

α -Melanocyte-Stimulating Hormone Plays an Important Role in the Onset of Pollinosis in a Pollen Allergy Mouse Model

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Key Words

α -Melanocyte-stimulating hormone • Melanocortin receptor 1 • Melanocortin receptor 5 • Immunoglobulin A • Pollen allergy model mice

Abstract

Background: α -Melanocyte-stimulating hormone (α -MSH) is a neuropeptide that controls melanogenesis in pigmented cells. In addition, its potent immunomodulatory activity has been recently described in cutaneous inflammatory disorders. However the mechanism of such pollen allergies remains to be elucidated. The purpose of this study was to investigate the role of α -MSH in a murine model of pollen allergy. **Methods:** Eight-week-old male BDF-1 mice were sensitized with Cry j I. After the last intranasal antigen, the number of sneezes was counted for 5 min. In addition, the serum levels of IgE and neuronal hormones were measured by ELISA. The expression of IgA, melanocortin receptor 1 (MC1R) and MC5R in the trachea were also observed by immunohistochemistry. **Results:** Both the concentration of α -MSH and adrenocorticotropin in plasma increase in pollen allergy model mice. Furthermore, MC5R increased in the trachea; however, MC1R did not increase in the trachea. In addition, the changes in sneezing and IgA expression in the pollen allergy model mice were suppressed by α -MSH antibody treatment, but they remained unchanged after MC1R

antagonist (agouti) treatment. **Conclusions:** These results indicate that sneezing due to pollen allergy is associated with an increased concentration of α -MSH and the expression of MC5R.

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Introduction

There has been an increase in the prevalence and incidence of allergic conditions [Japanese cedar (*Cryptomeria japonica*) pollinosis], which now affect approximately 15% of the Japanese population [1].

Patients with *C. japonica* pollinosis have symptoms such as sneezing, watery rhinorrhea and nasal blockage. Histological evaluation reveals a marked increase in eosinophils in the nasal submucosa and epithelium as well as chronic inflammation of the nasal mucosal membrane [2]. In addition to these allergen-specific symptoms, nasal hyper-responsiveness to nonspecific stimuli has been observed [3]. The majority of patients with allergic rhinitis are found to have high levels of allergen-specific IgE [4]. Many animal models for the study of allergic rhinitis have been reported [5–7]. Murine models of allergic rhinitis are especially useful for investigating the immunological basis of this disease.

Two major allergens, Cry j I and Cry j II, have been isolated from *C. japonica* pollen [8, 9], and an effective animal model using Cry j I has been developed [5–7, 10–12]. Antigen-induced nasal symptoms, late-phase nasal eosinophilia, and serum levels of antigen-specific IgE antibody were evaluated in an ovalbumin-sensitized BALB/c mouse model of allergic rhinitis [6, 12, 13].

This study examined sneezing and tracheal inflammation associated with pollinosis using the pollen allergy model of Nomiya et al. [12].

Materials and Methods

Animals

Specific pathogen-free, 8-week-old male BDF-1 mice (SLC, Hamamatsu, Japan), were subjected to experiments according to the animal care regulations of Osaka City University Medical School.

Sensitization and Nasal Antigen Challenge

These experiments employed the methods of Nomiya et al. [12]. The mice were sensitized with 10 µg of Cry j I (Hayashibara, Okayama, Japan) mixed with 4 mg of aluminum hydroxide gel (Sigma, St Louis, Mo., USA) by subcutaneous injection, which was administered 3 times every other day. One week after the final sensitization to Cry j I, the intranasal Cry j I challenge was performed for 7 consecutive days. Five days after the last intranasal antigen challenge, the number of sneezes was counted for 5 min. The sneezing behavior of the animals were observed as described previously [14]. Briefly, to evaluate sneezing, each time the animal rubbed or touched the area near the nose with its forepaws, it was counted as 1 event.

Anti-α-MSH Antibody or Agouti Treatment

Approximately 30 µg/day of anti-α-MSH polyclonal antibody (Acris Antibodies, Herford, Germany) in saline was injected intraperitoneally into some mice throughout the experimental period, while only saline was injected into the control mice. In another group, approximately 2.5 µg/day of the melanocortin receptor 1 (MC1R) antagonist agouti (Alpha Diagnostic International, San Antonio, Tex., USA) in saline was injected intraperitoneally in to the mice, while saline was injected into the control mice.

Preparation and Staining of Trachea

Two days after the last challenge, the tracheal specimens were fixed in phosphate-buffered paraformaldehyde (4%), embedded in frozen Tissue Tek, OCT compound, and cut into sections 5 µm thick. The expression of immunoglobulin A (IgA) was evaluated immunohistochemically under a fluorescent microscope as described previously [15]. To investigate the expression of MC1R and melanocortin receptor 5 (MC5R), the sections of trachea were washed in PBS, and then incubated with rabbit anti-MC1R and rabbit anti-MC5R (1:100) polyclonal antibody (Chemicon, Calif., USA) overnight at 4°C, respectively. The specimens were then washed in PBS and incubated at room temperature for 2 h with

Table 1. Number of sneezes and the plasma concentration of IgE, α-MSH and ACTH in the control and pollen allergy model mice

	Control	Pollen
Number of sneezes/5 min	2.0 ± 2.50	42.0 ± 20.5*
IgE, ng/ml	152.8 ± 120.5	1,086.4 ± 412.6*
α-MSH, ng/ml	56.4 ± 44.8	378.7 ± 180.1*
ACTH, ng/ml	22.6 ± 21.3	124.4 ± 50.6*

The number of sneezes and the plasma concentration of IgE, α-MSH and ACTH in pollen allergy model mice increase in comparison to be control mice. Values are presented as the mean ± SD of the values from 10 animals.

* p < 0.05, vs. control mice.

FITC-conjugated (MC1R) and TRITC-conjugated (MC5R) swine anti-rabbit immunoglobulin (1:30; Dako Cytomation, Denmark). The expression of MC1R and MC5R were evaluated immunohistochemically under a fluorescent microscope.

Quantification of Neuronal Hormones and IgE by ELISA

Blood samples were taken from the heart 5 days after the last intranasal antigen challenge, and then the plasma was fractionated. The plasma adrenocorticotrophic hormone (ACTH), α-MSH and IgE contents were determined using a commercial ELISA kit (ACTH and α-MSH; Phenix Pharmaceuticals, Calif., USA; IgE; Yamasa, Chiba, Japan) according to the manufacturer's instructions.

Statistical Analysis

All data were expressed as the mean ± SD. The results obtained from the animal groups were analyzed using either Student's t test or ANOVA using computer software. Differences were considered to be significant when p < 0.05.

Results

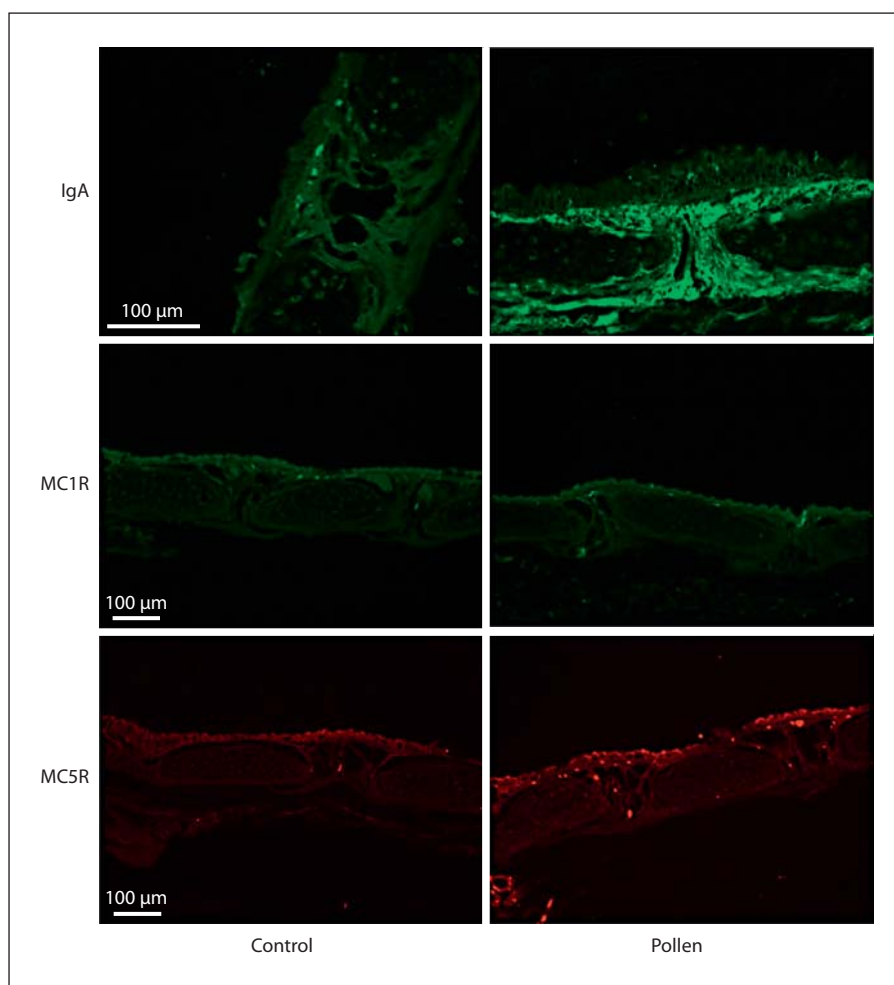
Effect of Cry j I Antigen on Sneezing, IgE and Neuronal Hormones

The number of sneezes significantly increased in the pollen allergy model mice group in comparison to the control group. Significant increases in the concentrations neuronal hormones (α-MSH and ACTH) and IgE in plasma were induced in the Cry j I sensitized mice (table 1).

IgA, MC1R and MC5R in the Trachea in the Pollen Allergy Model Mice

The expression of IgA, MC1R and MC5R in the tracheas of the Cry j I sensitized mice was analyzed. Figure 1 shows the IgA and MC5R expressions to increase in the

Fig. 1. IgA, MC1R and MC5R expression in the trachea of the control and pollen allergy model mice. IgA and MC5R are significantly increased in the pollen allergy model mice in comparison to the control mice. However, MC1R shows no significant changes in the trachea of the pollen allergy model mice. Histological data show 1 typical experiment from 10 animals.



trachea of the pollen allergy model mice; however, no increase in the expression of MC1R was observed in the trachea.

Effect of α -MSH Antibody or Agouti on Sneezing and the Expression of IgA in the Trachea

The pollen allergy mouse model showed significantly increased sneezing and expression of IgA in the trachea. However, α -MSH antibody treatment pollen allergy model mice showed significantly decreased sneezing and the expression of IgA in the trachea in comparison to the non-treatment pollen allergy model mice (fig. 2). On the other hand, regarding the agouti treatment, the number of sneezes and the expression of IgA did not change in comparison to the non-treatment pollen allergy mouse model (fig. 3).

Discussions

The present study demonstrate sneezing to be related to the increased expression of α -MSH (in the plasma) and MC5R (in the trachea) in pollen model mice.

In the model of pollen allergy, α -MSH was found to be related to the increase in the allergen specific IgE and IgA production. Based on the known effect of α -MSH on B cells, it is likely that B cells are among the targets of α -MSH action. Furthermore, α -MSH is a potent suppressor of bacterial endotoxin-mediated inflammation and the inflammatory activity of macrophages and neutrophils induced by IL-1, TNF- α and IFN- β [16–18].

α -MSH is a potent suppressor of bacterial endotoxin-mediated inflammation and the inflammatory activity of macrophages and neutrophils induced by IL-1, TNF- α , IFN- β [19–21]. The anti-inflammatory activities of α -MSH are important for regulating a normal inflamma-

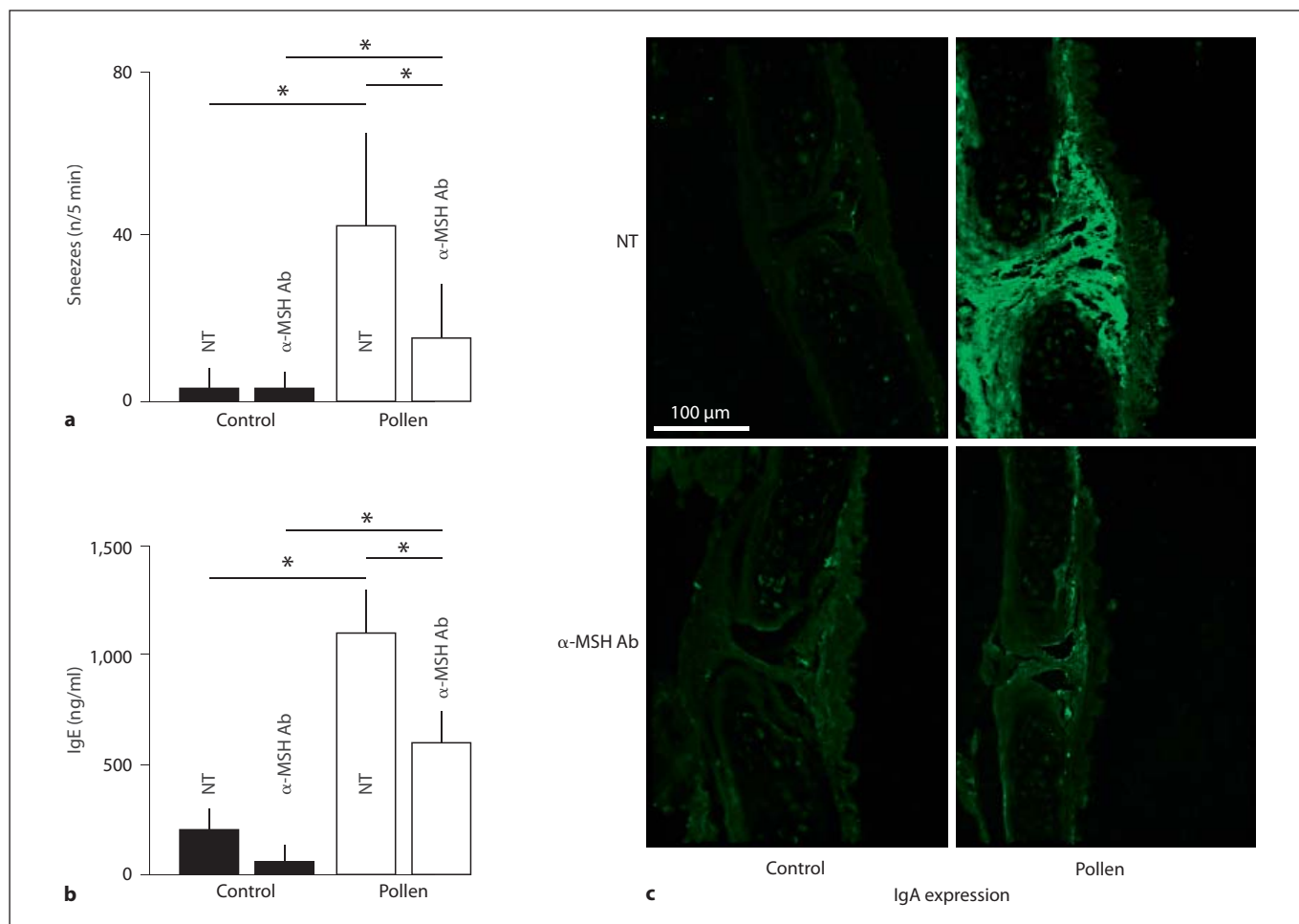


Fig. 2. Number of sneezes (a), plasma concentration of IgE (b) and IgA expression in the trachea (c). α -MSH antibody was injected intraperitoneally in the pollen allergy model mice. Values are increased in the pollen allergy model mice in comparison to the control mice. However, injection α -MSH antibody reduced the

increase in the number of sneezes, IgE level and expression of IgA. Values are presented as the mean $\pm$ SD from 10 animals. Histological data show 1 typical experiment from 10 animals. NT = No treatment. * $p < 0.05$.

tory response [22]. In addition, α -MSH suppresses IFN- β production but enhanced TGF- β 1 production [23]. In our experiment, the administration of α -MSH restricted increase of TGF- β 1 (data not shown). TGF- β is a cytokine with widely varying effects on the inflammatory and immunologic processes. As a result, even though it is a potent chemotactic factor for T lymphocytes and monocytes, which play a role in the initiation of inflammation, it also stimulates fibroblast proliferation, thus bringing the inflammatory processes to a close [24]. Regarding the TGF- β effects, it inhibits both T and B cell proliferation and differentiation [25, 26]. On the other hand, it up-regulates the expression of genes for cytokines, which have a positive effect on B cell growth [27, 28]. In addition, an-

other study suggested that TGF- β may play an isotype-specific role in the regulation of IgA B cell differentiation [29–31]. These studies have provided evidence that TGF- β induces large increases in IgA secretion while at the same time decreasing IgM and IgG secretion [29–31]. The α -MSH/TGF- β /IgA pathway has thus been suggested to affect pollinosis in these reports.

In addition, IgE is controlled like IgA by α -MSH in this examination too. However, the relationship between α -MSH and IgE remains to be clearly elucidated. We therefore herein examine the relationship of α -MSH and IgE by the pollinosis.

α -MSH has immunomodulatory effects on several cell types. It inhibits the production of a number of pro-in-

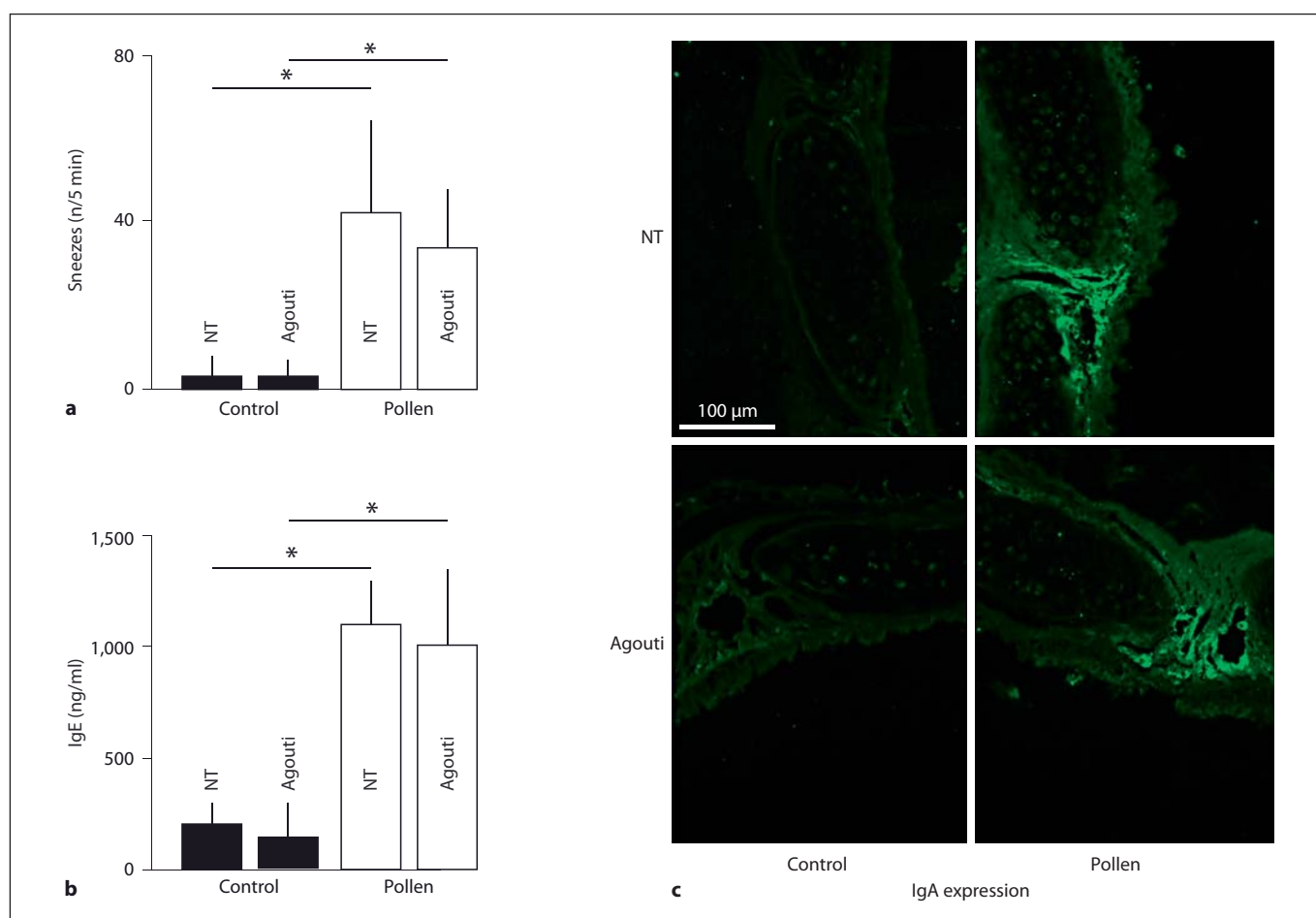


Fig. 3. Number of sneezes (**a**), plasma concentration of IgE (**b**) and IgA expression in the trachea (**c**). Agouti was injected intraperitoneally in the pollen allergy model mice. The values are increased in the pollen allergy model mice in comparison to the control mice. The pollen allergy model mice injected with agouti showed

no difference in comparison to the untreated pollen allergy model mice. Values are the mean + SD from 10 animals. Histological data show 1 typical experiment from 10 animals. NT = No treatment. * $p < 0.05$, vs. the control mice.

inflammatory cytokines [32, 33]. α -MSH seems to designate the macrophages as key target cells because they produce many cytokines [34]. In fact, MC1R, a high affinity receptor for α -MSH, has been found on macrophages and other cell populations [35]. However, in this study we observed no increase in the expression of MC1R in the trachea, but increased expression of MC5R was observed. In addition, the increased sneezing, IgE (in plasma) and the expression of IgA (in trachea) decreased. However, treatment with agouti, an antagonist of MC1R, had no effect on the pollen model mice.

The MC5R is similar to MC1R in its capacity to recognize α -MSH and ACTH. MC5R is ubiquitously expressed in peripheral tissues. The presence of MC5R in B and T

lymphocytes suggests a function in immune regulation. Indeed, recent data suggest that α -MSH participates in the B lymphocyte function via the activation of the Jak/STAT pathway, the intracellular phosphorylation pathway used by cytokines and growth factors, through specific binding to the MC5R [36]. Furthermore, α -MSH can induce CD25+CD4+ regulatory T cells through the MC5R expressed on primed T cells [37].

The results of the current study suggested that MC5R/ α -MSH is among the factors affecting immune modulation in the pollen allergy model mice. Further studies are called for to understand the signal transmission from sensitization to onset, such as that observed in sneezing.

References

- Kaneko Y, Motohashi Y, Nakamura H, Endo T, Eboshida A: Increasing prevalence of Japanese cedar pollinosis: a meta-regression analysis. *Int Arch Allergy Immunol* 2005; 136:365–371.
- Horak F, Stubner P: New trends in allergic rhinitis. *Allergy Clin Immunol Int* 1998;10: 81–87.
- Connell JT: Quantitative intranasal pollen challenges. 3. The priming effect in allergic rhinitis. *J Allergy* 1969;43:33–44.
- Hashimoto M, Nigi H, Sakaguchi M, Inouye S, Imaoka K, Miyazawa H, Taniguchi Y, Kurimoto M, Yasueda H, Ogawa T: Sensitivity to two major allergens (Cry j I and Cry j II) in patients with Japanese cedar (*Cryptomeria japonica*) pollinosis. *Eur J Pharmacol* 1995;25:848–852.
- Kim JH, Mun YJ, Aha SH, Park JS, Woo WH: Induction of oral tolerance to Japanese cedar pollen. *Arch Pharmacol Res* 2001;24:557–563.
- Murasugi T, Nakagami Y, Yoshitomi T, Hirahara K, Yamashita M, Taniguchi Y, Sakaguchi M, Ito K: Oral administration of a T cell epitope inhibits symptoms and reactions of allergic rhinitis in Japanese cedar pollen allergen-sensitized mice. *European J Pharmacol* 2005;510:143–148.
- Tsunematsu M, Yamaji T, Kozutsumi D, Murakami R, Nagai H, Kino K: A new murine model of allergic rhinitis by reported intranasal Cry j I challenge. *Biomed Res* 2008;29: 119–123.
- Komiyama N, Sone T, Shimizu K, Morikubo K, Kino K: cDNA cloning and expression of Cry j II the second major allergen of Japanese cedar pollen. *Biochem Biophys Res Commun* 1994;201:1021–1028.
- Sone T, Komiyama N, Shimizu K, Kusakabe T, Morikubo K, Kino K: Cloning and sequencing of cDNA coding for Cry j I, a major allergen of Japanese cedar pollen. *Biochem Biophys Res Commun* 1994;199:619–625.
- Takagi H, Hiroi T, Yang L, Tada Y, Yuki Y, Takamura K, Ishimitsu R, Kawauchi H, Kiyono H, Takaiwa F: A rice-based edible vaccine expressing multiple T cell epitopes induced oral tolerance for inhibition of Th2-mediated IgE responses. *Proc Natl Acad Sci USA* 2005;120:17525–17530.
- Tsunematsu M, Yamaji T, Kozutsumi D, Murakami R, Kimura S, Kino K: Establishment of an allergic rhinitis model in mice for the evaluation of nasal symptoms. *Life Sci* 2007; 80:1388–1394.
- Nomiya R, Okano M, Fujiwara T, Maeda M, Kimura Y, Kino K, Yokoyama M, Hirai H, Nagata K, Hara T, Nishizaki K, Nakamura M: CRTH2 plays an essential role in the pathophysiology of Cry j I-induced pollinosis in mice. *J Immunol* 2008;180:5680–5688.
- Sato J, Asakura K, Murakami M, Ueda T, Katakura A: Topical CTLA4-Ig suppresses ongoing mucosal immune response in pre-sensitized murine model of allergic rhinitis. *Int Arch Allergy Immunol* 1999;119:197–204.
- Takubo M, Inoue T, Jiang S, Tsumuro T, Ueda Y, Yatsuzuka R, Segawa S, Watari J, Kamei C: Effects of hop extracts on nasal rubbing and sneezing in BALB/c mice. *Biol Pharm Bull* 2006;29:689–692.
- Yamamoto M, Kweon MN, Rennert PD, Hiroi T, Fujihashi K, McGhee JR, Kiyono H: Role of gut-associated lymphoreticular tissues in antigen-specific intestinal IgA immunity. *J Immunol* 2004;173:762–769.
- Rajara N, Ceriani G, Catania A, Star RA, Murphy MT, Lipton JM: α -MSH production, receptor, and influence on neopterin in a human monocyte/macrophage cell line. *J Am Med Assoc* 1996;279:248–253.
- Star RA, Rajara N, Huang J, Chavei R, Catania A, Lipton JM: Evidence of autocrine modulation of macrophage nitric oxide synthase by α -MSH. *Proc Natl Acad Sci USA* 1995;90:8856–8860.
- Raap U, Brzoska T, Sohl S, Path Gunter, Emmel J, Herz U, Braun A, Luger T, Renz H: α -Melanocyte-stimulating hormone inhibits allergic airway inflammation. *J Immunol* 2003;170:353–359.
- Ichiyama I, Sakai T, Catania A, Barsh GS, Furukawa S, Lipton JM: Systemically administered α -melanocyte stimulating peptides inhibit NF- κ B activation in experimental brain inflammation. *Brain Res* 1999;836:31–37.
- Rajara N, Ceriani G, Catania A, Star RA, Murphy MT, Lipton JM: α -MSH production, receptor, and influence on neopterin in a human monocyte/macrophage cell line. *J Am Med Assoc* 1996;279:248–253.
- Star RA, Rajara N, Huang J, Chavei R, Catania A, Lipton JM: Evidence of autocrine modulation of macrophage nitric oxide synthase by α -MSH. *Proc Natl Acad Sci USA* 1995;90:8856–8860.
- Lipton JM, Catania A: Anti-inflammatory actions of the neuro-immunomodulator α -MSH. *Immunol Today* 1997;18:140–145.
- Namba K, Kitaichi N, Nishida T, Taylor AW: Induction of regulatory T cells by the immunomodulating cytokines α -melanocyte-stimulating hormone and transforming growth factor- β 2. *J Leukocyte Biol* 2002;72: 946–952.
- Wahl SM, Wong H, McCartney-Francis N: Role of transforming growth factors in inflammation and repair. *J Cell Biochem* 1989; 40:193–199.
- Kehrl JH, Wakefield LM, Roberts AB, Jakowlew S, Alvarez-Mon M, Deryneck R, Sporn MB, Fauci AS: Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. *J Exp Med* 1987;163: 1037–1050.
- Kehrl JH, Roberts AB, Wakefield LM, Jakowlew S, Sporn MB, Fauci AS: Transforming growth factor beta is an important immunomodulatory protein for human B lymphocytes. *J Immunol* 1986;137:3855–3860.
- Lee G, Ellingsworth LR, Gillis S, Walls R, Kincade PW: β transforming growth factors are potential regulators of B cells. *J Exp Med* 1987;166:1290–1299.
- Cross D, Cambier JC: Transforming growth factor β 1 has differential effects on B cell proliferation and activation antigen expression. *J Immunol* 1990;144:432–439.
- Coffman RL, Lebman DA, Shrader B: Transforming growth factor β specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. *J Exp Med* 1989;170:1039–1044.
- Sonoda E, Matsumoto R, Hitoshi Y, Ishii T, Sugimoto M, Araki S, Tominaga A, Yamaguchi N, Takatsu K: Transforming growth factor β induces IgA production and acts additively with interleukin 5 for IgA production. *J Exp Med* 1989;170:1415–1420.
- Kim PH, Kagnoff MF: Transforming growth factor β 1 is a costimulator for IgA production. *J Immunol* 1990;144:3411–3416.
- Cannon JG, Tatro JB, Reichlin S, Dinarello CA: α -Melanocyte stimulating hormone inhibits immunostimulatory and inflammatory actions of interleukin 1. *J Immunol* 1986;137:2232–2236.
- Hiltz ME, Catania A, Lipton JM: α -MSH peptides inhibit acute inflammation induced in mice by rIL-1 β , rIL-6, rIFN- α and endogenous pyrogen but not that caused by LTB4, PAF and rIL-8. *Cytokine* 1992;4:320–328.
- Luger TA, Brzoska T, Scholzen TE, Kalden DH, Sunderkotter C, Armstrong C, Ansel J: The role of α -MSH as a modulator of cutaneous inflammation. *Ann NY Acad Sci* 2000; 917:232–238.
- Bhardwaj R, Becher E, Mahnke K, Hartmeyer M, Schwarz T, Luger TA: Evidence for the differential expression of the functional α -melanocyte-stimulation hormone receptor MC-1 on human monocyte. *J Immunol* 1997; 158:3378–3384.
- Buggy JJ: Binding of α -melanocyte-stimulating hormone to its G-protect-coupled receptor on B-lymphocytes activates the Jak/STAT pathway. *Biochem J* 1998;331:211–216.
- Taylor A, Namba K: In vitro induction of CD25/CD4+ regulatory T cells by the neuropeptide α -melanocyte stimulating hormone (α -MSH). *Immunol Cell Biol* 2001;79:358–367.