

# Wasp venom allergy: effect of anti-IgE antibody on wasp venom anaphylaxis in a mouse model

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## Summary

**Background:** Although anti-IgE antibody (Ab) therapy was recently shown to be effective in patients with bronchial asthma, no study has reported the effect of IgE therapy in the prevention of wasp venom anaphylaxis. In this study, we used a mouse model of wasp venom allergy to investigate the effect of anti-IgE Ab on wasp venom anaphylaxis.

**Methods:** We developed a mouse model of wasp venom allergy by intraperitoneally (i.p.) injecting wasp venom into BALB/c mice twice on experimental day (day) 0 and 7. On day 20, a group of mice received an i.p. injection of mouse anti-IgE Ab as a pretreatment, and another group received rat anti-IgG1 Ab. On day 21, the animals were challenged by i.p. injection of wasp venom, and 30 min later, body temperature was measured and serum levels of leukotriene (LT) B4 and LTC4 were determined using enzyme immunoassay.

**Results:** The body temperature of mice treated with anti-IgE Ab and controls before and after wasp venom challenge was  $37.8 \pm 0.2$  vs  $37.7 \pm 0.3^\circ\text{C}$  before challenge and  $37.8 \pm 0.2$  vs  $37.1 \pm 0.3^\circ\text{C}$  after challenge, respectively, showing that anti-IgE Ab treatment significantly prevented body temperature from falling ( $p < 0.05$ ). Furthermore, anti-IgE Ab treatment reduced total serum IgE levels in the treated mice ( $42.2 \pm 15.9$  pg/ml), compared with controls ( $105.9 \pm 23.1$  pg/ml,  $p < 0.05$ ), and inhibited the secretion of LTC4 in the treated mice ( $32.0 \pm 18.8$  pg/ml), but not in the controls ( $162.4 \pm 12.4$  pg/ml,  $p < 0.05$ ), following challenge with wasp venom.

**Conclusion:** The results of the present study indicate that anti-IgE Ab treatment is an effective preventive measure against wasp venom-induced anaphylaxis. (*Asian Pac J Allergy Immunol* 2013;31:115-24)

**Key words:** Hymenoptera allergy, wasp venom, LTC4, mouse model, Anti-immunoglobulin E

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## Abbreviations

LTC4	=	Leukotriene C4
ELISA	=	Enzyme-linked immunosorbent assay
EIA	=	Enzyme immunoassay
Ig	=	Immunoglobulin
NSAIDs	=	Non-steroid anti-inflammatory drugs
Igh-C	=	Immunoglobulin heavy chain
BLT1	=	Leukotriene B4 receptor 1
PAF	=	Platelet-activating factor
IFN $\gamma$	=	Interferon-gamma
OVA	=	Ovalbumin
Fc epsilon R1	=	High-affinity immunoglobulin E receptor
IL	=	Interleukin



## Introduction

Immediate-type allergic reactions are represented by allergic rhinitis and conjunctivitis, bronchial asthma, atopic dermatitis, and reactions triggered by drugs, food, wasp venom, and latex. Among them, allergic reactions due to drug, food, wasp venom, and latex are mediated by immunoglobulin (Ig) E and are associated with a variety of symptoms. Upon exposure to an antigen (Ag), chemical messengers such as histamine and serotonin are released IgE-dependently from mast cells and basophils in the skin, the conjunctiva, the mucosa of the oral cavity, nose, and airway, and the lining of the digestive tract. In addition, within 30 min of allergen exposure, bronchial smooth muscle contraction, dilated blood vessels, and increased vascular permeability develop because of the synthesis and secretion of lipid mediators, including leukotriene (LT) B<sub>4</sub>, LTC<sub>4</sub>, and prostaglandin (PG), due to activation of arachidonic acid, a cell membrane lipid. On the other hand, development of symptoms associated with allergic retinitis, bronchial asthma, and atopic dermatitis requires the presence of local chronic allergic inflammation mediated mostly by T cells in addition to IgE-dependent reactions. Therefore, different pathological mechanisms are likely to exist, even within IgE-dependent immediate-type allergies.

To understand such pathology, different mouse models of IgE-dependent immediate-type allergy have been developed using allergens such as ovalbumin (OVA),<sup>1</sup> mite Ag (Der p2),<sup>2</sup> *Aspergillus fumigatus* extract,<sup>3</sup> cedar pollen,<sup>4</sup> latex Ag (Hev b5),<sup>5</sup> wheat (gliadin),<sup>6</sup> and penicillin.<sup>7</sup> However, almost no studies have investigated the pathogenesis of wasp venom allergy in detail using an mouse model.

Recently, therapy targeting IgE has been established, and its efficacy towards bronchial asthma,<sup>8</sup> pollinosis,<sup>9</sup> and food allergy<sup>10</sup> has been shown in mice and humans. However, the effect of anti-IgE therapy in wasp venom allergy has remained unreported. Therefore, we developed a mouse model of wasp venom allergy using a wasp venom extract as an allergen to elucidate the pathogenesis of wasp venom allergy and the efficacy of anti-IgE antibody (Ab).

## Methods

### Animals

Female 6-week-old BALB/c mice were purchased from Japan SLC (Hamamatsu, Japan) and

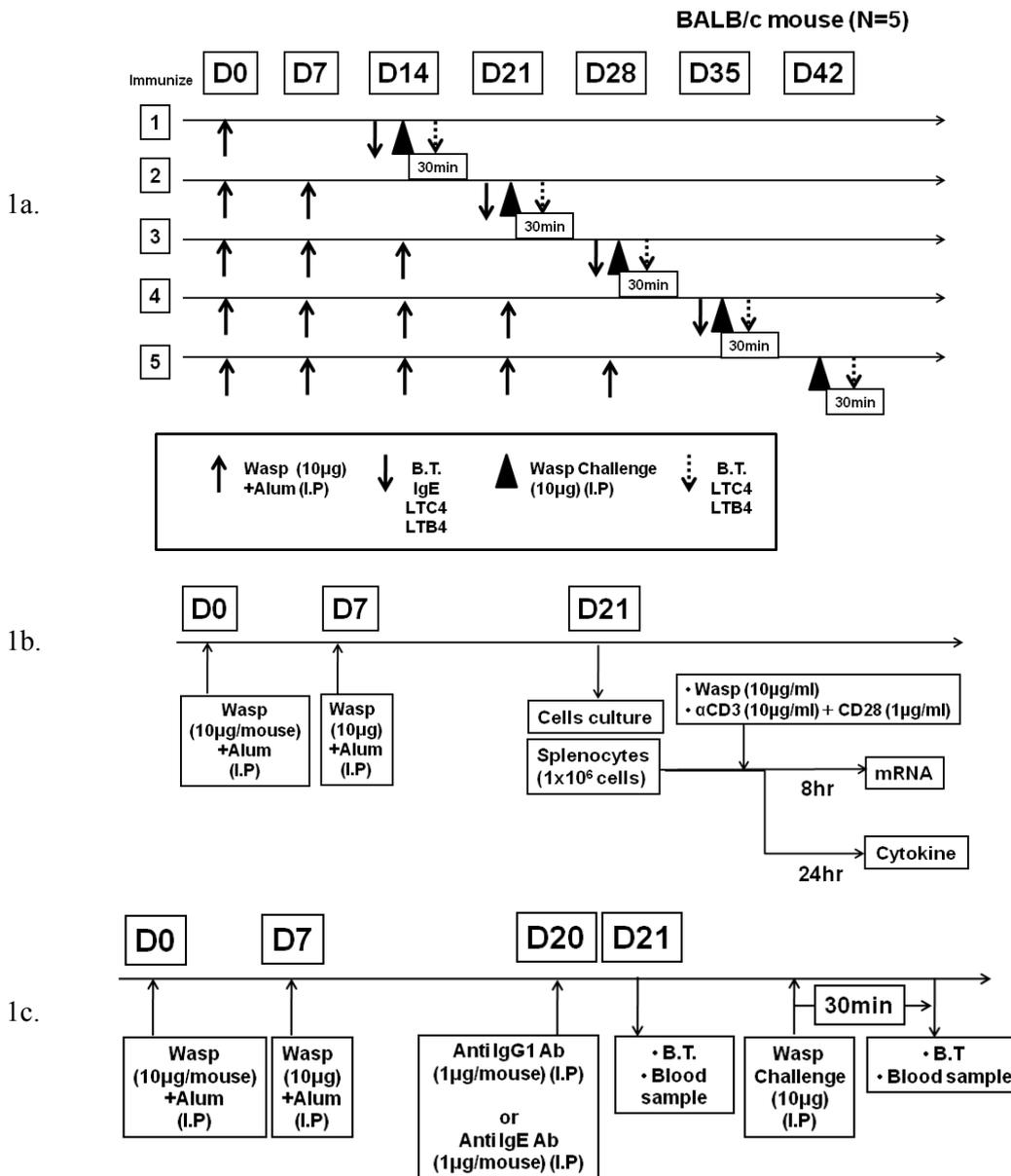
maintained under specific pathogen-free conditions. The Animal Ethics Committee of Dokkyo University School of Medicine approved all in vivo manipulations.

### Sensitization of mice with wasp venom

Wasp venom (10 µg, 20 µg/ml, 0.5 ml/mouse; Hollister-Stier Laboratories, Spokane, WA) adsorbed in 5 mg aluminum hydroxide gel (aluminium potassium sulfate (Alum); 20 mg/ml, 0.25 ml/mouse; Wako Pure Chemical Industries, Osaka, Japan) was intraperitoneally (i.p.) injected into BALB/c mice to develop a mouse model of wasp venom allergy (Figure 1a). Another group of mice were injected with a vehicle (0.25 ml adjuvant + 0.25 ml physiological saline/mouse) as non-sensitized controls. Mice injected with wasp venom were further divided into 5 groups based on the number of immunizations: once on experimental day (day) 0 (one-sensitization group), twice on days 0 and 7 (two-sensitization group), 3 times on days 0, 7, and 14 (three-sensitization group), 4 times on days 0, 7, 14, and 21 (four-sensitization group), and 5 times on days 0, 7, 14, 21, and 28 (five-sensitization group). Five mice were used in each experimental and control group. Wasp venom challenge was performed via i.p. injection of 10 µg of wasp venom (20 µg/ml, 0.5 ml/mouse) 14 days after the last immunization in each group. Rectal temperature was measured before and after wasp venom challenge using a digital thermometer (OMRON Corporation, Kyoto, Japan). Blood was collected through the tail vein immediately before challenge and from the aorta (1 ml) using a syringe immediately after cervical dislocation following challenge. Blood was centrifuged at 3000 rpm for 10 min at 4 °C to separate the serum which was subsequently stored at -80 °C until measurement of total IgE, LTB<sub>4</sub>, and LTC<sub>4</sub> levels.

### Expression of cytokine in spleen cell cultures

The spleen was removed from mice sensitized twice with wasp venom and aluminum adjuvant on days 0 and 7 to culture splenocytes in RPMI1640 medium (Sigma-Aldrich, St. Louis, MO) + 10% FCS (Sigma-Aldrich). Cells ( $1 \times 10^6$  cells/ml) were not stimulated, stimulated non-specifically with anti-CD3 Ab (10 µg/ml; Ancell Corporation, Bayport, MN) + anti-CD28 Ab (1 µg/ml; Becton Dickinson Biosciences, Franklin Lakes, NJ), or stimulated specifically with wasp venom (10 µg/ml). The mRNA levels of interleukin (IL)-4, IL-5, IL-13, and interferon-gamma (IFN-γ) were measured using



**Figure 1.** Schematic of the experimental procedures used in the present study.

(a) BALB/c mice were immunized via i.p. injection of wasp venom + aluminium potassium sulfate (Alum); 20 mg/ml, 0.25 ml/mouse; (10 µg + 5 mg, respectively/mouse; sensitization group) or Alum + saline (5 mg + 0.25 ml, respectively/mouse; non-sensitized group). Mice were further divided into five groups sensitized 1 to 5 times in interval of one weeks. Wasp venom challenge (10 µg/mouse) was performed via i.p. injection 2 weeks after the last immunization in each group. Total IgE Ab levels in the serum were measured before challenge, and body temperature (B.T.) and the levels of LTC4 and LTB4 were measured before and 30 min after challenge.

(b) On day 21, the spleen was removed from mice immunized twice on day 0 and 7 with wasp venom and Alum to establish splenocyte culture cells. Cells were stimulated with or without anti-CD3 Ab + anti-CD28 Ab or specifically with wasp venom. Both mRNA and protein levels of IL-4, 5, 13, and IFN- $\gamma$  were determined by real-time PCR or by ELISA after stimulation, respectively.

(c) Mice sensitized twice with wasp venom were divided into the sensitized and non-sensitized group. On day 20, mice were Ag-specifically treated with mouse anti-IgE Ab or non-specifically with rat anti-IgG1K isotype Ab. Another group of mice did not receive Ab as a control. All groups were challenged with wasp venom on day 21, and B.T, total IgE levels in the serum before challenge, and LTC4 levels were measured before and 30 min after challenge. Five mice were used in each experimental and control group. i.p.: intraperitoneally.

real-time PCR 8 h after stimulation, and their protein levels in culture medium supernatant were determined by the enzyme linked immunosorbent assay (ELISA) 24 h after stimulation (Figure 1b).

#### **Expression of cytokine mRNA in splenocytes**

At each time point, splenocyte culture supernatants were removed, washed with PBS, and stored at  $-80^{\circ}\text{C}$  for a few days. Splenocyte culture supernatants were thawed at  $0^{\circ}\text{C}$  in 1 mL of TRIzol reagent, a monophasic solution of phenol and guanidine isothiocyanate (Invitrogen, Carlsbad, CA), then homogenized using a microhomogenizer ( $10\,000 \times \text{g}$ , 90 s). Total RNA was extracted by the modified guanidine isothiocyanate-phenol-chloroform method, as recommended by the manufacturer. Total RNA ( $5\ \mu\text{g}$ ) was immediately reverse-transcribed using Superscript III reverse transcriptase (Invitrogen) and oligo(dT) primers (Amersham Pharmacia Biotech, Piscataway, NJ) as recommended by the manufacturers. The resultant cDNA products were diluted in Tris-EDTA buffer and used for real-time quantitative PCR.

#### **Real-time quantitative PCR**

Quantification of cytokine mRNA expression was performed by real-time RT-PCR. PCR was performed on an ABI/PRISM 7000 sequence detection system and analyzed using ABI/PRISM 7000 SDS v1.0 software (Applied Biosystems, Foster City, CA).  $\beta$ -Actin was used as an internal control. PCR conditions were as follows:  $4\ \mu\text{L}$  of cDNA was mixed with  $12.5\ \mu\text{L}$  of Power SYBR Green PCR Master Mix (Applied Biosystems) and  $1.5\ \mu\text{L}$  of primer mix ( $15\ \text{pmol}$ ) and adjusted to a final volume of  $25\ \mu\text{L}$ . The thermal cycler conditions were as per the manufacturer's instructions. Standard curves for cytokine mRNA were generated using cDNA from the splenocyte of naïve BALB/c mice. Amplification of standard cDNA and sample cDNA was carried out in 96-well reaction plates. Each plate contained the same standard cDNA. Cycling conditions included a hot start at  $95^{\circ}\text{C}$  for 10 min, followed by 40 cycles at  $95^{\circ}\text{C}$  for 15 s, and  $60^{\circ}\text{C}$  for 1 min. Accumulation of product was monitored by SYBR green fluorescence. The data from two runs were averaged and relative expression levels were determined from a standard curve of serial dilutions of cDNA samples. Cytokine mRNA levels were expressed as absolute numbers of copies, after normalization to  $\beta$ -actin mRNA levels. The primers for real-time quantitative RT-PCR were as follows: mouse  $\beta$ -actin, 5'-CCAGCCTTCCTTCTTGGGTAT (forward),

5'-TGGCATAGAGGTCTTTACGGATGT (reverse). Mouse IL-4, IL-5, IL-13 and interferon (IFN)- $\gamma$  primers were obtained from Takara Bio (Madison, WI).

#### **Measurement of total IgE in mice serum**

Concentrations of total IgE in serum were measured using ELISA kits (YAMASA Corporation, Tokyo, Japan). The sensitivity of each assay exceeded  $10\ \text{ng/mL}$ .

#### **Measurement of chemical mediators and cytokines in splenocyte culture supernatants**

Concentrations of LTB<sub>4</sub>, LTC<sub>4</sub> (Cayman, Ann Arbor, MI) in splenocyte culture supernatants were assessed by enzyme immunoassay, which had sensitivities of  $>3.9$  and  $7.8$ , respectively. Concentrations of IL-4, IL-5, IL-13, and IFN- $\gamma$  in splenocyte culture supernatants were measured using ELISA kits (R&D Systems, Minneapolis, MN). The sensitivity of each assay exceeded  $7.8$ ,  $15.6$ ,  $7.8$ , and  $9.4\ \text{pg/mL}$ , respectively.

#### **Anti-IgE Ab**

Mice sensitized twice with wasp venom were divided into two groups: a sensitized group ( $n=5$ ) and non-sensitized group ( $n=5$ ). On day 20, mice were pretreated with mouse anti-IgE Ab ( $1\ \mu\text{g}/\text{mouse}$ ; Becton Dickinson Bioscience) that recognizes the Igh-C(a) and Igh-C(b) haplotypes. Controls received rat anti-IgG1 $\kappa$  isotype Ab ( $1\ \mu\text{g}/\text{mouse}$ ; Becton Dickinson Bioscience). On day 21, wasp venom challenge was performed via i.p. injection, and body temperature and the levels of total IgE and LTC<sub>4</sub> in the serum were measured 30 min after challenge (Figure 1c) by the method described above.

#### **Statistical analysis**

Data are expressed as mean  $\pm$  SD values. Statistical significance between groups was assessed by the Student's t-test, at the same time point if the data were normally distributed. An overall significance level of 0.05 was used for all statistical comparisons.

## **Results**

#### **Change in body temperature following immunization of wasp venom**

Following wasp venom challenge, the body temperature of mice in the one-, two-, three-, and four-sensitization groups changed from  $37.4 \pm 0.2$  to  $35.8 \pm 0.4$ ,  $37.6 \pm 0.2$  to  $35.7 \pm 0.4$ ,  $38.1 \pm 0.2$  to  $35.6 \pm 0.3$ , and  $37.6 \pm 0.3$  to  $36.9 \pm 0.3$  ( $^{\circ}\text{C}$ ), respectively (Figure 2). On the other hand, body temperature barely

changed in mice in the five-sensitization group ( $38.2\pm 0.3$  to  $38.4\pm 0.2$  °C) and the non-sensitized controls ( $37.3\pm 0.5$  to  $37.4\pm 0.3$  °C). These results show that the body temperature of mice immunized one–four times dropped significantly compared with the controls ( $p < 0.05$ ).

#### **Change of serum IgE level by immunization of wasp venom**

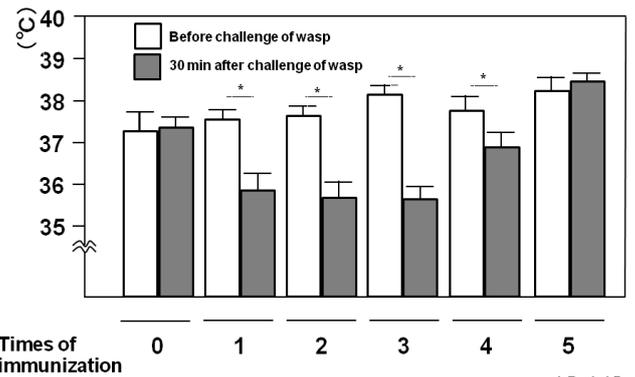
Two weeks after the last immunization with wasp venom, the total serum IgE levels were  $17.5\pm 26.0$  pg/ml in the two-sensitization group,  $36.7\pm 16.6$  pg/ml in the three-sensitization group, and  $121.6\pm 31.2$  pg/ml in the five-sensitization group, demonstrating that the levels were higher with increasing number of immunizations.

#### **Levels of serum $LTB_4$ and $LTC_4$ by venom challenge**

The serum levels of  $LTB_4$  and  $LTC_4$  were determined 30 min after wasp venom challenge in the two-sensitization group. Although  $LTB_4$  levels barely changed between before ( $8.7\pm 3.3$  nM) and after ( $11.7\pm 6.3$  nM) challenge, wasp venom challenge significantly increased the level of  $LTC_4$  from  $52.4\pm 11.0$  to  $124.0\pm 28.4$  pg/ml ( $p < 0.05$ ). Even after challenge,  $LTB_4$  and  $LTC_4$  were undetectable in the non-sensitized group.

#### **Expression of cytokine mRNA and protein in splenocytes by stimulation with $\alpha CD3$ and $\alpha CD28$ or wasp venom**

To elucidate the patterns of cytokine production in the wasp venom allergy model, the expression of IL-4, IL-5, IL-13, and IFN- $\gamma$  in splenocytes due to Ag-specific stimulation was studied at the mRNA (Figure 3a) and protein (Figure 3b) levels. While non-specific stimulation resulted in the expression of IL-4 in the cells from both controls and sensitized mice, Ag-specific stimulation increased IL-4 only in the cells derived from sensitized mice at the mRNA (relative expression of mRNA  $8.8\pm 8.6$  vs  $105.2\pm 26.1$ ) and protein (value not determined vs  $33.4\pm 8.2$  pg/ml) levels. The mRNA and protein levels of IL-5 (relative expression of mRNA  $8.9\pm 10.3$  vs  $116.4\pm 14.3$ ; value not determined vs  $125.0\pm 52.2$  pg/ml) and IL-13 (relative expression of mRNA  $9.1\pm 12$  vs  $30.9\pm 15$ ; value not determined vs  $184.2\pm 74.3$  pg/ml) were significantly increased by non-specific and Ag-specific stimulation in the mouse model ( $p < 0.05$ ). In contrast, neither type of stimulation increased IFN- $\gamma$ .



**Figure 2.** Change in body temperature (B.T) following wasp venom challenge. Mice in the non-sensitized group and the one to five-sensitization groups or Alum were challenged by wasp venom 14 days after the last immunization in each group, and B.T was measured before and 30 min after challenge. White and black bars represent before challenge and 30 min after challenge, respectively. Data are expressed as mean  $\pm$  SD values for each group. Significance was set at  $p < 0.05$  for comparison before challenge of wasp.

#### **Effects of anti IgE Ab on serum total IgE level**

We investigated the effect of anti-IgE Ab on serum total IgE levels (Figure 4). While the level was  $105.9\pm 23.1$  pg/ml in the mouse model of wasp venom allergy, the levels were  $42.2\pm 15.9$  and  $112.1\pm 23.4$  pg/ml in mice treated with anti-IgE and anti-IgG1 Ab, respectively, demonstrating that the administration of anti-IgE Ab significantly inhibited the rise in the serum total IgE levels.

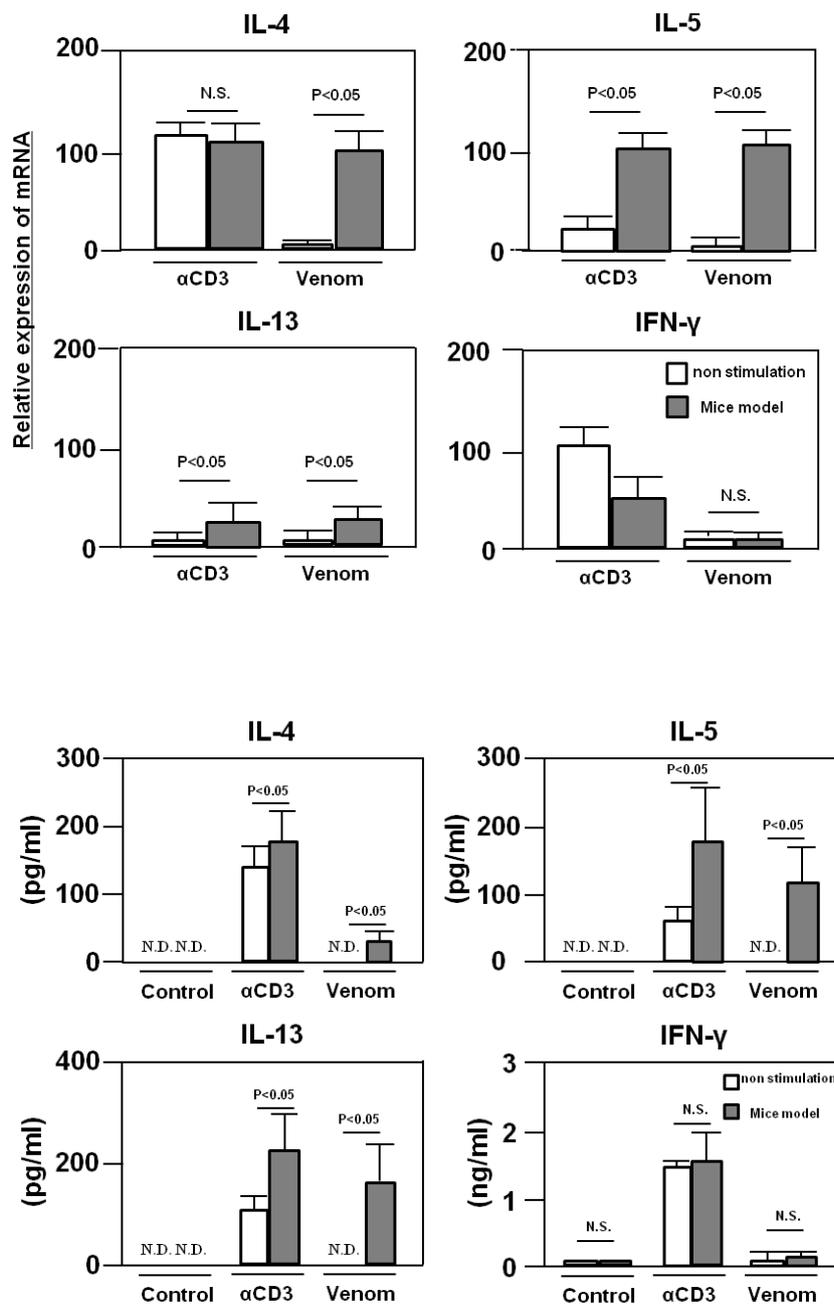
#### **Effects of body temperature by anti IgE Ab**

We then measured body temperature to reveal the effect of anti-IgE Ab on anaphylactic reactions in the mouse model (Figure 5).

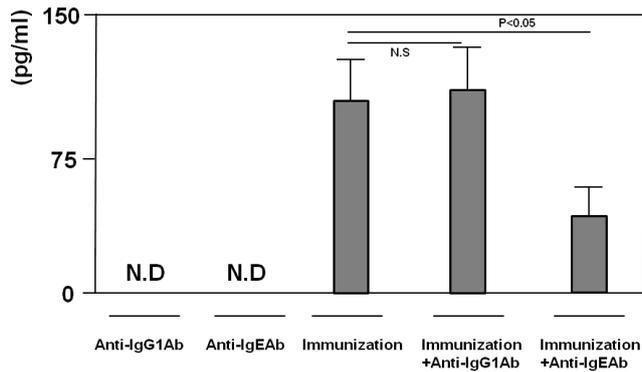
Wasp venom challenge did not significantly change the body temperature of mice treated with anti-IgG1 Ab ( $37.7\pm 0.1$  to  $37.1\pm 0.3$  °C). In mice treated with anti-IgE Ab, the temperature did not change between before and after challenge ( $37.8\pm 0.2$  and  $37.8\pm 0.2$  °C). These results show that the administration of anti-IgE Ab significantly inhibited the drop in body temperature compared with mice not treated with the Ab ( $p < 0.05$ ).

#### **Effects of serum $LTC_4$ levels by anti IgE Ab**

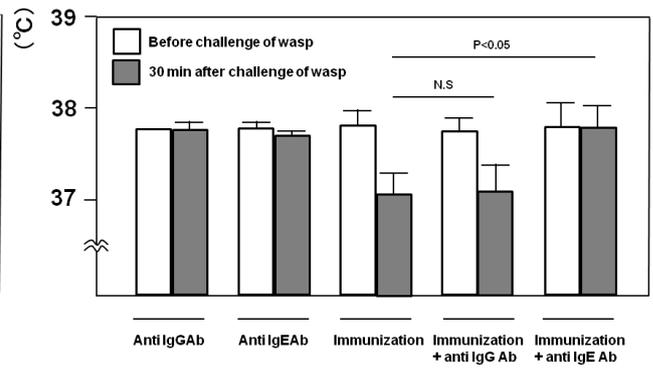
We also investigated the effect of anti-IgE Ab on the serum levels of  $LTC_4$  in the mouse model (Figure 6). In mice not treated with Ab and in those



**Figure 3.** Expression of cytokines in splenocytes derived from mice with wasp venom allergy (a) Splenocyte culture cells ( $1 \times 10^6$  cells/ml) derived from mice in the non-sensitized group and those sensitized twice on days 0 and 7 were stimulated with  $10 \mu\text{g/ml}$  anti-CD3 Ab +  $1 \mu\text{g/ml}$  anti-CD28 Ab or  $10 \mu\text{g/ml}$  wasp venom. The mRNA levels of cytokines were determined 8 h after stimulation. (b) Splenocytes were prepared and stimulated as described above, and the levels of IL-4,-5, 13, and IFN- $\gamma$  in the supernatant were determined using ELISA 24 h after stimulation. White and black bars represent non-sensitized mice (control) and sensitized mice (mice model), respectively. Data are expressed as mean  $\pm$  SD values for each group. Significance was set at  $p < 0.05$  for comparison without wasp venom or with non-sensitized mice. N.S.: not significant; N.D.: not detected.



**Figure 4.** Alteration by anti-IgE Ab of the effect of wasp venom challenge on total IgE in a mouse model of wasp venom allergy. On day 20, mice sensitized twice and those in the non-sensitized group were treated with mouse anti-IgE Ab (1  $\mu$ g/mouse) or rat anti-IgG1K isotype Ab (1  $\mu$ g/mouse) as a control. On day 21, mice were challenged with wasp venom, and serum total IgE levels before challenge were determined by ELISA. Data are expressed as mean  $\pm$  SD values for each group. Significance was set at  $p < 0.05$  for comparison of twice-sensitized mice lacking anti IgG Ab and anti IgE Ab. N.S.: not significant; N.D.: not detected.



**Figure 5.** Alteration by anti-IgE Ab of the effect of wasp venom challenge on body temperature (B.T) in a mouse model of wasp venom allergy. On day 20, mice sensitized twice and those in the non-sensitized group were treated with mouse anti-IgE Ab (1  $\mu$ g/mouse) or rat anti-IgG1K isotype Ab (1  $\mu$ g/mouse) as a control. On day 21, mice were challenged with wasp venom, and B.T was measured before and after challenge. White and black bars represent before challenge and 30 min after challenge of wasp, respectively. Data are expressed as mean  $\pm$  SD values for each group. Significance was set at  $p < 0.05$  for comparison without both anti IgG Ab and anti IgE Ab in twice sensitized mice 30 min after challenge with wasp venom. N.S.: not significant; N.D.: not detected.

treated with anti-IgG1 Ab, LTC4 levels increased significantly after wasp venom challenge (not treated with Ab group:  $32.0 \pm 11.4$  to  $162.4 \pm 11.4$  pg/ml,  $p < 0.05$ , Anti-IgG1 Ab treated group:  $29.6 \pm 14.4$  to  $165.4 \pm 13.8$  pg/ml,  $p < 0.05$ ) On the other hand, LTC4 production was significantly prevented in mice treated with anti-IgE Ab (before and after challenge,  $30.2 \pm 15.0$  pg/ml and  $32.0 \pm 18.8$  pg/ml) compared with mice not treated with the Ab ( $p < 0.05$ ).

## Discussion

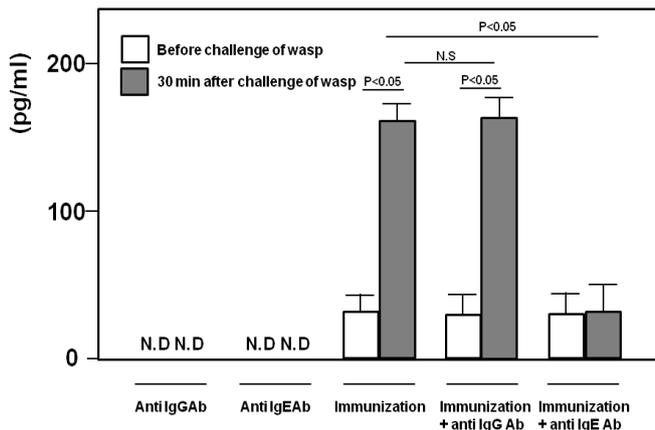
Anti-IgE Ab has demonstrated its effectiveness against asthma<sup>8</sup>, allergic rhinitis<sup>9</sup>, and food allergy<sup>10</sup> in humans and in animal models. Here we show for the first time that anti-IgE Ab suppresses the onset of anaphylaxis. However, the efficacy of anti-IgE Ab on wasp venom anaphylaxis in humans requires further investigation.

Histamine<sup>11</sup> and serotonin released from mast cells and basophils play an important role in the pathology of anaphylaxis, and mediators such as LTB4,<sup>12</sup> LTC4,<sup>13</sup> and PG<sup>14</sup> are also known to be involved in the mechanism. To elucidate the pathology of anaphylaxis, various mouse models have been developed using OVA,<sup>1</sup> chemical substance (hapten TNP),<sup>15</sup> wheat-dependent exercise-

induced anaphylaxis (gliadin),<sup>16</sup> and antimicrobial agent (penicillin G).<sup>7</sup> Anaphylactic reactions in mice are reportedly mediated by two pathways: an IgE-dependent pathway with IgE-mast cells as the major player<sup>16</sup> and another pathway mediated by IgG1 with macrophages and basophils as the major players.<sup>17</sup>

In this study, while body temperature decreased significantly in the one- to three-sensitization groups, the reduction was increasingly inhibited as the number of immunizations increased beyond four. These results suggest that, although mice were fully sensitized after one to three immunizations, hyposensitization resulted from more than four immunizations, presumably because of the depletion of histamine and the production of blocking Ab (wasp venom-specific IgG4).<sup>18</sup>

We then investigated how the number of immunizations affects the production of IgE. A previous study reported the use of an aluminum adjuvant and recombinant major wasp venom allergen to successfully produce specific IgE Ab in the mouse model.<sup>19</sup> In the present mouse model, we also used an aluminum adjuvant to sensitize mice with wasp venom, and we observed a production of total IgE Ab in the previous study.<sup>19</sup> IgE was



**Figure 6.** Alteration of the effect of wasp venom challenge on LTC4 by anti-IgE Ab in a mouse model of wasp venom allergy. On day 20, mice in the sensitization and non-sensitized groups were treated with mouse anti-IgE Ab (1  $\mu$ g/mouse) or rat anti-IgG1K isotype Ab (1  $\mu$ g/mouse) as controls. On day 21, mice were challenged with wasp venom, and EIA was used to determine the serum levels of LTC4 before and 30 min after challenge. White and black bars represent before challenge and 30 min after challenge with wasp venom, respectively. Data are expressed as mean  $\pm$  SD values for each group. Significance was set at  $p < 0.05$  for comparison of twice-sensitized mice lacking anti IgG Ab and anti IgE Ab 30 min after challenge with wasp venom. N.S.: not significant; N.D.: not detected.

detectable after the second immunization, and the production increased with increasing number of immunizations after the third immunization. These results suggest that the production of IgE Ab was dependent on wasp venom allergen.

Histamine is frequently used as an indicator of mediators in IgE-dependent immediate-type anaphylaxis. However, we could not accurately assess the level of histamine in the present study because histamine was detected as a non-specific reaction due to the high histamine content of wasp venom. We therefore used LTB4 and LTC4-secreted from neutrophils and basophils or from eosinophils, respectively, as indicators to measure the pre- and post-challenge levels of total IgE in mice sensitized twice. While the levels of LTB4 did not change after wasp venom challenge, LTC4 levels increased significantly. LTB4 production due to the stimulation of platelet-activating factor (PAF) is reportedly involved in the pathogenesis of anaphylaxis, and intravenous injection of PAF is known to attenuate anaphylactic reaction in an inflammatory model using mice

deficient in leukotriene B4 receptor 1 (BLT1), a receptor for LTB4.<sup>20</sup> However, the involvement of LTB4 in anaphylactic reactions was not clear in the present study. Because LTC4 is also known to be produced via an IgE-dependent pathway in food-dependent aspirin-induced anaphylaxis,<sup>21</sup> LTC4 elevation seen after wasp venom challenge in this study likely plays a role in the pathogenesis of wasp venom-associated anaphylaxis.

When elucidating the patterns of Th cytokine production in our mouse model in response to splenocyte culture cells, we observed that the Ag-specific stimulation did not change the expression of IFN- $\gamma$ , but rather, it significantly upregulated the Th2 cytokines IL-4, IL-5, and IL-13, suggesting the involvement of Th2 type responses in our wasp venom allergy model. We also detected elevated levels of IL-4 in the controls after non-specific stimulation with  $\alpha$ CD3. A previous study reported that even in the naïve Th state against foreign Ag, Th2 responses are preferentially induced in BALB/c mice compared with other mouse strains,<sup>22</sup> and this might have been the cause of the high levels of IL-4 after non-specific stimulation in the present study.

In recent years, a number of studies have reported the development of anti-IgE Ab, including omalizumab, in several allergic diseases<sup>9,23-25</sup>, including asthma<sup>8</sup> and food allergy<sup>10</sup>. By binding to the secreted form of human IgE Igh-C (c) (C $\epsilon$ 3), omalizumab inhibits the interaction between IgE and high-affinity immunoglobulin E receptor (Fc $\epsilon$ R1) present on the surface of mast cells and basophils. The drug also attenuates IgE-dependent immediate- and delayed-type allergic reactions in humans through the inhibition of histamine release<sup>26</sup> and the production of chemical mediators, such as leukotriene,<sup>27</sup> reduction of free IgE,<sup>28</sup> down-regulation of Fc $\epsilon$ R1,<sup>29</sup> and suppression of Th2 cytokine induction.<sup>30</sup>

At the same time, many studies have investigated the use of mouse anti-IgE Ab in mouse models of allergic diseases, such as chronic asthma induced with OVA.<sup>31</sup> These studies reported IgE Ab-dependent attenuation of allergic reactions, including a reduction in total IgE,<sup>31</sup> inhibition of Th2 cytokine induction,<sup>31</sup> and inhibition of histamine release.<sup>32</sup> To reveal the efficacy of anti-IgE Ab in our mouse model of wasp venom anaphylaxis, we pretreated the mice with anti-IgE Ab that recognizes the Igh-C (a) and Igh-C (b) haplotypes or with anti-IgG1 Ab prior to the measurement of body temperature and total IgE Ab levels. We observed increased levels of total IgE Ab in the serum and decreased body temperature in mice treated with anti-IgG1 Ab and

controls. On the other hand, inhibition of total IgE upregulation and prevention of a body temperature drop were observed in mice treated with anti-IgE Ab. We also measured the serum levels of LTC4 to investigate the effect of the Ab on chemical mediators. While no changes in the levels of LTC4 were detected in the controls or mice treated with anti-IgG1 Ab, the expression of LTC4 was significantly inhibited in mice treated with anti-IgE Ab. The anti-mouse IgE Ab used in the present study reacts with the Igh-C(a) and (b) haplotypes and is thus capable of binding to free and membrane bound IgE. It is therefore likely that the Ab functioned as a neutralizing Ab by binding to free IgE and competitively inhibiting their interaction with FcεR1 on mast cells and basophils. The Ab might have also inhibited the cross-linking of wasp venom allergens to membrane-bound IgE by binding to the latter.

The results of the present study suggest that an IgE-dependent pathway plays a major role in the onset of anaphylaxis in the mouse model of wasp venom allergy. It is therefore possible that anaphylactic reactions following exposure to the allergen can be blocked by the administration of anti-IgE Ab. Specific immunotherapy for wasp venom anaphylaxis has been established,<sup>33</sup> and its efficacy and safety have been shown in clinical settings. Similarly, the present study showed that anti-IgE Ab can be an effective tool for preventing wasp venom-induced anaphylaxis. While anti-IgE Ab directly inhibits the activity of IgE Ab, specific immunotherapy is thought to exert its effects indirectly by increasing the production of IgE-specific IgG Ab that acts as a blocking Ab. Immunotherapy is generally effective; however, even patients who undergo immunotherapy for five consecutive years are known to have an anaphylaxis recurrence rate of 10–15% after discontinuation<sup>34</sup>. Anti-IgE Ab is also believed to be effective during treatment, but because its inhibitory effect is temporary, anaphylaxis is expected to recur after discontinuation. In addition, although the cost-effectiveness of immunotherapy has recently been reported<sup>35</sup>, it is difficult to compare the economic impacts of immunotherapy with anti-IgE Ab because each country handles health insurance and medical practice differently. Furthermore, despite the cost of anti-IgE Ab therapy not being covered by health insurance in Japan, the use of anti-IgE Ab is recommended during seasons known for frequent Hymenoptera stings and in posts-immunotherapy

patients as well as immunotherapy patients with severe anaphylactic reactions.

In summary, we developed a mouse model of wasp venom allergy and investigated the pathology of anaphylaxis and the efficacy of anti-IgE Ab on such reactions. The results demonstrate that an IgE-dependent pathway(s) play a primary role in the anaphylactic onset caused by wasp venom and that the administration of anti-IgE Ab can prevent the onset. These findings suggest the potential clinical application of anti-IgE Ab therapy in humans.

### Conflict of interest

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### References

1. Arumugam M, Ahrens R, Osterfeld H, Kottyan LC, Shang X, MacLennan JA, et al. Increased susceptibility of 129SvEvBrd mice to IgE-Mast cell mediated anaphylaxis. *BMC Immunol.* 2011;12:14.
2. Herz U, Botchkarev VA, Pausz R, Renz H. Increased airway sponsiveness, allergy type-I skin responses and systemic anaphylaxis in a humanized-severe combined immuno-deficiency mouse model. *Clin Exp Allergy.* 2004;34:478-87.
3. Hoselton SA, Samarasinghe AE, Seydel JM, Schuh JM. An inhalation model of airway allergic response to inhalation of environmental *Aspergillus fumigatus* conidia in sensitized BALB/c mice. *Med Mycol.* 2010;48:1056-65.
4. Nabe T, Shimizu K, Mizutani N, Saeki Y, Yamamura H, Takenaka H, et al. A new model of experimental allergic rhinitis using Japanese cedar pollen in Guinea pigs. *J Pharmacol Sci.* 1997;75:243-51.
5. Hardy CL, Kenins L, Drew AC, Rolland JM, O'Hehir RE. Characterization of a mouse model of allergy to a major occupational latex glove allergen Hev b 5. *Am J Respir Crit Care Med.* 2003;167:1393-9.
6. Tanaka M, Nagano T, Yano H, Matsuda T, Ikeda TM, Haruma K, et al. Impact of ω-5 Gliadin on Wheat-Dependent Exercise-Induced Anaphylaxis in mice. *Biosci, Biotechnol, Biochem.* 2011;75:313-17.
7. Muranaka M, Suzuki S, Koizumi K, Igarashi H, Okumura H, Takeda K, et al. Benzylpenicillin preparations can evoke a systemic anaphylactic reaction in guinea pigs. *J Allergy Clin Immunol.* 1978; 62:276-82.
8. Ohta K, Miyamoto T, Amagasaki T, Yamamoto M. 1304 Study Group. Efficacy and safety of omalizumab in an Asian population with moderate-to-severe persistent asthma. *Respirology.*

- 2009;14:1156-65.
9. Casale TB, Bernstein IL, Busse WW, LaForce CF, Tinkelman DG, Stoltz RR, et al. Use of anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. *J Allergy Clin Immunol* 1997;100:110-21.
  10. Leung DY, Sampson HA, Yunginger JW, Burks AW Jr, Schneider LC, Wortel CH, et al. Effect of anti-IgE therapy in patients with peanut allergy. *N Engl J Med* 2003;348:986-93.
  11. Bochner BS, Lichtenstein LM. Anaphylaxis. *N Engl J Med* 1991;324:1785-1790.
  12. Haribabu B, Verghese MW, Steeber DA, Sellars DD, Bock CB, Snyderman R. Targeted disruption of the leukotriene B(4) receptor in mice reveals its role in inflammation and platelet-activating factor-induced anaphylaxis. *J Exp Med*. 2000;192:433-8.
  13. Suzuki Y, Ra C. Analysis of the mechanism for the development of allergic skin inflammation and the application for its treatment: aspirin modulation of IgE-dependent mast cell activation: role of aspirin-induced exacerbation of immediate allergy. *J Pharmacol Sci*. 2009;110:237-44.
  14. Higashi N, Mita H, Ono E, Fukutomi Y, Yamaguchi H, Kajiwara K, et al. Profile of eicosanoid generation in aspirin-intolerant asthma and anaphylaxis assessed by new biomarkers. *J Allergy Clin Immunol*. 2010;125:1084-91.
  15. Matsuoka K, Taya C, Kubo S, Toyama-Sorimachi N, Kitamura F, Ra C, et al. Establishment of antigen-specific IgE transgenic mice to study pathological and immunobiological roles of IgE in vivo. *Int Immunol*. 1999;11:987-94.
  16. Fred D, Finkelman. Anaphylaxis: Lessons from mouse models. *J Allergy Clin Immunol*. 2007;120:506-15.
  17. Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet JP, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and FcγRIII. Assessment of the cardiopulmonary changes, mast cell de-granulation, and death associated with active or IgE- or IgG1- dependent passive anaphylaxis. *J Clin Invest*. 1997;99:901-14.
  18. Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci U S A*. 1993;90:3730-4.
  19. Birgit W, Caroline B, Ulla S, Michael DS, Christof E, Ursula W. Allergen-specific immunosuppression by mucosal treatment with recombinant Ves v5, a major allergen of *Vespula vulgaris* venom, in a murine model of wasp venom allergy. *Immunology*. 2003;110:376-85.
  20. Repa A, Wild C, Hufnagl K, Winkler B, Bohle B, Pollak A, et al. Influence of the route of sensitization on local and systemic immune responses in a murine model of type I allergy. *Clin Exp Immunol*. 2004;137:12-8.
  21. Togo K, Suzuki Y, Yoshimaru T, Inoue T, Terumi T, Ochiai T, et al. Aspirin and salicylates modulate IgE-mediated leukotriene secretion in mast cells through a dihydropyridine receptor-mediated Ca<sup>2+</sup> influx. *Clin Immunol*. 2009;131:145-56.
  22. Kuroda E, Sugiura T, Zeki K, Yoshida Y, Yamashita U. Sensitivity Difference to the Suppressive Effect of Prostaglandin E2 Among Mouse Strains: A Possible Mechanism to Polarize Th2 Type Response in BALB/c Mice. *J Immunol*. 2000;164:2386-95.
  23. Tartibi HM, Majmundar AR, Khan DA. Successful use of omalizumab for prevention of fire ant anaphylaxis. *J Allergy Clin Immunol*. 2010;126:664-5.
  24. David Amrol. Anti-Immunoglobulin E in the Treatment of Refractory Atopic Dermatitis. *South Med J*. 2010;103:554-8.
  25. Boyce JA. Successful treatment of cold-induced urticaria/anaphylaxis with anti-IgE. *J Allergy Clin Immunol*. 2006;117:1415-8.
  26. Noga O, Hanf G, Kunkel G. Immunological and Clinical Changes in Allergic Asthmatics following Treatment with Omalizumab. *Int Arch Allergy Immunol*. 2003;131:46-52.
  27. Kopp MV, Stenglein S, Kamin W, Friedrichs F, von Berg A, Zielen S, et al. Omalizumab (Xolair) in children with seasonal allergic rhinitis: leukotriene release as a potential in vitro parameter to monitor therapeutic effects. *Pediatr Allergy Immunol*. 2007;18:523-7.
  28. Ohta K, Miyamoto T, Amagasaki T, Yamamoto M. 1304 Study Group. Efficacy and safety of omalizumab in an Asian population with moderate-to-severe persistent asthma. *Respirology*. 2009;14:1156-65.
  29. MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, et al. Down-regulation of Fc (epsilon) RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE anti-body. *J Immunol*. 1997;158:1438-45.
  30. Djukanović R, Wilson SJ, Kraft M, Jarjour NN, Steel M, Chung KF, et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med*. 2004;170:583-93.
  31. Kang JY, Kim JW, Kim JS, Kim SJ, Lee SH, Kwon SS, et al. Inhibitory effects of anti-immunoglobulin E antibodies on airway remodeling in a murine model of chronic asthma. *J Asthma*. 2010;47:374-80.
  32. Haak-Frendscho M, Saban R, Shields RL, Jardieu PM. Anti-immunoglobulin E antibody treatment blocks histamine release and tissue contraction in sensitized mice. *Immunology*. 1998;94:115-21.
  33. Krishna MT, Ewan PW, Diwakar L, Durham SR, Frew AJ, Leech SC, et al. Diagnosis and management of hymenoptera venom allergy: British Society for Allergy and Clinical Immunology (BSACI) guidelines. *Clin Exp Allergy*. 2011;41:1201-20.
  34. Golden DB. Long-term outcome after venom immunotherapy. *Curr Opin Allergy Clin Immunol*. 2010;10:337-41.
  35. Hockenhull J, Elremeli M, Cherry MG, Mahon J, Lai M, Darroch J, et al. A systematic review of the clinical effectiveness and cost-effectiveness of Pharmedgen® for the treatment of bee and wasp venom allergy. *Health Technol Assess*. 2012;16:1-110.