatory cell infiltration and remodelling [3]. Although glucocorticosteroids (GC) are highly effective in mitigating inflammation, their potent action often causes severe adverse effects [4,5]. To decrease the potential for adverse effects, intranasal glucocorticosteroid (INS) formulations with low systemic availability have been developed for the treatment of allergic rhinitis [6].

In this review, we discuss the pathophysiology of allergic rhinitis and the mechanism of action of GC, including the induction of regulatory T cells (T_{regs}), in the pathogenesis of AR. We also discuss the usefulness and pitfalls of INS in the clinical setting and assess the current status of INS for the treatment of AR.

Pathophysiology of AR

Pathogenesis of AR

Most causal antigens for AR are inhalant allergens. House dust mite, animal dander and pollens are the principal allergens. Many allergens, including the major house dust-mite allergen, Der p 1, have protease activity that impairs epithelial barrier function and facilitates the penetration of allergens into nasal mucosa [7]. Following nasal exposure to the inhalant allergens, professional antigen-presenting cells in the nasal mucosa, such as dendritic cells (DC), capture the allergens and provide two distinct signals, the allergen-derived peptide/MHC complex and co-stimulatory molecules such as CD80 and CD86, to naive T cells [8–10]. Allergen-specific T helper type 2 (Th2) cells are generated in patients with AR, whereas allergen-specific Th1 cells are

generated in healthy individuals [11,12]. Early interleukin (IL)-4 and thymic stromal lymphopoietin (TSLP) produced by basophils in response to allergens with protease activity may contribute to Th2 differentiation [12]. Th2 cells produce IL-4/IL-13 and express CD40L, which promote the class-switching of B cells to immunoglobulin (Ig)E [13,14]. When sensitized subjects inhale antigens, the antigens pass through the epithelial tight junctions in the nasal mucosa to bind IgE on the surface of mast cells in the epithelial layer of the nasal mucosa, inducing the release of chemical mediators including histamine, prostaglandins and cysLTs by aggregation of FcεRI. Histamine regulates tight junctions via the coupling of H1 receptors and increases paracellular permeability [15]. This increased permeability allows DC to penetrate epithelial tight junctions easily and enhance antigen presentation to T cells [16]. The early-phase response, which consists of sneezing, rhinorrhoea and nasal congestion, is caused by interactions between chemical mediators and the sensory nerve terminals and blood vessels in the nasal mucosa [17].

After the nasal exposure to allergen, infiltration of inflammatory cells, such as activated eosinophils and Th2 cells, into the nasal mucosa is induced by cytokines, chemical mediators, chemokines and growth factors [18,19]. Cytokines such as IL-5, IL-4, IL-13 and granulocyte–macrophage colony-stimulating factor (GM-CSF) are produced mainly in Th2 cells and mast cells; however, eosinophils also have the potential to produce these cytokines [18,20,21]. Chemical mediators such as platelet-activating factor (PAF), leukotriene B4 (LTB_4), cysteinyl leukotrienes (cysLTs) and thromboxane A_2 (TXA_2) are also released mainly from mast cells and eosinophils [17,20]. Chemokines such as eotaxin, regulated upon activation normal T cell expressed and secreted (RANTES) and thymus and activation regulated chemokine (TARC) are produced mainly in fibroblasts, epithelial cells and vascular endothelial cells [22]. Proinflammatory cytokines such as tumour necrosis factor (TNF)-α from mast cells and eosinophil-derived granules such as eosinophil cationic proteins are also produced and participate in allergic inflammation [23,24]. The sensitivity of the nasal mucosa to different stimulants increases along with the progress of allergic inflammation in the nasal mucosa; this increased sensitivity is referred to as the priming effect [25]. The secondary reaction with inflammatory cells and their mediators, especially the cysLTs produced by eosinophils, causes oedema of the nasal mucosa [26]. This inflammation, which develops 6–10 h after the allergen challenge, is referred to as the late-phase response [17]. Management of allergic rhinitis should be determined based on its mechanism (Fig. 1).

Onset of three major AR symptoms

Sneezing. Sensory nerves containing substance P (SP) and calcitonin gene-related peptide (CGRP) are distributed throughout the epithelial and subepithelial layers of the nasal mucosa [27]. Sensory nerve terminals are located in the epithelial junctions and subepithelial layers. In the guinea pig model of allergic rhinitis, the sneezing reflex following allergen challenge is inhibited significantly by pretreatment with capsaicin, which depletes SP and CGRP from the nasal mucosa [28]. When various chemical mediators are applied to the nasal mucosa, histamine is the only mediator that induces a significant sneezing reflex [28,29]. Therefore, the sneezing reflex following allergen challenge is a respiratory reflex induced by the interaction between histamine and the H1 receptor at the sensory nerve terminals containing SP and CGRP and might be a sensory stimulation response amplified by hyperreactivity in the nasal mucosa [25].

Rhinorrhoea. Synchronously with the sneezing reflex, sensory stimulation on the nasal mucosa induces excitation reflexively in the parasympathetic centre. After allergen challenge on the hemilateral nasal mucosa of patients with allergic rhinitis, the weight of rhinorrhoea induced in both sides of nasal cavities is correlated with the number of sneezes. In addition, the weight of rhinorrhoea in the nasal cavity with allergen challenge is correlated with that on the opposite side. Therefore, rhinorrhoea can be regarded as the secretion from the mucous glands by parasympathetic stimulation [30]. Furthermore, allergic inflammation induced by nasal allergen exposure augments this 'naso-nasal' reflex [31]. Possible mechanisms for sensory nerve hyperresponsiveness include the increased release of nerve growth factor during allergic inflammation [32].

Chemical mediators including histamine, cysLTs, and PAF induce plasma exudation directly from the blood vessels in the nasal mucosa, which constitutes a part of rhinorrhoea. However, only 4–15% of total rhinorrhoea is attributed to plasma exudation, according to calculations based on the albumin concentration in the rhinorrhoea induced by allergen challenge [33].

Nasal congestion. The underlying causes of nasal congestion in the early phase of allergic rhinitis are the relaxation of the smooth muscle layer of capacitance vessels in the nasal mucosa and the interstitial oedema induced by plasma exudation. Swelling of the nasal turbinate is induced by the parasympathetic reflex and the axon reflex through the nerve centre and the direct effects of the chemical mediators on the vascular system. Dilation of the capacitance vessels and plasma exudation after excitation of the parasympathetic centre are caused by the nitric oxide (NO) released from parasympathetic terminals and vascular endothelial cells [34]. However, the participation of the nerve reflex in nasal turbinate swelling after allergen challenge is minor compared with the direct effects of chemical mediators, such as histamine, cysLTs, PAF and prostaglandin D_2 (PGD_2) and kinin, on the vascular system in the nasal mucosa [35,36]. Nasal congestion in the late phase is induced by the allergic inflammation, as described above.

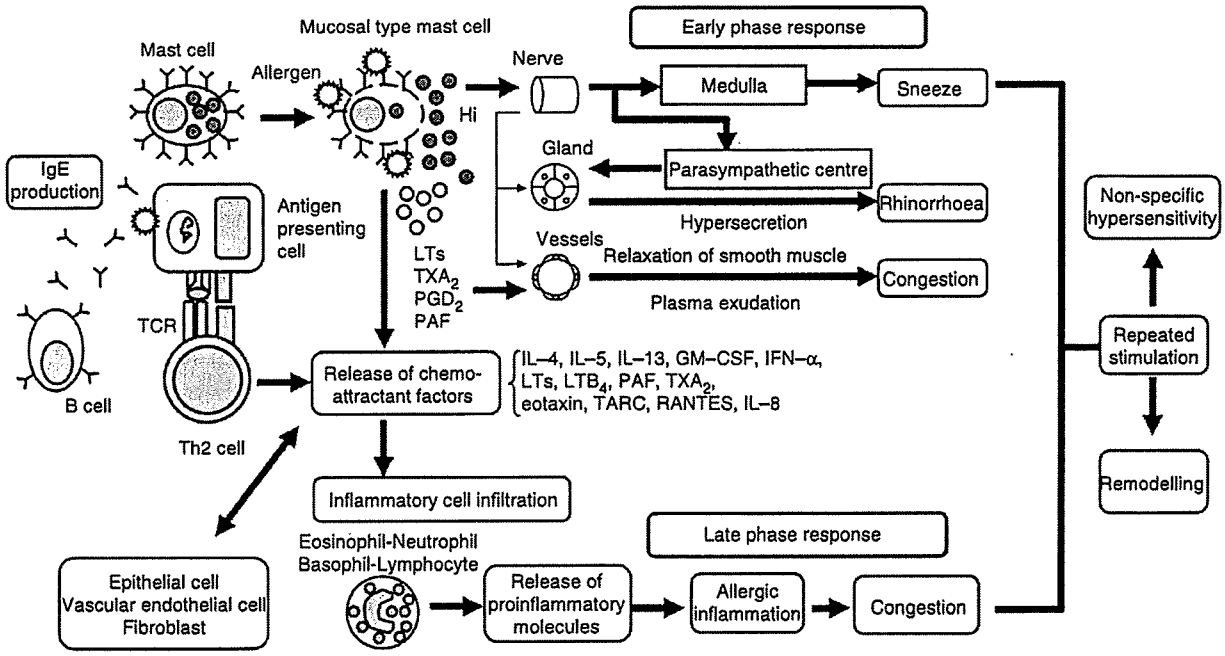

Fig. 1. Pathophysiology of allergic rhinitis as described in Practical Guideline for Management of Allergic Rhinitis in Japan (PG-MARJ). After allergens are inhaled into the nasal mucosa of sensitized subjects, they bind to immunoglobulin (Ig)E on the surface of mast cells, inducing the release of chemical mediators including histamine, prostaglandins and cysteinyl leukotrienes (cysLTs) by aggregation of FcεRI. Histamine regulates tight junctions by coupling the H1 receptor, which increases paracellular permeability. The early-phase response, which is characterized by sneezing, rhinorrhoea and nasal congestion, is the response of the sensory nerve terminals and blood vessels on the nasal mucosa to these chemical mediators. After the nasal exposure to allergen, infiltration of inflammatory cells, such as activated eosinophils and T helper type 2 (Th2) cells, into the nasal mucosa is induced by chemoattractant factors such as cytokines including interleukin (IL)-5, chemical mediators including cysLTs and chemokines including eotaxin. Oedema of the nasal mucosa develops as a secondary reaction with inflammatory cells. This inflammation, referred to as the late-phase response, develops 6–10 h after allergen challenge and causes prolonged nasal congestion.

Mechanisms of glucocorticosteroid

Molecular level

At the molecular level, the effects of GC begin when GC crosses the cell membrane and binds to the intracellular glucocorticosteroid receptor (GR) [37]. Cytoplasmic GR is maintained in an inactive form by heat shock protein (hsp)90 and hsp70 [38,39]. Binding of GC dissociates the hsps, allowing the GR complex to translocate into the nucleus or interact with cytoplasmic transcriptional factors. An alternative splicing variant, GRβ, lacks the ability to bind GC [40]. GRβ forms heterodimers with the wild-type GR (GRα) and may act as an inhibitor of GRα. In atopic nasal tissue, staphylococcal enterotoxin induces GRβ expression and steroid resistance [41].

GC exerts its anti-inflammatory effects through at least two pathways, transactivation and transrepression [42]. Transactivation occurs when the receptor complex binds to the glucocorticosteroid-response elements (GRE) in the promoter regions of glucocorticosteroid-responsive genes, which encode anti-inflammatory genes such as annexin 1, IκB and CD163 [43]. Alternatively, the GR complex represses the transcription of proinflammatory genes by protein–protein interactions such as GR–nuclear factor kappa B (NFκB) and GR–activator protein 1 (AP-1) [44]. Evidence for a co-activator competition model of transrepression involving CBP/p300 was first provided for GR transrepression of AP-1 target genes [45].

Cellular level (Fig. 2)

GC inhibits the functions of infiltrating inflammatory cells and their recruitment into the nasal mucosa. GC inhibits the maturation, cytokine production, FcεRI expression and mediator release of mast cells [46,47]. GC inhibits histamine release from basophils [48,49], induces apoptosis of eosinophils [50] and reduces the recruitment of antigen-presenting cells such as Langerhans cells [51]. GC decreases the numbers of GATA-3$^+$ Th2 cells and the production of Th2 cytokines, such as IL-4, IL-5, IL-6 and IL-13, while having little effect on T-bet$^+$ Th1 cells and the production of Th1 cytokines such as IL-2, IL-12 and interferon (IFN)-γ [52,53]. Although the inhibitory effect of GC on B cell recruitment is limited, GC inhibits class-switching to IgE in the nasal mucosa [51,54].

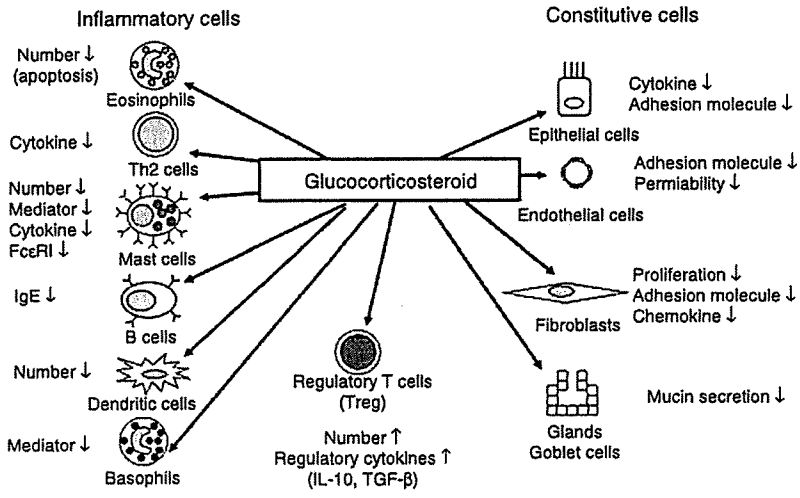

Fig. 2. Effect of glucocorticosteroids (GC) on nasal mucosa. The anti-inflammatory effects of GC on allergic rhinitis are mediated not only by inflammatory cells such as eosinophils, T helper type 2 (Th2) cells, mast cells, B cells, dendritic cells and basophils, but also by nasal constitutive cells such as epithelial cells, endothelial cells, fibroblasts and glands/goblet cells. In addition, treatment with GC can induce regulatory T cells.

GC also has anti-inflammatory effects on nasal constitutive cells, such as epithelial cells, fibroblasts, vascular endothelial cells and glands. GC inhibits intercellular adhesion molecule 1 (ICAM-1) expression [49] and GM-CSF production [55] by nasal epithelial cells. GC down-regulates nasal fibroblast functions, including basic fibroblast growth factor (bFGF)-induced proliferation, TNF-α-induced ICAM-1 expression, TNF-α- or IL-4-stimulated eotaxin release [56], TNF-α-induced matrix metalloproteinase production [57] and TNF-α-induced vascular endothelial growth factor (VEGF) and bFGF production [58]. GC inhibits TNF-α- or IL-1β-stimulated E-selectin expression on nasal vascular endothelial cells [59]. The effect of GC on vascular cel adhesion molecule 1 (VCAM-1) expression on nasal vascular endothelial cells is controversial [60,61]. The effect of GC on vascular permeability reflects the inhibition of cellular inflammatory processes indirectly rather than the direct effect on nasal vascular endothelial cells [62].

Induction of regulatory cytokines and T_{regs}

Among the cells with regulatory functions such as $CD8^+$, $CD4^-CD8^-$ and γδ T cells, $CD4^+CD25^+$forkhead box P3 ($FoxP3^+$) T_{reg} cells play a central role in immune tolerance and immune homeostasis [63]. T_{regs} are derived from the thymus and the periphery [64]. The suppressive effect of T_{regs} is associated with expression of the transcription factor FoxP3, which is used as a T_{reg} marker [65]. In addition, T_{regs} express high-affinity IL-2 receptor (CD25), and IL-2 is vital for the development and survival of T_{regs} [64]. T_{regs} regulate effector cells by cell-to-cell contact, the production of inhibitory cytokines such as IL-10 and transforming growth factor (TGF)-β, cytotoxicity mediated by perforins and granzymes, and competition for T cell growth factors, especially IL-2 [66].

The impaired expression or function of T_{regs} is involved in the pathogenesis of allergic rhinitis. For example, regulatory $CD4^+CD25^+$ T cells from patients with birch pollinosis but not healthy controls were defective in down-regulating birch pollen-induced IL-13 and IL-5 production by $CD4^+CD25^-$ T cells during the pollen season, while their capacity to suppress IFN-γ production and proliferation was retained [67]. The ratio of $FoxP3^+$/GATA binding protein 3 ($GATA\text{-}3^+$) cells in nasal mucosa was decreased significantly in patients with pollinosis as compared with healthy controls outside the pollen season, and the ratio was decreased further during the pollen season in allergic patients [53]. In addition, T_{regs} are induced in both peripheral blood and nasal mucosa following allergen-specific immunotherapy [68,69].

Treatment with GC induces T_{regs}. FoxP3 mRNA expression in $CD4^+$ cells was increased significantly in adult asthmatic patients receiving GC, and systemic GC treatment led to an early increase in FoxP3 mRNA and T_{reg} expression in patients with asthma [70]. Paediatric asthma patients treated with GC also had an increased frequency of T_{regs} in $CD4^+$ cells from peripheral blood and bronchoalveolar lavage fluid (BALF). In addition, T_{regs} in the BALF of asthmatic patients failed to suppress proliferation and production of Th2-associated cytokines by responder T cells, which was restored after inhalation of GC [71]. FoxP3 and IL-10 were down-regulated in nasal polyps compared with control mucosa, and their expression was increased after intranasal GC treatment [72]. We have demonstrated that GC induced $CD4^+$ $CD25^+$ $FoxP3^+$ T_{regs} in dispersed nasal polyp cells in the presence of IL-2. In fact, combined treatment with GC and IL-2 expands T_{regs} *in vivo*, and the induced T_{regs} suppress the proliferation of responder T cells in mice [73]. GC leads to the production of glucocorticosteroid-induced leucine zipper (GILZ) by dendritic cells; GILZ is critical for commitment of DCs to differentiate into regulatory DCs and for the generation of antigen-specific T_{regs} [74]. The detailed mechanism by which GC induces T_{regs} has not been elucidated.

Practical Guideline for Management of Allergic Rhinitis in Japan (PG-MARJ)

To address the classification, epidemiology, pathophysiology and management of allergic rhinitis in Japan, a practical guideline for the management of this condition, PG-MARJ, was first released in 1993. Based on the latest basic and clinical evidence, the sixth edition of PG-MARJ was published in 2008 [2]. The following discussion summarizes the PG-MARJ guidelines regarding the positioning of INS and systemic GC for the management of allergic rhinitis.

INS are potent agents indicated for the treatment of allergic rhinitis. In the treatment of type I allergy, INS are used as anti-inflammatory drugs. INS exert anti-inflammatory effects by the following mechanisms: inhibiting the local infiltration of effector cells of allergic inflammation such as mucosal-type mast cells, eosinophils and lymphocytes; inhibiting the production and release of cytokines; inhibiting vascular permeability and mucus gland secretion; and down-regulating the production of leukotrienes and prostaglandins by inhibiting arachidonic acid cascades. INS are not effective in controlling acute-phase allergic reactions but are effective for late-phase allergic reactions. However, INS are effective in controlling acute-phase allergic reactions when administered continuously.

Beclomethasone propionate, fluticasone propionate, mometasone furoate and fluticasone furoate are currently available as nasal sprays in Japan. These INS have potent local effects at small doses; they are not absorbed easily into the systemic circulation and are metabolized rapidly when absorbed [75]. Thus, the incidence of systemic adverse effects is low, even in patients receiving these drugs for ≥ 1 year, and reliable clinical effects can be expected with their use [76,77]. In addition, INS with lower bioavailability are believed to show fewer systemic adverse effects [78]. Because these drugs are administered locally, mild nasal irritation, dry nose and nasal bleeding may develop in winter when the air is dry.

The onset of the effects of INS is rapid, with efficacy observed in as little as 1 day [79]. Efficacy increases as the treatment period is prolonged. These drugs are effective even in patients with severe allergic rhinitis; their effects are clearly observable, and many patients obtain excellent results. INS are effective for the treatment of nasal obstruction that is unresponsive to H_1-receptor antagonists, for aiding withdrawal from vasoconstrictive nose drops (α-sympathetic stimulants) and for the treatment of vasomotor rhinitis [80].

Oral GC may be used in patients who do not respond to INS (such as those with severe, very severe and intractable allergic rhinitis). Celestamine® (a mixture of H_1-receptor antagonist *d*-chlorpheniramine maleate and betamethasone) is used relatively widely in Japan; however, no placebo-controlled trials have been reported. In addition, evidence regarding a suitable dosage of this drug is lacking. Among oral GC, only methylprednisolone tablets are confirmed as an effective treatment for allergic rhinitis by a placebo-controlled trial; this trial showed that a daily dosage of 24 mg of methylprednisolone was necessary to obtain a significant improvement in all nasal symptoms [81]. Thus, the use of oral GC corresponding to 20–30 mg of prednisolone should be limited to a brief period of time (within 1 week) when treating patients with allergic rhinitis. Caution is needed to avoid adverse effects including adrenal cortical suppression and difficulty in withdrawing GC following prolonged administration (longer than 2 weeks) [82].

Although some physicians use intramuscular injection with depot glucocorticosteroids for the treatment of pollinosis [83], these injections may induce systemic adverse effects. Therefore, a careful examination including the serum cortisol level and blood glucose level should be performed both before and after treatment. Because adverse effects such as moon face, skin/skin appendage disorders, menstrual disorder, application site disorders, including atrophy, and adrenal cortical hypofunction may develop, depot glucocorticosteroids are not recommended for patients with pollinosis [84].

Based on the above observations, glucocorticosteroids are recommended for patients with moderate-to-severe perennial allergic rhinitis (Table 1) and mild-to-severe pollinosis, except for prophylactic treatment (Table 2).

Effect of INS on ocular symptoms in patients with allergic rhinitis

Regarding statements on the mechanisms and efficacy of intranasal glucocorticosteroids, the Japanese guideline (PG-MARJ) has many similarities with Allergic Rhinitis and its Impact on Asthma (ARIA), the evidence-based international guideline for allergic rhinitis [1]. However, there are differences between these two guidelines, such as different conclusions regarding the efficacy of INS for ocular symptoms. According to the PG-MARJ, INS are effective only against nasal symptoms [2]. However, the updated ARIA documented that INS are effective not only for nasal but also ocular symptoms in patients with pollinosis.

Bernstein *et al.* performed a double-blind, double-dummy, randomized study comparing fluticasone propionate aqueous nasal spray 200 μg once daily, oral loratadine 10 mg once daily or placebo for the treatment of seasonal allergic rhinitis and found that fluticasone propionate reduced ocular symptoms, especially ocular itching, tearing and redness, compared with not only placebo but also oral loratadine [85]. More recently, Fokkens *et al.* performed a multi-centre, randomized, double-blind, placebo-controlled, parallel group study of fluticasone furoate 110 μg once daily nasal spray *versus* placebo for the treatment of seasonal allergic rhinitis caused by grass pollen, and they found that fluticasone furoate is significantly effective for not only nasal symptoms and quality of life but also

Table 1. Management for perennial allergic rhinitis in PG–MARJ.

Grade type	Mild	Moderate		Severe	
		Sneeze/discharge type	Congestion type	Sneeze/discharge type	Congestion type
Management	① H1 RA ② CMRI ③ Th2 CS Either ①, ② or ③	① H1 RA ② CMRI ③ Th2 CS ④ INS Either ①, ②, ③ or ④ Combination of ④ with ①, ② or ③	① LT RA ② PGD_2/TXA_2 RA ③ INS Either ①, ②, or ③ Combination of ③ with ① or ②	HIS + H1 RA	INS + LT RA or PGD_2/TXA_2 RA Topical decongestant for 5–7 days at initial treatment if necessary
				Corrective surgery of nasal cavity	
		Allergen-specific immunotherapy			
		Allergen avoidance/elimination			

H1 RA, second generation H1 receptor antagonists; CMRI, chemical mediator release inhibitors; LT RA, leukotriene receptor antagonists; PGD_2/TXA_2 RA, PGD_2/TXA_2 receptor antagonist (ramatroban); T helper type 2 (Th2) C, Th2 cytokine suppressor (suplatast); INS, intranasal glucocorticosteroids.

ocular symptoms including eye itching/burning, eye tearing/watering and eye redness [86]. The efficacy of fluticasone furoate nasal spray against ocular symptoms was also confirmed in patients with ragweed allergy [87].

Although the precise mechanism remains unclear, several explanations regarding the effectiveness of INS drugs for the treatment of ocular symptoms have been proposed. Because of low bioavailability, systemic absorption and circulation is negligible among second-generation INS drugs [75]. The symptoms of itchy and watery eyes and bilateral ocular secretion weights increase after ipsilateral nasal challenge with allergen, suggesting that the ocular symptoms associated with allergic rhinitis arise, in part, from a naso-ocular reflex [88]. The reduced nasal inflammation caused by INS may lead to a normalization or modification of the naso-ocular reflex. In addition, the reduced inflammation in the nose may lessen the release of inflammatory mediators that can cause inflammation in neighbouring tissues including the conjunctiva. Reduction of oedema and inflammation surrounding the opening of the nasolacrimal duct might also reduce the retention of allergen in the conjunctiva.

Effect of INS on impaired performance

Allergic rhinitis itself impairs performance by causing daytime sleepiness and disrupting cognitive functions such as learning ability [89,90]. Nasal congestion due to allergic reaction and inflammation seems to be the major causative

Table 2. Management for pollinosis in PG–MARJ.

Grade type	Prophylactic	Mild	Moderate		Severe	
			Sneeze/discharge type	Congestion type	Sneeze/discharge type	Congestion type
Management	① CMRI ② H1 RA ③ LT RA ④ Th2 CS ⑤ PGD_2/TXA_2 RA Either ①, ②, ③, ④ or ⑤	① H1 RA ② INS Start with ① with eye drops Add ② if necessary	H1 RA + INS	LT RA + INS + H1 RA	INS + H1 RA	INS + LT RA + H1 RA Topical decongestant for 7–10 days at initial treatment if necessary Short-term administration (4–7 days) of oral glucocorticoids may be chosen for patients with extremely severe congestion
		Eye drops of either H1 RA or CMRI			Eye drops of either H1 RA, CMRI or glucocorticoids Corrective surgery of nasal cavity	
		Allergen-specific immunotherapy				
		Allergen avoidance/elimination				

H1 RA, second generation H1 receptor antagonists; CMRI, chemical mediator release inhibitors; LT RA, leukotriene receptor antagonists; PGD_2/TXA_2 RA, PGD_2/TXA_2 receptor antagonist (ramatroban); T helper type 2 (Th2) CS, Th2 cytokine suppressor (suplatast); INS, intranasal glucocorticosteroids.

factor of daytime sleepiness, as this symptom can cause obstructive sleep apnoea and microarousals during sleep [91]. Symptomatic seasonal allergic rhinitis has been associated with significant detrimental effects on examination performance in young people [90].

Treatment with sedating H_1-receptor antagonists exacerbates impaired performance [90,92]; students taking these medications on examination days exhibited a significant tendency to unexpectedly drop a grade [90].

On the other hand, INS can improve impaired performance in allergic rhinitis patients [93,94]. Craig *et al.* [93] showed that intranasal budesonide 128 μg/day, flunisolide 200 μg/day and fluticasone 200 μg/day were each effective in improving sleep and daytime fatigue and somnolence, although significant changes in polysomnography did not always occur. Moreover, treatment with intranasal fluticasone propionate 200 μg once daily significantly improved not only nasal symptoms and daytime sleepiness but also cognitive performance, as measured by the test of variables of attention (TOVA) in patients with seasonal allergic rhinitis [94].

Efficacy of INS for prophylactic (initial) treatment of pollinosis

The PG-MARJ recommends that patients who experience severe symptoms of pollinosis every year should receive prophylactic treatment immediately after the start of pollen release or the onset of symptoms [2,95]. Considering the amount of pollen release expected during the season and the type and severity of symptoms usually experienced by patients during the peak pollen season, physicians should determine the drug regimen for each individual patient by selecting from among chemical mediator–release inhibitors, second-generation H_1-receptor antagonists, leukotriene receptor antagonists, Th2 cytokine inhibitor (suplatast) and PGD_2/TXA_2 receptor antagonist (ramatroban) [2]. Patients with sneezing/rhinorrhoea-type rhinitis should receive chemical mediator–release inhibitors or second-generation anti-histamines, whereas patients with congestion-type disease should be treated with leukotriene receptor antagonist, Th2 cytokine inhibitor or PGD_2/TXA_2 receptor antagonist.

Several reports suggest that INS drugs are effective for the prophylactic treatment of pollinosis. One study of prophylactic treatment with mometasone furoate 200 μg once daily aqueous nasal spray, beclomethasone dipropionate 168 μg b.i.d. aqueous nasal spray or placebo was initiated in patients with ragweed pollinosis 4 weeks before the estimated start of pollen season. Both the proportion of minimal symptom days from start of ragweed season and the number of days from start of ragweed season to first non-minimal symptom day were significantly higher in patients treated with either mometasone furoate or beclomethasone dipropionate compared with placebo [96]. Yokoo [97] compared the efficacy of prophylactic treatment with intranasal fluticasone propionate 200 μg twice daily *versus* the second-generation oral H_1-antagonist olopatadine 10 mg twice daily in patients with Japanese cedar pollinosis and found that fluticasone propionate delayed the onset of nasal symptoms significantly compared with olopatadine. In addition, treatment with fluticasone suppressed symptoms significantly during peak pollen season. Okubo *et al.* [98] reported that initial treatment with fluticasone propionate 100 μg b.i.d. prevented exacerbation of nasal symptoms in paediatric patients with seasonal allergic rhinitis. Indeed, nasal symptoms disappeared in 44·0% of patients who had mild symptoms at initiation of treatment.

As described above, one of the pathways of the anti-inflammatory effect of GC is the down-regulation of proinflammatory genes by several mechanisms such as protein–protein interactions that sequester protein kinase A and cAMP enhancer binding protein (CREB)-binding protein from NF-κB [44,45]. The interaction between NF-κB, CREB and CREB-binding protein leads to the acetylation of chromatin and the subsequent transcription of proinflammatory genes, such as genes encoding cytokines, inflammatory enzymes, adhesion molecules and inflammatory receptors [99]. Thus, GC may be more effective for prophylactic treatment compared with post-onset treatment because increased levels of NF-κB in the nose after the onset of pollinosis can attenuate protein–protein interaction by glucocorticosteroids.

Conclusions

In addition to the novel information that appeared in the sixth edition of the PG-MARJ in 2008, considerable evidence supports the use of GC against allergic rhinitis. GC can induce regulatory cytokines and $FoxP3^+$ T_{regs} in the nose. The appropriate use of INS may improve nasal symptoms, ocular symptoms and impaired performance. Moreover, INS can be used for the first-line prophylactic treatment of pollinosis. These recent findings may provide additional information for incorporation into future editions of guidelines for allergic rhinitis treatment, including the PG-MARJ. On the other hand, several issues remain unsolved. For example, although inhaled GC have not been incriminated as teratogens in humans and are used commonly by pregnant women who have asthma, there are no placebo-controlled, randomized, double-blind studies of INS during the first trimester of pregnancy.

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References

1 Bousquet J, Khaltaev N, Cruz AA *et al.* Allergic rhinitis and its impact on asthma (ARIA) 2008 update. Allergy 2008; 63 (Suppl. 86):8–160.

2 Editorial Committee for PG-MARJ. Practical guideline for the management of allergic rhinitis in Japan, 6th edn. Tokyo: Life Science, 2008.

3 Bousquet J, Jacquot W, Vignola M, Bachert C, Van Cauwenberge P. Allergic rhinitis: a disease remodeling the upper airways? J Allergy Clin Immunol 2004; 113:43–9.

4 Kimmerle R, Rolla AR. Iatrogenic Cushing's syndrome due to dexamethasone nasal drops. Am J Med 1985; 79:535–7.

5 Masuyama K. Merits and demerits of steroid – different whole body and limited part administering. J Jpn Immunol Allergol Otolaryngol 2000; 18:6–11.

6 Dorendorf H, Meltzer EO. Molecular and clinical pharmacology of intranasal corticosteroids: clinical and therapeutic implications. Allergy 2008; 63:1292–300.

7 Wan H, Winton HL, Soeller C *et al.* Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. J Clin Invest 1999; 104:123–33.

8 Hottori H, Okano M, Yoshino T *et al.* Expression of costimulatory CD80/CD86-CD28/CD152 molecules in nasal mucosa of patients with perennial rhinitis. Clin Exp Allergy 2001; 31:1242–9.

9 Okano M, Azuma M, Yoshino T *et al.* Differential role of CD80 and CD86 molecules in the induction and effector phases of allergic rhinitis in mice. Am J Respir Crit Care Med 2001; 164:1501–7.

10 Kleinjan A, Willart M, van Rijt LS *et al.* An essential role for dendritic cells in human and experimental allergic rhinitis. J Allergy Clin Immunol 2006; 118:1117–25.

11 Akdis M, Verhagen A, Taylor F *et al.* Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med 2004; 199:1567–75.

12 Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat Immunol 2008; 9:310–18.

13 Okano M, Yoshino T, Nishizaki K *et al.* Interleukin-4 independent production of Th2 cytokines by nasal lymphocytes and nasal eosinophilia in murine allergic rhinitis. Allergy 2000; 55:723–31.

14 Hattori H, Okano M, Kariya S, Nishizaki K, Satoskar AR. Signals through CD40 play a critical role in the pathophysiology of *Schistosoma mansoni* egg antigen-induced allergic rhinitis in mice. Am J Rhinol 2006; 20:165–9.

15 Flynn AN, Itani OM, Moninger TO, Welsh MJ. Acute regulation of tight junction ion selectivity in human airway epithelia. Proc Natl Acad Sci USA 2009; 106:3591–6.

16 Takano K, Kojima T, Go M *et al.* and CD11c-positive dendritic cells penetrate beyond well-developed epithelial tight junctions in human nasal mucosa of allergic rhinitis. J Histochem Cytochem 2005; 53:611–19.

17 Gelfand EW. Inflammatory mediators in allergic rhinitis. J Allergy Clin Immunol 2004; 114:S135–8.

18 Nomiya R, Okano M, Fujiwara T *et al.* CRTH2 plays an essential role in the pathophysiology of Cry j 1-induced pollinosis in mice. J Immunol 2008; 180:5680–8.

19 Choi GS, Pard HJ, Hur GY *et al.* Vascular endothelial growth factor in allergen-induced nasal inflammation. Clin Exp Allergy 2009; 39:655–61.

20 Durham SR, Ying S, Varney VA *et al.* Cytokine messenger RNA expression for IL-4, IL-4, IL-5, and granulocyte/macrophage colony stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J Immunol 1992; 148:2390–4.

21 Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P. Eosinophil: biological properties and role in health and disease. Clin Exp Allergy 2008; 38:709–50.

22 Takahashi N, Yamada T, Narita N, Fujieda S. Double-stranded RNA induces production of RANTES and IL-8 by human nasal fibroblasts. Clin Immunol 2006; 118:51–8.

23 Marcucci F, Sensi LG, Migali E, Coniglio G. Eosinophilic cationic protein and specific IgE in serum and nasal mucosa of patients with grass-pollen-allergic rhinitis and asthma. Allergy 2001; 56:231–6.

24 Iwasaki M, Saito K, Tekemura M *et al.* TNF-alpha contributes to the development of allergic rhinitis in mice. J Allergy Clin Immunol 2003; 112:134–40.

25 Bousquet J, Vignola AM, Campbell AM, Michel FB. Pathophysiology of allergic rhinitis. Int Arch Allergy Immunol 1996; 110:207–18.

26 Fujita M, Yonetomi Y, Shimouchi K *et al.* Involvement of cysteinyl leukotriens in biphasic increase of nasal airway resistance of antigen-induced rhinitis in guinea pigs. Eur J Pharmacol 1999; 369:349–56.

27 Heppt W, Dinh QT, Cryer A *et al.* Phenotypic alteration of neuropeptide-containing nerve fibers in seasonal intermittent allergic rhinitis. Clin Exp Allergy 2004; 34:1105–10.

28 Imanura T, Kambara T. Substance P as a potent stimulator of sneeze responses in experimental allergic rhinitis of guinea pigs. Agents Actions 1992; 37:245–9.

29 Nabe T, Tsuzuike N, Ohtani Y *et al.* Important roles of tachykinines in the development of allergic nasal hyperresponsiveness in guinea-pigs. Clin Exp Allergy 2008; 39:138–46.

30 Baroody FM, Ford S, Lichtenstein LM, Kagey-Sobotka A, Naclerio RM. Physiologic responses and histamine release after nasal antigen challenge. Effect of atropine. Am J Respir Crit Care Med 1994; 149:1457–65.

31 Sheaham P, Walsh RM, Walsh MA, Costello RW. Induction of nasal hyper-responsiveness by allergen challenge in allergic rhinitis: the role of afferent and efferent nerves. Clin Exp Allergy 2005; 35:45–51.

32 Bresciani M, Laliberte F, Laliberte MF, Gramiccioni C, Bonini S. Nerve growth factor localization in the nasal mucosa of patients with persistent allergic rhinitis. Allergy 2009; 64:112–17.

33 Konno A, Terada N, Okamoto Y, Togawa K. The role of chemical mediators and mucosal hyperreactivity in nasal in nasal hypersecretion in nasal allergy. J Allergy Clin Immunol 1987; 79:620–7.

34 Struben VM, Wieringa MH, Feenstra L, de Jongste JC. Nasal nitric oxide and nasal allergy. Allergy 2006; 61:665–70.

35 Sikoff PE, Roth Y, McClean P, Cole P, Chapnik J, Zamel N. Nasal nitric oxide does not control basal nasal patency or acute congestion following allergen challenge in allergic rhinitis. Ann Otol Rhinol Laryngol 1999; 108:368–72.

36 Tanaka Y, Mizutani N, Fujii M, Nabe T, Kohno S. Different mechanisms between thromboxane A2- and leukotriene D4-induced nasal blockage in guinea-pigs. Prostagalandins Other Lipid Mediat 2006; 80:144–54.

37 Mangelsdorf DJ, Thummel C, Beato M *et al.* The nuclear receptor superfamily: the second decade. Cell 1995; 83:835–9.

38 Pratt WB, Morishima Y, Murphy M, Harrel M. Chaperoning of glucocorticoid receptors. Handb Exp Pharmacol 2006; 172:111–38.

39 Grad I, Picard D. The glucocorticoid responses are shaped by molecular chaperones. Mol Cell Endocrinol 2007; 274:2–12.

40 Oakley RH, Jewell CM, Yudt MR, Bofetiado DM, Cidlowski JA. The dominant negative activity of the human glucocorticoid receptor beta isoform. Specificity and mechanisms of action. J Biol Chem 1999; 274:27857–66.

41 Fakhri S, Tulic M, Christodoulopoulos P *et al.* Microbial superantigens induce glucocorticoid receptor β and steroid resistance in a nasal explants model. Laryngoscope 2004; 114:887–92.

42 Barnes PJ. Corticosteroid effects on cell signaling. Eur Respir J 2006; 27:413–26.

43 Graversen JH, Madsen M, Moestrup SK. CD163: a signal receptor scavenging haptoglobin–hemoglobin complex from plasma. Int J Biochem Cell Biol 2002; 34:309–14.

44 Pascual G, Glass CK. Nuclear receptors versus inflammation: mechanisms of transrepression. Trends Endocrinol Metab 2006; 17:321–7.

45 Saito M, Takayanagi R, Goto K *et al.* The presence of both the amino- and carboxyl-terminal domains in the AR is essential for the completion of a transcriptionally active form with coactivators and intranuclear compartmentalization common to the steroid hormone receptors: a three-dimensional imaging study. Mol Endocrinol 2002; 16:694–706.

46 Smith SJ, Piliponsky AM, Rosenhead F, Elchalal U, Nagler A, Levi-Schaffer F. Dexamethasone inhibits maturation, cytokine production and Fc epsilon RI expression of human cord blood-derived mast cells. Clin Exp Allergy 2002; 32:906–13.

47 Crocker IC, Zhou CY, Bewta AK, Kreutner W, Townley RG. Glucocorticosteroids inhibit leukotriene production. Ann Allergy Asthma Immunol 1997; 78:497–505.

48 Stellato C, Atsuta J, Bickel CA, Schleimer RP. An *in vitro* comparison of commonly used topical glucocotricoid preparations. J Allergy Clin Immunol 1999; 104:623–9.

49 Ciprandi G, Tosca MA, Passalocqua G, Canonica GW. Intranasal mometasone furoate reduces late-phase inflammation after allergen challenge. Ann Allergy Asthma Immunol 2001; 86:433–8.

50 Meagher LC, Cousin JM, Seckl JR, Haslett C. Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. J Immunol 1996; 156:4422–8.

51 Till SJ, Jacobson MR, O'Brien F *et al.* Recruitment of CD1a+Langerhans cells to the nasal mucosa in seasonal allergic rhinitis and effects of topical corticosteroid therapy. Allergy 2001; 56:126–31.

52 Erin EM, Zacharasiewicz AS, Nicholson GC *et al.* Topical corticosteroid inhibits interleukin-4, -5, and -13 in nasal secretions following allergen challenge. Clin Exp Allergy 2005; 35:1608–14.

53 Malmhall C, Bossios A, Pullertis T, Lotvall J. Effects of pollen and nasal glucocorticoids on FOXP3+, GATA-3+ and T-bet+ cells in allergic rhinitis. Allergy 2007; 62:1007–13.

54 Durham SR, Gould HJ, Thiense CP *et al.* Expression of epsilon germ-line transcripts and mRNA for the epsilon heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. Eur J Immunol 1997; 27:2599–906.

55 Mullol J, Roca-Ferrer J, Xaubet A, Raserra J, Picado C. Inhibition of GM-CSF secretion by topical corticosteroids and nedocromil sodium. A comparison study using nasal polyp epithelial cells. Respir Med 2000; 94:428–31.

56 Silvestri M, Sabatini F, Scarso L, Cordone A, Dasic G, Rossi GA. Fluticasone propionate downregulates nasal fibroblast functions involved in airway inflammation and remodeling. Int Arch Allergy Immunol 2002; 128:51–8.

57 Namba M, Asano K, Kanai K, Watanabe S, Hisamitsu T, Suzaki H. Suppression of matrix metalloproteinase production from nasal fibroblasts by fluticasone propionate *in vitro*. Acta Otolaryngol 2004; 124:964–9.

58 Uchida J, Kanai K, Asano K, Watanabe S, Hisamitsu T, Suzaki H. Influence of fluticasone propionate on the production of vascular endothelial growth factor and basic fibroblast growth factor from nasal fibroblasts *in vitro*. In Vivo 2004; 18:767–70.

59 Yamamoto Y, Ikeda K, Watanabe M *et al.* Expression of adhesion molecules in cultured human nasal mucosal microvascular endothelial cells activated by interleukin-1 beta or tumor necrosis factor-alpha: effects of dexametasone. Int Arch Allergy Immunol 1998; 117:68–77.

60 Hamilos DL, Thawley SE, Kramper MA, Kamil A, Hamid Q. Effect of intranasal fluticasone on cellular infiltration, endothelial adhesion molecule expression, and proinflamatory cytokine mRNA in nasal polyp disease. J Allergy Clin Immunol 1999; 103:79–87.

61 Tingsgaard PK, Bock T, Larsen PL, Tos M. Topical budesonide treatment reduces endothelial expression of intracellular adhesion molecules (vascular cell adhesion molecule-1 and P-selectin) and eosinophil infiltration in nasal polyps. Acta Otolaryngol 1999; 119:362–8.

62 Greiff L, Andersson M, Svensson C, Alkner U, Persson CGA. Glucocorticoids may not inhibit plasma exudation by direct vascular antipermeability effects in human airways. Eur Respir J 1994; 7:1120–4.

63 Shevach EM, DiPaolo RA, Andersson J, Zhao DM, Stephens GL, Thornton AM. The lifestyle of naturally occurring CD4+CD25+Foxp3+ regulatory T cells. Immunol Rev 2006; 212:60–73.

64 Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008; 133:775–87.

65 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003; 299:1057–61.

66 Ryanna K, Stratigou V, Safinia N, Hawrylowicz C. Regulatory T cells in bronchial asthma. Allergy 2009; 64:335–47.

67 Grindebacke H, Wing K, Andersson AC, Suri-Payer E, Rak S, Rudin A. Defective suppression of Th2 cytokines by CD4CD25 regulatory T cells in birch allergics during birch pollen season. Clin Exp Allergy 2004; 34:1364–72.

68 Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K, Akdis CA. IL-10 and TGF-beta cooperate in the regulatory T cell responses to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003; 33:1205–14.

69 Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+CD25+ cells in the nasal mucosa. J Allergy Clin Immunol 2008; 121:1467–72.

70 Karagiannidis C, Akdis M, Holopainen P *et al.* Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. J Allergy Clin Immunol 2004; 114:1425–33.

71 Hartl D, Koller B, Mehlhorn AT *et al.* Quantitative and functional impairment of pulmonary CD4+ CD25high regulatory T cells in pediatric asthma. J Allergy Clin Immunol 2007; 119:1258–66.

72 Li HB, Cai KM, Liu Z *et al.* T regulatory cells (Tregs) are increased in nasal polyps (NP) after treatment with intranasal steroid. Clin Immunol 2008; 129:394–400.

73 Chen X, Oppenheim JJ, Winkler-Pickett R, Ortaldo JR, Zack

Howard OM. Glucocorticoid amplifies IL-2-dependent expansion of functional FoxP3+CD4+CD25+ T regulatory cell *in vivo* and enhances their capacity to suppress EAE. Eur J Immunol 2006; 36:2139–49.

74 Hamdi H, Godot V, Maillot MC *et al.* Induction of antigen-specific regulatory T lymphocytes by human dendritic cell expressing glucocorticoid-induced leucin zipper. Blood 2007; 110:211–19.

75 Benninger MS, Ahmad N, Marple BF. The safety of intranasal steroids. Otolaryngol Head Neck Surg 2003; 129:739–50.

76 Allen DB, Melzer EO, Lemanske RF Jr *et al.* No growth suppression in children treated with the maximum recommended dose of fluticosone propionate aqueous nasal spray for one year. Allergy Asthma Proc 2002; 23:407–13.

77 Schenkel EJ, Skoner DP, Bronsky EA *et al.* Absence of growth retardation in children with perennial allergic rhinitis after one year of treatment with mometasone furoate aqueous nasal spray. Periatrics 2000; 105:e22.

78 Daley-Yates PT, Kunka RL, Yin Y, Andrews SM, Callejas S, Ng C. Bioavailability of flutiasone propionate and mometasone furoate aqueous nasal sprays. Eur J Clin Pharmacol 2004; 60:265–8.

79 Okubo K, Nakashima M, Miyake N, Komatsubara M, Okuda M. Comparison of fluticasone furoate and fluticasone propionate for the treatment of Japanese cedar pollinosis. Allergy Asthma Proc 2009; 30:84–94.

80 Arikan OK, Koc C, Kendi T, Muluk NB, Ekici A. CT assessment of the effect of fluticasone propionate aqueous nasal spray treatment on lower turbinate hypertrophy due to vasomotor rhinitis. Acta Otolaryngol 2006; 126:37–42.

81 Brooks CD, Karl KJ, Francom SF. Oral methylprednisolone acetate (Medrol tablets) for seasonal allergic rhinitis: examination of dose and symptom response. J Clin Pharmacol 1993; 33:816–22.

82 Vargas R, Dockhorn RJ, Findlay SR, Korenblat PE, Field EA, Kral KM. Effect of fluticasone propionate aqueous nasal spray versus oral prednisone on the hypothalamic–pituitary–adrenal axis. J Allergy Clin Immunol 1998; 102:191–7.

83 Mygind N, Laursen LC, Dahl M. Systemic corticosteroid treatment for seasonal allergic rhinitis: a common but poorly documented therapy. Allergy 2000; 55:11–15.

84 Nasser SMS, Ewan PW. Depot corticosteroid treatment for hay fever causing avascular necrosis of both hips. BMJ 2001; 322:1589–91.

85 Bernstein DI, Levy AL, Hampel FC *et al.* Treatment with intranasal fluticasone propionate significantly improves ocular symptoms in patients with seasonal allergic rhinitis. Clin Exp Allergy 2004; 34:952–7.

86 Fokkens WJ, Jogi R, Reinartz S *et al.* Once daily fluticasone furoate nasal spray is effective in seasonal allergic rhinitis caused by grass pollen. Allergy 2007; 62:1078–84.

87 Kaiser HB, Naclerio RM, Given J *et al.* Fluticasone furoate nasal spray: a single treatment option for the symptoms of seasonal allergic rhinitis. J Allergy Clin Immunol 2007; 119:1430–7.

88 Baroody FM, Foster KA, Markaryan A, deTineo M, Naclerio RM. Nasal ocular reflexes and eye symptoms in patients with allergic rhinitis. Ann Allergy Asthma Immunol 2008; 100:194–9.

89 Kremer B, den Hartog HM, Jolles J. Relationship between allergic rhinitis, disturber cognitive functions and psychological wellbeing. Clin Exp Allergy 2002; 32:1310–15.

90 Walker S, Khan-Wasti S, Fletcher M *et al.* Seasonal allergic rhinitis is associated with a detrimental effect on examination performance in United Kingdom teenagers: case–control study. J Allergy Clin Immunol 2007; 120:381–7.

91 Young T, Finn L, Kim H. Nasal obstruction as a risk factor for sleep-disordered breathing. The University of Wilcoxon Sleep and Respiratory Research Group. J Allergy Clin Immunol 1997; 99:S757–62.

92 Vuurman EPF, van Veggel LMA, Uiterwijk MMC *et al.* Seasonal allergic rhinitis and anti-histamine effect on children's learning. Ann Allergy 1993; 71:121–6.

93 Craig TJ, Hanks CD, Fisher LH. How do topical nasal corticoids improve sleep and daytime somnolence in allergic rhinitis? J Allergy Clin Immunol 2005; 116:1264–6.

94 Mansfield LE, Posey CR. Daytime sleepiness and cognitive performance improve in seasonal allergic rhinitis treated with intranasal fluticosone propionate. Allergy Asthma Proc 2007; 28:226–9.

95 Kawauchi H. Medication for allergic rhinitis. Otolaryngol Head Neck Surg 2004; 76:39–44.

96 Graft D, Aaronson D, Chervinsky P *et al.* A placebo- and active-controlled randomized trial of prophylactic treatment of seasonal allergic rhinitis with mometasone furoate aqueous nasal spray. J Allergy Clin Immunol 1996; 98:724–31.

97 Yokoo E. Prophylactic treatment of Japanese cedar pollinosis with intranasal coriticosteroids. Prog Med 2005; 25:3195–9.

98 Okubo K, Nagakura T, Usui H *et al.* Evaluation of efficacy, safety and effects on rhinitis-specific QOL by fluticasone propionate in pediatric patients with seasonal allergic rhinitis. Allergol Immunol 2005; 12:318–31.

99 Adcock IM, Ito K, Barnes PJ. Glucocorticoids: effects on gene transcription. Proc Am Thorac Soc 2004; 1:247–54.

厚生労働科学研究費補助金(免疫アレルギー疾患等予防・治療研究事業)
分担研究報告書

リアルタイムモニター花粉症の情報のあり方の研究と舌下ペプチド・アジュバンド療法の臨床研究
― スギ花粉症の舌下免疫療法における追加治療の研究 ―

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研究要旨
免疫療法は、長期寛解・治癒を望めることから、根治的治療と位置付けられている。しかし、従来の注射法による免疫療法であっても治療終了後に症状が再発するケースもある。この場合、改めて免疫療法を再開するのか、軽症であれば症状に応じて薬物療法で経過をみるか症例ごとに対応している。
今回は、舌下免疫療法を行い治療が完了していた症例での中で、症状が再発したもの（中等症以上）を対象とした。舌下免疫療法を再開することによって、経年的な上乗せ効果があるのか、投与スケジュールが妥当なものかどうかについて検討した。

A. 研究目的：
花粉症の治癒を望むには薬物療法では不十分で、抗原特異的皮下免疫療法(SCIT)が唯一の方法である。しかし SCIT はアナフィラキシーなどの副作用があり、それを減少させるため欧米では抗原投与ルートを変更させた代替免疫療法がかなり以前より行なわれている。その方法には経鼻、舌下、経口があり、日本では我々が舌下免疫療法(SLIT)での二重盲検比較試験(RCT)で有効性を証明したが、商業的には開発が行われていない。そこには効果のエフェクトサイズや後効果があるかどうか、再発時の対処はどうするのか、分かっていないことも理由だろう。今回我々は 1 度 SLIT を実施完了後に再発してしまったスギ花粉症患者に対し、数年を経たのちもう一度 SLIT を施行し、その有効性を評価検討した。

B. 方法：
一度 SLIT を行い、1 年以上無症状あるいは軽症であったのち、中等症以上に再発したスギ花粉症患者 71 症例に対して再び SLIT を行い、その臨床的効果を検証した。対照は RCT(初回 SLIT)でのプラセボ群 14 症例と実薬群 36 症例とした。投与スケジュールは初回の SLIT がスギ花粉抗原エキスを 1 週間目 2 JAU から 4 週目 2000JAU までは毎日で、5 週間目では最高濃度 20 滴を 1 週間のうち 2 回、6 週目以降は季節を通じて 1 週間に 1 回、2000 JAU/ml を 20 滴舌下に投薬した。一方、追加治療としての SLIT ではスギ花粉抗原エキスは 2000JAU の 1 週目は 5 滴、10 滴と 2 度行い、次の週から 1 週間に 1 回 20 滴の簡便的増量法とした。
季節中は鼻のかゆみ、くしゃみ、鼻水、鼻閉、目のかゆみなどの症状をアレルギー日記に記入してもらい、2 月、3 月、4 月と月 1 度ずつ QOL アンケートを実施し、症状と QOL の評価を行った。

C. 結果：
評価した 2009 年の東京都の花粉飛散数は 6527 個/cm^2/シーズンであった。RCT における実薬の効果は QOL の悪化の抑制でプラセボに有意に優る部分があったが、症状スコアでは有意な差は認められなかった。しかし追加 SLIT 群では QOL スコア、症状スコアともプラセボと比し有意にその悪化を抑制し、初めての実薬投与となった群と比較して有意にそのスコアを減少させた。副作用はアナフィラキシーショックや喘息発作はないが、初回 SLIT 実薬群の抗原投与時の舌や口腔の痒み、しびれ感、鼻汁増加、皮膚の痒みなどが 10%程度認められた。追加 SLIT では副作用は認められなかった。

D. 考察：
今回の結果では SLIT は初回治療でも効果を示すが、再発後の再度の追加 SLIT によりさらに有効性を増大させることが示唆された。いくつかの報告からも 1 年 8 カ月以上の SLIT がそれ以内の期間の SLIT より効果的であることが報告されており (Martin Penagos, et al. Ann Allergy Asthma Immunol.2006; 97:141-148.)、季節を跨いだとしても 2 度目の効果が大きいことが考えられた。また追加 SLIT では高濃度からエキスを用いてもなお、副作用は少ないことが考えられた。

E. 結論：
SLIT 治療後にスギ花粉症症状を再発した症例に

おいて、追加 SLIT は有用な方法であり、初回の SLIT での効果を上回る効果を得られることが示唆された。

F. 健康危険情報
該当項目なし

G. 研究発表
1. 論文発表
1) Okubo K, Gotoh M. Sublingual Immunotherapy for Japanese Cedar Pollinosis (review). Allergol Int. 2009 Jun;58(2):149-54. Epub 2009 Apr 25.
2) Okubo K, Gotoh M, Fujieda S, Okano M, Yoshida H, Morikawa H, Masuyama K, Okamoto Y, Kobayashi M. A randomized double-blind comparative study of sublingual immunotherapy for cedar pollinosis. Allergol Int. 2008 Sep;57(3):265-75. Epub 2008 Jul 1.
3) Gotoh M, Okubo K. Sublingual Immunotherapy for Japanese Cedar Pollinosis (case report). Allergology International 2005; 1: 167-171

2. 学会発表
1) 大久保公裕, 後藤　穣. 舌下免疫療法—スギ花粉症に対して—第 59 回日本アレルギー学会秋季学術大会シンポジウム 2009 年 10 月開催
2) 大久保公裕, 後藤　穣.花粉症に対する舌下免疫療法について. 第21回日本アレルギー学会春季臨床大会シンポジウム 2009 年 6 月開催
3) 大久保公裕, 廣井隆親. 東京都における花粉症の舌下減感作療法の臨床研究. 第 59 回日本アレルギー学会秋季学術大会ミニシンポジウム　2009 年 10 月開催

H. 知的財産権の出願・登録状況
1. 特許取得
該当項目なし
2. 実用新案登録
該当項目なし
3. その他
該当項目なし

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REVIEW ARTICLE

Sublingual Immunotherapy for Japanese Cedar Pollinosis

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ABSTRACT

The prevalence of pollinosis caused by cedar pollen has increased by 10% these ten years of 26.5% in the investigation of 2008 in Japan. The pharmacotherapy is a main treatment tool for pollinosis, and the surgical treatment is not acknowledged to the treatment of pollinosis internationally. Moreover, allergen immunotherapy enters a special treatment method, and is an important therapeutic procedure. The allergen immunotherapy is unique for having possibility of curing allergen specific allergic diseases. However the side effect of allergen subcutaneous immunotherapy (SCIT), such as anaphylaxis is kept at a distance in a medical situation in Japan. Then, a sublingual immunotherapy (SLIT) that was safer than it, developed in Europe for pollinosis induced by grass or ragweed, but not in Japan. As a result, the effect of SLIT was proven in the cedar pollinosis in Japan as high level evidence. A whole body immunity induction is thought in the appearance of the effect, and, in addition, it is necessary to be going to be cleared the accurate mechanism of the effect in the future. Moreover, the development of a special SLIT and the import of an overseas product are needed in Japan.

KEY WORDS

pollinosis, QOL, SCIT, sublingual immunotherapy (SLIT)

INTRODUCTION

After Dr Noon begins to appear the conventional allergen specific subcutaneous immunotherapy (SCIT) in 1911, and is continuing treatment method.[1] The effect of SCIT on pollinosis caused by cedar pollen is low though the high therapeutic gain is admitted for the perennial allergic rhinitis in Japan. It is because the effect of SCIT has decreased relatively because this depends on the amount of pollen to which the symptoms of pollinosis and the amount of dispersion increases in recent years or the administering allergen of SCIT is a little. The problem of anaphylaxis in cause that SCIT has not become general treatment though effectiveness is confirmed.[2] An alternative immunotherapy to change the allergen administering route in Europe and United States to decrease the number of side effects of SCIT is done considerably than before. There are alternative route via the nose, sublingual, and the oral in the method development is not done respectively in Japan as for the double blind test comparison examination though effectiveness has been proven either. Therefore, it explains around sublingual immunotherapy (SLIT) that we are doing without the relation of the pharmaceutical company in Japan.

DEVELOPMENT IN JAPAN

In SLIT, high effectiveness is shown in Europe, and the few reports of the anaphylaxis have shown in randomized double blind placebo controlled (RCT) comparison examination evaluation.[3-5] It was one asthma case, and it was one diarrhea case in the SLIT 115 cases in three theses. It is recorded that it is not an anaphylaxis though the asthmatic attack is not described detailed. Moreover, that has not arrived importantly though the reaction of one case's near anaphylaxis externals less than ten times of allergen dose administration was observed by a recent report.[6]

To receive a lot of these reports, and to make SLIT adjust to pollinosis caused by cedar pollen from which the amount of the dispersion pollen was thought most, the research was started. We did the ex vivo culture experiment of the first human mouth mucous membrane incised by the time of surgery for analysis of allergen aspiration to the mucosal membrane. The double of the amount of the allergen dose

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Table 1 Allergen administration schedule (increasing dosing)

	1st week (2 JAU)	2nd week (20 JAU)	3rd week (200 JAU)	4th week (2000 JAU)	5th week (2000 JAU)
1st day	1 drop	1 drop	1 drop	1 drop	20 drops
2nd day	2 drops	2 drops	2 drops	2 drops	
3rd day	3 drops	3 drops	3 drops	4 drops	
4th day	4 drops	4 drops	4 drops	8 drops	
5th day	6 drops	6 drops	6 drops	12 drops	20 drops
6th day	8 drops	8 drops	8 drops	18 drops	
7th day	10 drops	10 drops	10 drops	20 drops	

[After sixth week to pollen dispersed season, 20 drops of allergen extract was administered once a week sublingually. After pollen dispersed season, same dose was administered once in two weeks.]

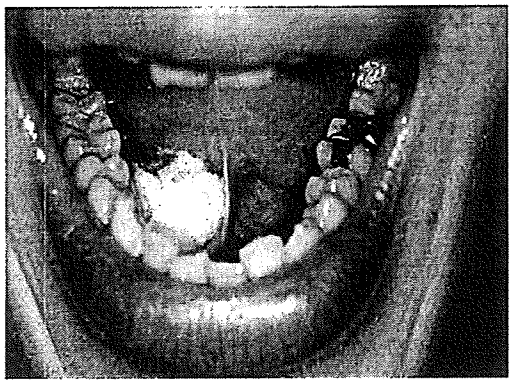

Fig. 1 How to be adapted the allergen extract and bit of bread.

in SCIT is almost the same dose aspirated by SLIT. So SLIT may act as the case of the SCIT is achieved is guessed by over the double dose of allergen at SCIT.[7]

HOW TO DO

The approval of the Nippon Medical School ethics committee was received to the pollinosis caused by cedar pollen patient and it went from some examinations including this basic experiment in SLIT. The allergen for SLIT, standardized Japanese cedar pollen allergen (2000 JAU [Japanese Allergology Unit]/ml, Torii Pharmaceutical, Tokyo, Japan), especially for SCIT products, was used for our SLIT trial. The allergen was able to be put on sublingual by using the bit of bread for the allergen to flow in actual sublingual and so as not to go out, then the allergen was kept to maintain at least for two minutes, and to present the antigen enough to the lymphatic tissue in the mouth.

The allergen administration was every day according the administration schedule from beginning to the forth week. On the first week, 2 JAU of allergen was administered from 1 drop to 10 drops, on the second week, 20 JAU of allergen was administered from 1 drop to 10 drops, on the third week, 200 JAU of allergen was administered from 1 drop to 10 drops, and then on forth week 2000 JAU of allergen was administered from 1 drop to 20 drops, as the final dose. On the fifth week twice a week after the sixth week, 2000 JAU/ml was administered to sublingual 20 drops as the final highest dose by once a week (Table 1, Fig. 1). There is tablet allergen for SLIT against grass pollinosis in Europe. There are some different allergen characters between Japanese cedar and grass. We cannot make the tablet allergen for SLIT of Japanese cedar pollinosis caused by its sticky character now.

THE EFFECT AND THE SIDE EFFECTS IN JAPANESE CEDAR POLLINOSIS

The Japanese cedar and cypress pollen dispersion was about 12000 grains, a large amount of dispersion in 2005 for these ten years. The RCT comparison by 60 cases was examined for making the first evidence in Japan. The SLIT group was intentionally low total symptom score (TSS) compared with the placebo (Fig. 2). This RCT of SLIT has shown to have lowered the symptom score more intentionally than the placebo in late pollen season.[8] SLIT had no significant difference with the drug therapy in the symptom score in the comparison research with the current drug therapy. However, the quality of life (QOL) score evaluated standardized Japanese Rhinitis Quality of Life Questionnaire (JRQLQ), is significantly decreased by SLIT group than placebo group, up to half level of score. QOL deterioration is significantly inhibited by SLIT (Fig. 3).

Moreover, it was confirmed though the side effect was completely fewer. Itchy of the tongue and the mouth when the antigen was administered, the feeling of numbness, nasal secretion increases, itchy of the skin, and hives were admitted at total of frequency of about 10% through the experiment, there were neither an anaphylaxis nor an asthmatic attack.

HOW TO ACT

The mechanism of the effect manifestation is known few up to the present time though the immunity induction of the limited part have some role on most of the effect of SLIT.[4] The mechanism of action for SCIT have been reported by the reduction of the effecter cells[9,10] and the increase of blocking antibody[11-14] in

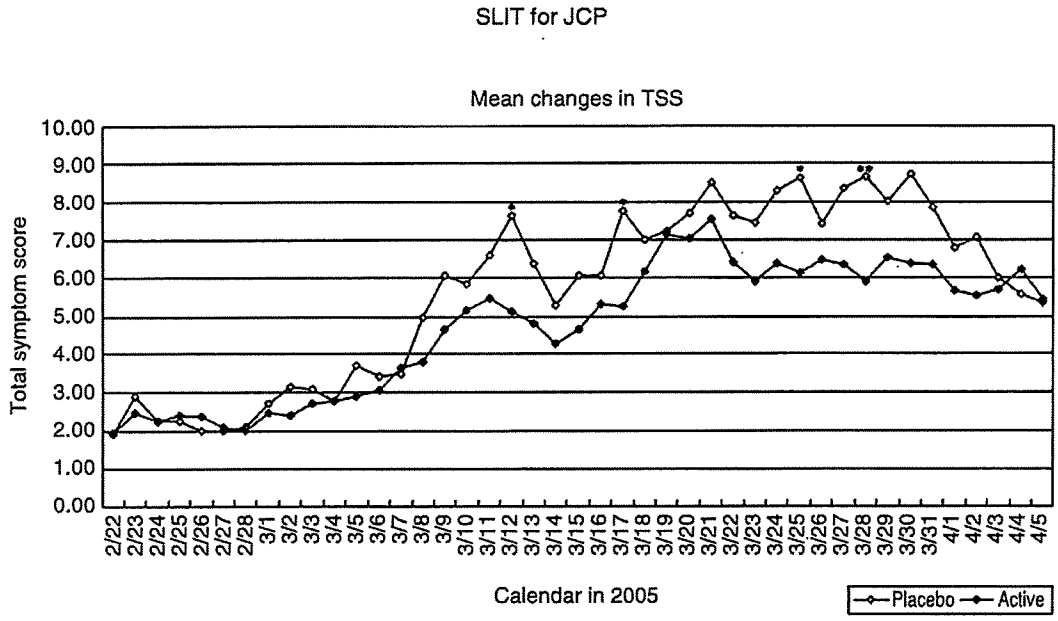

Fig. 2 Mean change of total nasal symptom score by SLIT and placebo group.

the conventional theories ten years ago. Recently, however, it has become widely accepted that SCIT may modify the T cell response to natural allergen because of T cell anergy and/or immune deviation[15-18] and regulatory T cell enhancement.[19]

For SLIT in particular, allergen administered to the oral mucosa accumulates in the sub-mandible lymph node, in which the immune response occurs[20] and peaks at approximately 2 h after administration.[21] An increase in stimulation index (SI) of PBMC at the early stage of the SLIT shows that the immunity induction of a sublingual allergen was at least caused in the general reaction.[22] It tried to reduce the side effect by reducing the effect throughout the body compared with past SCIT in SLIT. However, it has been understood that this result causes a general immunity induction. One more study of SLIT for Japanese cedar pollinosis was published by Chiba group also expressed the SLIT controlled the general Cry j-specific Th2 clone size.[23] The regulatory T cell enhancement in general by SLIT has reported in some papers recently.[24-26] So SLIT may act on generally, not just locally. It is necessary to clarify the exact effect mechanism of SLIT from the examination of the regional lymph node etc. by a similar examination that increased the number of cases or a detailed basic examination on animals in near future.

FOR THE FUTURE IN JAPAN

Approximately 15% of the Japanese population is affected by Japanese cedar pollinosis in 2002[27] and increase up to 26.5% in 2008.[28] The proportion of severe status patients is higher than with grass or ragweed pollinosis, which is the representative condition in other countries. The symptoms of Japanese cedar pollinosis persist for about 3 months, becoming a social issue. When the amount of pollen increases, patients show more severe symptoms, and the number of severe status patients is greatest in mid-March when the pollen count reaches its peak. Substantial antigen exposure enhances the antigen-antibody reaction in the airways (airway hypersensitivity), which is the mechanism involved in severe pollinosis, and immunotherapy with antigen-specific effects may control the exacerbation of the symptoms in the latter half of the cedar pollen season by inhibiting antigen-related enhancement of nasal mucosal hypersensitivity.

In SCIT for pollinosis treatment, the comments and responses of WHO are that the effect is verified from a lot of RCT comparison examinations.[29] However, it is a treatment method to which the medical treatment of Japan is kept at a distance because of the complexity, the possibility of the side effects, the cost and the enforcement under the present situation. The drug therapy is a main current in Japan where the allergy clinic has not been established from these problems for pollinosis. However, the immunotherapy that is fundamental treatment is an important method in the allergy management. The new SLIT shows the effect in pollinosis by cedar pollen was clarified in our examination in Japan. Any QOL fields and items became half QOL deterioration by the placebo in the evaluation using JRQLQ No1. This QOL questionnaire developed in Japan in the symptom score though the difference with the placebo was small in pharmacological treatment.[30] SLIT strongly controls the QOL deterioration in pollinosis rather than the symptom score to do effect is thought. Of the local immunotherapy modalities and SLIT is the most effective with a lower incidence of side effects, which complies with the WHO position paper on allergen

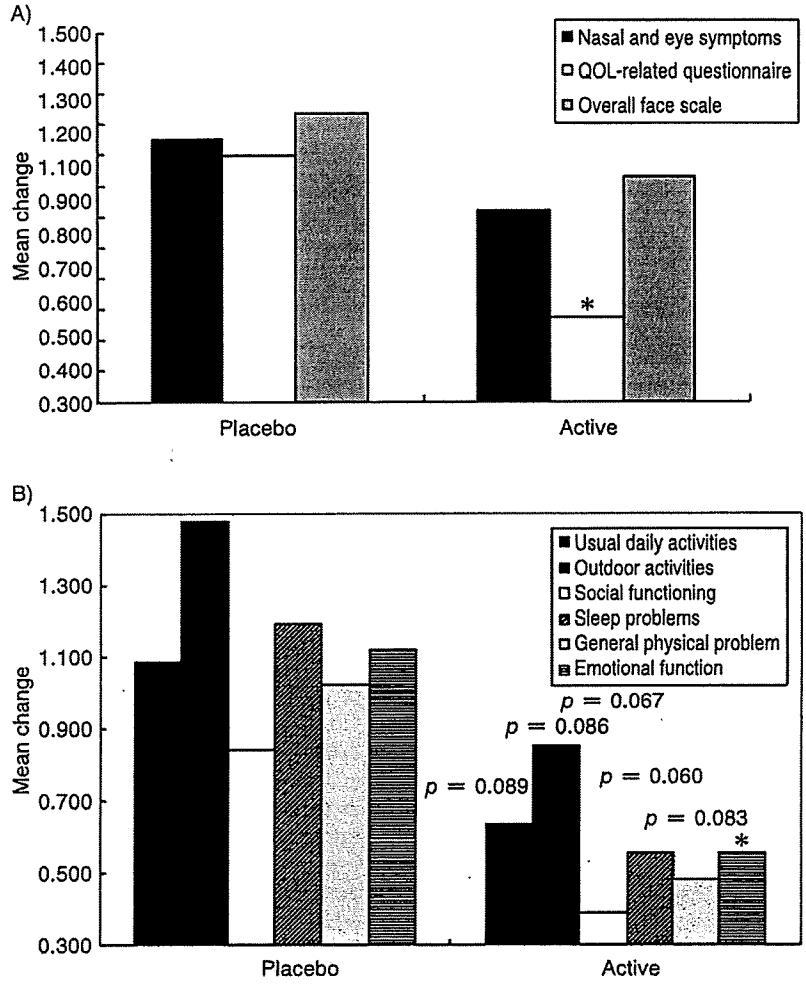

Fig. 3 The mean change of QOL score. **A)**, total QOL scores; **B)**, health related QOL fields.

immunotherapy requiring a new route of administration, such as local immunotherapy, and treatment that does not cause anaphylaxis, such as peptide therapy.[31]

In the comparison of double blinds RCT of the immunotherapy by a SLIT and the SCIT examination, the report is still few.[32] As for the level of the side effect frequency and the effect, it is uncertain. The score of the symptom medicine passes low through the pollen dispersion all seasons. This shows that the drug use decreases in SLIT and corresponding to the result of the RCT examination that uses the placebo.[33] It is thought that the effect equal with the drug use is shown, and a SLIT from which the use of the medicine is decreased is useful in economy. In SLIT studies in Japan, SLIT both inhibited the exacerbation of symptoms in the latter half of the season and reduced their severity throughout the season. Furthermore, there were neither local nor systemic side effects, as reported elsewhere for other antigens.

SLIT for cedar pollinosis is a new therapy and in the future SLIT may by indicated for patients with nasal allergy caused by other allergens such as house dust mites or animal dander through improvement of the administration schedule and establishing the dose at which the most potent effects are achieved.

It is the Ministry of Health, Labour and Welfare science research expense subsidy immunity and an allergy prevention treatment research grant (H14-immunity-001), (H17-immunity-general-001), as for development in this SLIT Japan. It is now progressing as a multicenter study in "Research of the ideal way of information on the real-time monitor pollen count and clinical research on sublingual peptide and the adjuvant therapy (H20-immunity-general-003)".

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REFERENCES

1. Noon L. Prophylactic inoculation against hay fever. *Lancet* 1991;**1**:1572-4.
2. Bousquet J, Locky RF, Malling HJ *et al.* Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American academy of Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol* 1998;**81**:401-5.
3. Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double-blind study. *Allergol Immunopathol* 1990;**18**:277-84.
4. Sabbah A, Hassoun S, Le Sellin J, André C, Sicard H. A double-blind, placebo-controlled trial by the sublingual route of immunotherapy with a standardized grass pollen extract. *Allergy* 1994;**49**:309-13.
5. Horak F, Stübner P, Berger UE, Marks B, Toth J, Jäger S. Immunotherapy with sublingual birch pollen extract. A short-term double-blind placebo study. *J Investig Allergol Clin Immunol* 1998;**8**:165-71.
6. Rodríguez-Pérez N, Ambriz-Moreno Mde J, Canonica GW, Penagos M. Frequency of acute systemic reactions in patients with allergic rhinitis and asthma treated with sublingual immunotherapy. *Ann Allergy Asthma Immunol* 1998;**101**:304-10.
7. Okubo K, Akimoto R, Okuda M *et al.* [Base of sublingual immunotherapy to hay fever caused by cedar pollen: Cryptomeria major allergen index of absorption in human oral mucous membrane]. *Jibi Meneki* 1997;**15**:21-6 (in Japanese).
8. Okubo K, Gotoh M, Fujieda S *et al.* A randomized double-blind comparative study of sublingual immunotherapy for cedar pollinosis. *Allergol Int* 2008;**57**:265-75.
9. Kimura I, Tanizaki Y, Goda Y, Komagoe H, Kitani H. Decrease in reactivity of basophils by immunotherapy with housedust extract. *Clin Allergy* 1985;**15**:1-7.
10. Otsuka H, Mezawa A, Ohnishi M, Okubo K, Seki H, Okuda M. Changes in nasal metachromatic cells during allergen immunotherapy. *Clin Exp Allergy* 1991;**21**:115-9.
11. Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998;**102**:157-64.
12. van Neerven RJ, Wikborg T, Lund G *et al.* Blocking antibodies induced by specific allergy vaccination prevent the activation of $CD4^+$ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 1999;**163**: 2944-52.
13. Golden DB, Meyers DA, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Clinical relevance of the venom-specific immunoglobulin G antibody level during immunotherapy. *J Allergy Clin Immunol* 1982;**69**:489-93.
14. Van der Zee JS, Aalberse RC. The role of IgG in immediate-type hypersensitivity. *Eur Respir J Suppl* 1991; **13**:91S-6.
15. Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. *J Exp Med* 1993;**178**:2123-30.
16. Lamb JR, Skidmore BJ, Green N, Chiller JM, Feldmann M. Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza hemagglutinin. *J Exp Med* 1983;**157**:1434-47.
17. Fasler S, Aversa G, Terr A, Thestrup-Pedersen K, de Vries JE, Yssel H. Peptide-induced anergy in allergen-specific human Th2 cells results in lack of cytokine production and B cell for IgE synthesis: reversal by IL-2, not by IL-4 or IL-13. *J Immunol* 1995;**155**:4199-206.
18. Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Müller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. *J Immunol* 1995;**154**:4187-94.
19. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. *J Allergy Clin Immunol* 2008;**121**:1467-72.
20. Muller D, Ruitenberg EJ, Elgersma A. The influence of different immunization pathways on the immunological response in the oral mucosa. *Br J Exp Pathol* 1983;**64**:367-72.
21. Bagnasco M, Passalacqua G, Villa G *et al.* Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers. *Clin Exp Allergy* 2001;**31**:54-60.
22. Okubo K, Gotoh M, Shimada K *et al.* [Sublingual immuno-therapy for Japanese cedar pollinosis: pilot study]. *Jpn J Rhinol* 2002;**41**:30-5 (in Japanese).
23. Horiguchi S, Okamoto Y, Yonekura S *et al.* A randomized controlled trial of sublingual immunotherapy for Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2008;**146**:76-84.
24. Ciprandi G, Cirillo I, Tosca MA, Marseglia G, Fenoglio D. Sublingual immunotherapy-induced IL-10 production is associated with changed response to the decongestion test: preliminary results. *Allergy Asthma Proc* 2007;**28**: 574-7.
25. Burastero SE, Mistrello G, Falagiani P *et al.* Effect of sublingual immunotherapy with grass monomeric allergoid on allergen-specific T-cell proliferation and interleukin 10 production. *Ann Allergy Asthma Immunol* 2008;**100**:343-50.
26. Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 2007;**120**:707-13.
27. Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. *Ann Allergy Asthma Immunol* 2003;**91**: 288-96.
28. Baba K, Nakae K. [Epidemiological survey of nasal allergy in Japan 2008]. *Progress in Medicine* 2008;**28**:2001-12 (in Japanese).
29. Lockey RF. "ARIA": Global guidelines and new forms of allergen immunotherapy. *J Allergy Clin Immunol* 2001; **108**:497-9.
30. Okuda M, Ohkubo K, Goto M *et al.* Comparative study of two Japanese rhinoconjunctivitis quality-of-life questionnaires. *Acta Otolaryngol* 2005;**125**:736-44.
31. World Health Organization. Allergen immunotherapy: therapeutic vaccines for allergic diseases (WHO position

paper). *J Allergy Clin Immunol* 1998;**102**:558-62.
32. Mungan D, Misirligil Z, Gurbuz L. Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma: a placebo controlled study. *Ann Allergy Asthma Immunol* 1999;**82**: 485-90.
33. Pradalier A, Basset D, Claudel A *et al.* Sublingual-swallow immunotherapy (SLIT) with a standardized five-grass-pollen extract (drops and sublingual tablets) versus placebo in seasonal rhinitis. *Allergy* 1999;**54**:819-28.

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Efficacy of Oral Administration of a Heat-Killed *Lactobacillus gasseri* OLL2809 on Patients of Japanese Cedar Pollinosis with High Japanese-Cedar Pollen-Specific IgE

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A randomized, double-blind, placebo-controlled clinical trial was conducted to determine whether oral administration of heat-killed *Lactobacillus gasseri* OLL2809 would affect the immune response and reduce the symptoms of Japanese cedar pollinosis (JCP) in subjects with JCP. Following a 1-week pre-observation period, the subjects were randomly divided into two groups and were orally administered a placebo or tablets containing 100 mg of *L. gasseri* OLL2809 per d for 8 weeks during the pollen season in 2007. The results showed no obvious differences between the groups. Supplementary subgroup analysis revealed that the OLL2809 subgroups with CAP-RAST scores of 4 or 5 exhibited improvement in nasal symptoms scores and serum allergy-related items, including Japanese cedar pollen-specific IgE levels. *L. gasseri* OLL2809 was found to be effective in reducing symptoms in subjects with a high predisposition to allergies by modulating systemic immune systems.

Key words: Japanese cedar pollen; Japanese cedar pollinosis; *Lactobacillus gasseri*; probiotics; Th1/Th2

In the past few decades, the incidence of Japanese cedar pollinosis (JCP) has been increasing, and a current paper suggests that at least one-sixth of the Japanese population is affected by this allergic disease.[1] Thus far, several medicines, including antihistamines, leukotriene inhibitors, anti-inflammatory cytokines, and corticosteroids, have been developed that greatly contribute to reduction of the symptoms of allergic disease.[2–7] However, because allergic diseases are chronic, continuous treatment is required, and the cost spent on treatment is enormous. In addition, most of the available treatments are symptomatic.[8] Therefore, in view of the high prevalence of JCP and the adverse effects that accompany long-term treatment,[4,9] more effective treatment methods are required.

The increase in the prevalence of allergic diseases has been explained by the hygiene hypothesis proposed by Strachan.[10] Strachan suggested that limited exposure to bacterial and viral pathogens during early childhood leads to insufficient stimulation of the Th1 direction of the immune system and primes an overactive Th2 reaction, leading to allergic disease. The mechanisms underlying this phenomenon are considered to include defective maturation or an absence of regulatory T cells and an inappropriate Th1/Th2 balance.[11] A number of reports suggest correlations between the incidence of allergic diseases and intestinal microbiota, which might serve as stimuli to develop appropriate immune systems.[12,13]

Lactobacilli are gram-positive anaerobic bacteria commensal to humans and animals.[14] For many years, they have been consumed worldwide through foods such as fermented milk. Consequently, they are known to be very safe microorganisms. Moreover, a recent double-blind placebo-controlled clinical study revealed that *Lactobacillus rhamnosus* GG administration suppressed the incidence of atopic diseases in high-risk children by approximately 50%.[15] Hence, the use of lactobacilli might be an easy and effective way to prevent or treating allergies without any side effects.

L. gasseri OLL2809, which was isolated from a human subject, has been selected from approximately 300 *Lactobacillus* strains on the basis of its immunoregulatory effect.[16] We have found using mouse experimental allergy models that when orally administered, heat-killed *L. gasseri* OLL2809 exhibit suppressive effects on antigen-specific IgE and eosinophilia *via* modulation of the Th1/Th2 balance.[16–18] This suggests that heat-killed *L. gasseri* OLL2809 can reduce the clinical symptoms of JCP. In this study, we examined the clinical efficacy of heat-killed *L. gasseri* OLL2809 as to JCP.

Methods

Subjects. Subjects ($n = 107$) aged 20 to 50 years were enrolled in the clinical study. Subjects with JCP who fulfilled the following criteria were enrolled: (i) subjects who experienced JCP symptoms for

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Abbreviations: JCP, Japanese cedar pollinosis; QOL, quality of life; SMS, symptom medication score; IFN, interferon; IL, interleukin

over 2 years, (ii) subjects with serum cedar pollen-specific IgE levels at scores 2 to 5 by the CAP-radioallergosorbent test (CAP-RAST score), (iii) subjects with a moderate total symptom score (comprising symptoms of sneezing, runny nose, nasal congestion, itchy and watery eyes, scored for JCP in the past 2 years) according to the "Practical Guidelines for the Management of Allergic Rhinitis in Japan," 5th edition.[19] Subjects who lived or worked in the suburbs of Tokyo were preferred as study subjects.

The following subjects were excluded from the study: (i) those whose symptoms had developed before the cedar pollen season, (ii) those with nasal disease that might have affected efficacy evaluation in this trial (perennial allergic rhinitis, acute and chronic rhinitis, *etc.*), (iii) those who planned to travel to Hokkaido, Okinawa, or abroad, and (iv) others whom the physician in charge judged unfit as study subjects.

Prior to participation, written consent was obtained from all the subjects after the physician in charge had explained the study to the group. In addition, the study received the approval of the Ethics Committee of the Division of Research and Development of Meiji Dairies Corporation, and it was performed in accordance with the Declaration of Helsinki.

Study design. The study was a randomized, double-blind, placebo-controlled clinical trial performed at a single institution in Tokyo between January 10, 2007 and April 6, 2007. The study protocol is summarized in Fig. 1. After obtaining informed consent from the subjects, they were screened to confirm compliance with the inclusion and exclusion criteria and to examine the physical condition of each individual. Subject background, clinical laboratory analysis (hematology, blood biochemistry, and serology), and physical examination were included in the screening.

Following a 1-week pre-observation period, the subjects were randomly divided into two groups: one group of subjects who were orally administered tablets containing 100 mg (approximately 1×10^{10} cells) of heat-killed (75 °C for 60 min) *L. gasseri* OLL2809 per d and the other, who received placebo tablets. The placebo tablets contained dextrin instead of heat-killed *L. gasseri* OLL2809, and were identical in color and taste to the OLL2809 tablets. Each subject received tablets for an 8-week course (from February 5 to April 6).

On dividing the groups, a controller who was not directly involved in the study was responsible for group allocation. The subjects were divided randomly into the active (OLL2809) and placebo groups according to the total symptom scores for JCP in the past 2 years, the Japanese-cedar-pollen specific IgE not to be different between the groups. A group allocation number was given to each subject. To prevent leakage of information, this number was closely guarded jointly by the controller and a member of the ethical committee who was not directly involved in the study, until accessed with the key after completion of the study.

The physician in charge examined each subject a total of 3 times: during the pre-observation period, and 4 and 8 weeks after treatment. The subjects were asked to fill out the Japanese Allergic Rhinitis QOL standard Questionnaire (JRQLQ) during the pre-observation period, and 4 and 8 weeks after treatment. They were asked to record pollinosis symptoms (sneezing, runny nose, nasal congestion, itchy eyes, watery eyes, and interference with daily life) and compliance with the administration schedule in the subject diary.

Evaluation items.

Nasal cavity findings. Nasal examinations were conducted by rhinoscopy during the pre-observation period and after 4 and 8 weeks of treatment. Mucosal swelling of the inferior turbinate and the amounts of watery rhinorrhea were scored on a 4-point scale of severity (0 = none to 3 = severe).[19]

Allergy diary. Allergy diaries were kept by the subjects to self-assess items from a list, including sneezing (number of attacks per d), runny nose (number of incidences of nose blowing per d), nasal congestion, and itchy and watery eyes. These symptoms were scored subjectively on a 5-point severity scale, where 0 indicated no symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms; and 4, very severe symptoms.[19] The total scores for sneezing, runny nose, and nasal congestion were counted as the nasal symptom score, while the total scores for itchy and watery eyes were counted as the ocular symptom score. The medication score was recorded as described elsewhere.[19] Severity during the season was scored daily as nasal or ocular symptom medication scores (SMS), and the mean SMS each week was compared between the placebo and OLL2809 groups.

Japanese allergic rhinitis QOL standard questionnaire (JRQLQ). JRQLQ was used for evaluation of the subjects' QOL during the pollen season. The questionnaire is composed of three parts: nasal and eye symptoms (JRQLQ-I), 17 questions regarding the QOL (JRQLQ-II), and a comprehensive evaluation (face scale).[20]

Nasal and ocular symptoms included the following six categories: runny nose, sneezing, nasal congestion, itchy nose, and itchy and watery eyes. The symptoms of each subject were evaluated on a 5-point scale, 0 denoting no symptoms; 1, mild; 2, moderate to severe; 3, severe; and 4, very severe.

The QOL-related questionnaire included 17 items concerning (i) reduced productivity at work/home; (ii) poor mental concentration; (iii) reduced thinking power; (iv) impaired reading book/newspaper; (v) reduced memory; (vi) limitation of outdoor life (*e.g.*, sports, picnic); (vii) limitation of going out; (viii) hesitation visiting friend or relatives; (ix) reduced contact with friends or others by telephone or conversation; (x) not an easy person to be around; (xi) impaired sleeping; (xii) tiredness; (xiii) fatigue; (xiv) frustration; (xv) irritability; (xvi) depression; and (xvii) unhappiness. Each item was evaluated on a 5-point scale, 0 denoting no significant problem; 1, a mild problem; 2, moderately severe; 3, severe; and 4, very severe.

Blood examination. Blood samples were collected 3 times: at the pre-observation period and after 4 and 8 weeks of treatment. The samples were used to determine the concentrations of total and Japanese cedar pollen-specific IgE levels and number of eosinophils. The ratio of Th1 to Th2 cells (Th1/Th2 ratio) was determined 2 times: in the pre-observation period and after 8 weeks of treatment. The blood examinations described above were performed at SRL (Tokyo).

Assessment of outcomes. The primary efficacy outcome was the difference in the nasal and the ocular symptom medication scores over the 8-week administration period between the OLL2809 and placebo groups. The secondary assessments were based on the QOL-related scores on JRQLQ at 4 and 8 weeks after treatment.

Statistical analysis. A blind data review was performed before decoding, and decisions concerning the handling of drop-outs were made on the basis of blinded results. The assessment of efficacy was based on all the subjects who completed the study. Subgroup analyses based on CAP-RAST scores were done additionally after decoding.

Data were expressed as mean ± SE. Statistical differences between the placebo and OLL2809 groups and subgroups were analyzed by Student's t test or Mann-Whitney's U test. The differences between pre-observation and 4 and 8 weeks after treatment were analyzed in each group and subgroup by Student's paired-t test with Bonferroni's correction. Differences were considered significant when the p-value was less than 0.05.

Results

Background characteristics of subjects and cedar pollen count

Among 107 subjects (placebo $n = 54$, OLL2809 $n = 53$) enrolled, seven subjects withdrew from the study citing personal reasons and 100 (placebo $n = 53$, OLL2809 $n = 47$) successfully completed it. The baseline characteristics of the subjects were similar in the placebo and OLL2809 groups in terms of age, sex, duration of JCP, nasal cavity findings, nasal and ocular symptom medication scores in the allergy diary, and a blood examination (data not shown).

The Japanese cedar pollen season started on January 31 and continued to the end of April in 2007. The total pollen count during the study period was 1,263 grains/cm^2 in Chiyoda-ku, central Tokyo, slightly higher than the pollen count for 2006 (874 grains/cm^2)