



Allergenicity potential of red kidney bean (*Phaseolus vulgaris* L.) proteins in orally treated BALB/c mice and passively sensitized RBL-2H3 cells



Sandeep Kumar^{a,b}, Akanksha Sharma^{a,c}, Alok K. Verma^{a,c}, B.P. Chaudhari^d, Mukul Das^{a,c}, S.K. Jain^b, Premendra D. Dwivedi^{a,*}

^a Food Toxicology Division, CSIR-Indian Institute of Toxicology Research (Formerly – Industrial Toxicology Research Centre), Mahatma Gandhi Marg, P.O. Box No. 80, Lucknow 226 001, India

^b Department of Biotechnology, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi 110 062, India

^c Academy of Scientific & Innovative Research (AcSIR), New Delhi, India

^d Central Pathology Laboratory, CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Gehru Campus, Lucknow 226 001, India

ARTICLE INFO

Article history:

Received 17 December 2012

Accepted 1 July 2013

Available online 12 July 2013

Keywords:

Legumes

Allergy

Transcription factors

Allergic mediators

Thymic stromal lymphopoietin

ABSTRACT

Red kidney bean (*Phaseolus vulgaris* L.) is one the most commonly consumed legumes that requires an in depth understanding of its allergenicity. Therefore, the aim of this study was to explore the allergenicity of red kidney bean proteins following oral exposure in BALB/c mice and elucidate the levels of Th1/Th2 transcription factors induced by red kidney bean proteins in rat basophilic leukemia cells (RBL-2H3 cells) passively sensitized with the sera of red kidney bean sensitized mice. Red kidney bean proteins showed enhanced levels of total and specific IgE, anaphylactic symptoms, thymic stromal lymphopoietin (TSLP) and peritoneal albumin over control. Enhanced release of β -hexosaminidase along with up regulated expressions of GATA-3, STAT-6, T-bet, c-MAF and NFAT were observed in the RBL-2H3 cells exposed with red kidney bean proteins when compared to that of the controls. Taken together, exposure of red kidney bean proteins may cause allergic symptoms in mice and the ambivalent effect on Th2/Th1 transcription factors in RBL-2H3 cells.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Food, especially legume allergy is a raising concern which may cause several health complications ranging from sneezing, rhinitis, bronchial asthma, adipose tissue inflammation and systemic metabolic alterations to life threatening anaphylaxis and in extreme cases death [1,2]. Being a major source of vegetarian proteins, leguminous foods are consumed throughout the world and due to its high consumption; the chances of legume allergy are also very high. Allergenicity of several legumes including peanut, soybean, lentil, chickpea, green gram and red gram have been well studied [3]. But, most of the in depth and mechanistic studies are limited to peanut and soybean, and not explored in several leguminous foods including red kidney bean (*Phaseolus vulgaris* L.), a commonly consumed legume worldwide. Most of the food allergic reactions are caused by IgE mediated immediate type hypersensitivity reactions, but non IgE mediated reactions have also been reported [4]. The late eczematous reaction in the patients of cow milk allergy is a common example of a

non-IgE-mediated food allergy. Recently, mixed type of allergic reactions has also been reported in the several legumes including peanut and chickpea [5,6]. The Th1/Th2 balance plays a very crucial role in the determination of immune reactions. The transcription factors like signal transducer and activator of transcription 6 (STAT-6), trans-acting T-cell-specific transcription factor GATA-3, T-bet, NFAT and transcription factor Maf or c-maf have been found to be actively involved in Th1/Th2 balance [7]. Food allergic reactions generally induce in the individuals with impaired oral tolerance and controlled by several immune molecules including Foxp3 [8,9].

Therefore, the aim of this study was to explore the effect of red kidney bean proteins following oral exposure in BALB/c mice and also to determine effect on Th1/Th2 transcription factors following exposure of red kidney bean proteins in rat basophilic leukemia cells (RBL-2H3 cells).

2. Methods

2.1. Extraction of crude protein extracts

Red kidney bean crude protein extract (abbreviated as red kidney bean) and peanut crude protein extract (abbreviated as peanut) were prepared according to the earlier described method [10].

* Corresponding author. Address: Food, Drug and Chemical Toxicology Group, CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Mahatma Gandhi Marg, P.O. Box No. 80, Lucknow 226 001, India. Fax: +91 522 2628227.

E-mail addresses: sandeep_sant@yahoo.co.in (S. Kumar), pddwivedi@yahoo.com (P.D. Dwivedi).

2.2. Animal

Healthy 6–8 week old female BALB/c mice (22 ± 3 g) were obtained from the animal breeding colony of Gheru campus, CSIR-IITR, Lucknow, India. Animals were maintained under standard laboratory conditions in pathogen free environment and on red kidney bean free diet. Animal studies were carried out after approval of the animal ethics committee of CSIR-IITR, Lucknow.

2.3. Animal treatment protocol

In brief, mice were randomly divided into three groups ($n = 15$ /group). Control, red kidney bean and peanut groups of mice were gavaged daily up to 42 days with phosphate buffer saline or PBS, pH 7.4 (100 μ L), red kidney bean (100 μ g in 100 μ L PBS) and peanut (100 μ g in 100 μ L PBS), respectively. Blood samples were withdrawn from the retro-orbital sinus to estimate serum allergic parameters on day 15th, 43rd and 59th. On day 60th, control, red kidney bean and peanut groups ($n = 10$ /group) were challenged with 1 ml of 5 mg/ml of the respective proteins.

2.4. Serum immunoglobulins

Total IgE levels in the sera of control and red kidney bean treated mice were estimated by OptEIA mouse IgE kit (BD Biosciences, Germany) as per the manufacturer's instructions. Specific IgE levels were estimated according to the earlier described methods [11].

2.5. Anaphylactic symptoms

The clinical symptoms induced after challenge in the control and red kidney bean sensitized mice was observed as per the earlier described scoring system [12]. The core body temperature or rectal temperature was measured before and after the challenge in the control and red kidney bean treated groups using a digital rectal thermometer (Bioseb, France).

2.6. Serum TSLP level

The serum TSLP level was determined in the sera of control and red kidney bean treated mice using an ELISA kit (Ebioscience, Inc. CA, USA) following manufacturers' instructions.

2.7. Vascular permeability assay

Vascular permeability or vascular leakage was studied 40 min after the challenge as per the earlier described method [13]. In brief, 3 ml of PBS, 10 mM EDTA injected into the peritoneal cavity of control as well as red kidney bean treated mice. Following, a 1 min abdominal massage, peritoneal lavage fluids were aspirated, gently. Further, peritoneal lavage fluids were centrifuged at 600 rpm for 6 min at 4 °C and the supernatant was collected. The albumin content in the supernatants was estimated by bicinchoninic acid kit (Thermo Scientific, USA). Albumin levels of control mice challenged with the same amount of red kidney bean was used as background level. Bovine serum albumin (BSA) was used as standard to calculate the concentrations of albumin.

2.8. Histopathology of the intestine

For histopathology, the intestinal tissues of control and red kidney bean treated mice were fixed in 10% formalin in PBS, embedded in paraffin, and cut into 3–5- μ m thick sections.

2.9. Fluorescence histochemistry for Foxp3 level in the intestine

The expression of Foxp3 in the intestine of control and red kidney bean treated and challenged mice was studied with the help of fluorescent histochemistry following the earlier described protocol [14]. Briefly, goat antimouse Foxp3 (Santa Cruz Biotechnology, Inc. USA) in dilution 1:200 was used as primary antibody while anti-goat-Alexa Fluor-488 antibody (Invitrogen, USA) was used as secondary antibody. Prolonged gold antifade reagent with DAPI (Invitrogen, USA) was used as mounting media. The images were captured by confocal microscope (Leica Micosystem, Germany).

2.10. Passive cutaneous anaphylaxis (PCA)

The PCA was carried out according to the previously described method with slight modifications [15]. Briefly, the abdomens of naïve mice were shaved and intradermally injected with 10 μ L of serum from red kidney bean treated and control groups, respectively. Following 48 h of serum administration, each mouse received an intravenous dose of 100 μ L of the PBS and red kidney bean (2.5 μ g/ μ L) in the presence of 0.5% Evans blue dye (Sigma Aldrich, Mo, USA) in PBS. The appearance of wheal was observed within 30 min and a reaction diameter of >3 mm was considered a positive PCA response.

2.11. Serum cytokine levels using flowcytometry

At day 43, the levels of cytokines IL-2, IL-4, IL-6, IL-10, IFN- γ and TNF- α were estimated in the serum of red kidney bean treated and control mice by Th1/Th2 CBA cytokines kit (BD Biosciences, San Jose, California) using flowcytometry (BD FACS Canto II).

2.12. Priming and sensitization of RBL-2H3 cells

Rat basophilic leukemia cells (RBL-2H3 cells) were obtained from American type cell culture (ATCC, Manassas, VA). The priming of RBL-2H3 cells with the sera (dilution 1:10) of red kidney bean sensitized mice was carried out according to the earlier described method [16]. The cells were challenged with different concentrations (20, 40, 60, 80, 100 and 120 μ g) of red kidney bean for 90 min. The control group was challenged either with PBS or lysis buffer (0.1% Triton-X 100). The plate was put on the ice to terminate the ongoing reactions. The supernatant were kept for the estimation of β -hexosaminidase release assays.

2.13. β -hexosaminidase release assay

The release of β -hexosaminidase was estimated according to the earlier described protocol [17]. In brief, 75 μ L from aforesaid supernatants and substrate buffer for β -hexosaminidase (1.2 mM 4-methylumbelliferyl-N-acetyl- β -D-glucosaminide in 0.05 M CH₃COONa, pH 4.4) were mixed in a 96 well plate, and incubated at 37 °C for 90 min. The quenching buffer (0.1 M Glycine, 0.1 M Na₂CO₃, pH 10.0) was used to stop the reaction. The observance was taken at 405 nm (ELISA plate reader, Biotek, Power Wave XS2). The percentage of β -hexosaminidase release was calculated as per the formula given below:

$$\% \text{Release of } \beta\text{-hexosaminidase} = \frac{\{(\text{Sample } \beta\text{-hexosaminidase}) - (\text{Background } \beta\text{-hexosaminidase})\}}{\{(\text{Total } \beta\text{-hexosaminidase}) - (\text{Background } \beta\text{-hexosaminidase})\}} \times 100. \quad (1)$$

2.14. Immunofluorescence for transcription factors

The expressions of Th1/Th2 transcription factors GATA-3, STAT-6, T-bet, c-MAF and NFAT were observed by immunofluorescence

according to the protocol described online [18]. In brief, RBL-2H3 cells (1×10^6 cells) were primed and challenged with a dose of 100 μ g red kidney bean as per described in the above section. Formaldehyde was used to fix the cells while 5% goat serum was used for blocking the nonspecific sites. The antibodies for GATA-3, STAT-6, c-MAF, NFAT and T-bet were obtained from the BD Biosciences, San Jose, California and Santa Cruz biotechnology, Inc. Santa Cruz, CA, respectively. These antibodies were used as primary antibodies (dilution 1:200) while Alexa Fluor® 488 Goat anti-Mouse IgG (Invitrogen, Carlsbad, CA, USA) was used as secondary antibody. Further, Prolong gold antifade reagent with 4', 6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI; Invitrogen, Carlsbad, CA, USA) was used as mounting media. Images were captured by confocal microscope (Leica Microsystems, Germany).

2.15. Western blots for transcription factors

The levels of Th1/Th2 transcription factors GATA-3, STAT-6 (BD Biosciences, San Jose, California), T-bet, NFAT and c-MAF (Santa Cruz Biotechnology, Inc. Santa Cruz, CA) in RBL-2H3 cells were detected by immunoblotting [19]. The β -tubulin (Santa Cruz Biotechnology, Inc. Santa Cruz, CA) was used as internal control.

2.16. Statistical analysis

The statistical significance of the data obtained was determined using a software package from InStat version 3.0 (Graph pad, San Diego, CA, USA; <http://www.graphpad.com>) using the Bonferroni analysis of variance (ANOVAs) test. Values for all measurements have been expressed as means $\pm$ SEM. Differences between groups were considered significant when $p < 0.05$.

3. Results

3.1. Allergic symptoms in BALB/c mice following oral exposure of red kidney proteins

The level of total IgE was found significantly increased on 15 ($p < 0.01$), 43 ($p < 0.001$) and 59 days ($p < 0.01$) in the sera of red kidney bean treated mice over control mice (Fig. 1A). Further, the levels of specific IgE were also found significantly enhanced in 15 ($p < 0.05$), 43 ($p < 0.001$) and 59 ($p < 0.01$) days in the sera of red kidney bean treated mice over control (Fig. 1B). The mice sensitized and challenged with red kidney bean showed scratching around the nose and mouth (anaphylactic score 2; 30%), forced respiration (score 3, 30%); and 30% showed symptoms of score 4, like, severe diarrhea including unconsciousness or negligible response despite the gentle touch and score 5 in 10% (Fig. 1C). A decrease of 2–3 $^{\circ}$ C in the rectal temperature of red kidney bean sensitized and challenged mice was noticed after 40 min of challenge while control mice failed to show any decrease in the rectal temperature (Fig. 1D). Enhanced level ($p < 0.05$) of the peritoneal albumin was observed in red kidney bean treated and challenged mice as compared to control (Fig. 1E). Furthermore, significant ($p < 0.001$) increase in the serum TSLP level was found in red kidney bean treated mice when compared to that of control (Fig. 1F).

3.2. Foxp3 level and histopathology of the intestine

An insignificant increase in the mean intensity of the intestinal Foxp3 level was found in red kidney bean treated group when compared to the control (Fig. 2A). Further, the histopathological studies of the intestine of red kidney bean treated and challenged mice explored exfoliations in the intestinal wall as well as infiltration of leukocytes (Fig. 2B).

3.3. Positive PCA against red kidney beans

The appearance of a blue wheal at the site of intra-dermal serum injection was observed within 30 min following antigen challenge, and a reaction diameter of 3.9 mm was observed in red kidney bean treated naïve mice, while no spot was found at the site of post intra-dermal injection in the control (Fig. 2C).

3.4. Red kidney bean exposure demonstrated higher cytokines secretion

Significantly enhanced levels of IL-4 ($p < 0.05$), IL-6 ($p < 0.001$), IL-10 ($p < 0.05$), IFN- γ ($p < 0.05$) and TNF- α ($p < 0.01$) were observed in red kidney bean exposed group over the control group (Fig. 3A–E).

3.5. Enhanced mediator release and transcription factors in RBL-2H3 cells

A significant ($p < 0.05$) release of β -hexosaminidase was observed following exposure of different doses of red kidney bean in RBL-2H3 cells. The percentage release were 58, 64, 68, 72, 75 and 74% in 20, 40, 60, 80, 100 and 120 μ g red kidney bean exposed group, respectively (Fig. 4A). Further, the significantly enhanced mean intensities of GATA-3 ($p < 0.001$), STAT-6 ($p < 0.001$), c-MAF ($p < 0.01$), T-bet ($p < 0.001$) and NFAT ($p < 0.05$) were found in red kidney bean treated group when compared to that of the controls (Fig. 4B–F). Furthermore, western blot analysis showed significantly enhanced levels of STAT-6 ($p < 0.05$), GATA-3 ($p < 0.001$), T-bet ($p < 0.05$), c-MAF ($p < 0.001$) and NFAT ($p < 0.05$) in red kidney bean treated RBL-2H3 cells when compared to control (Fig. 5).

4. Discussion

Most of the food allergy reactions have been reported to cause by “Big Eight” foods that include peanut, tree nut, soybean, egg, wheat, crustaceans, fish and milk. About 25% most allergic foods come from leguminous foods. The red kidney bean has been reported to cause allergic reactions in BALB/c mice and allergic rhinitis patients [11]. Previously, red kidney bean allergenicity was assessed in BALB/c mice using the intraperitoneal route of sensitization [11]. Since, oral route is the natural route, which is not comparable to other modes of exposure owing to distinct mechanisms of tolerance/allergenicity, therefore, in this study, oral exposure of red kidney bean was administered to the BALB/c mice to understand the mechanism of sensitization following oral route of exposure.

The approach of this study was very similar to that of the mouse model of rice and egg allergies with simple oral sensitization without the use of adjuvant [20,21]. In the present study, along with total and specific IgE, clinical symptoms were also found enhanced that are frequently reported parameters in food allergy. Similar outcomes like higher levels of serum anti-ovalbumin IgE and anaphylaxis after oral challenge have also been reported in OVA allergy [21,22]. The results obtained following oral exposure of red kidney bean in BALB/c mice showed similar outcomes of elevated total and specific IgE, systemic anaphylaxis score and rectal temperature but, to a lesser extent.

The level of the peritoneal albumin is an *in vivo* marker of mast cell degranulation and vascular permeability during the anaphylaxis reactions [13,23]. In this study, increased level of peritoneal albumin in the red kidney bean treated group indicated the increased vascular leakage in the intestine and severity of anaphylaxis. Such observation has been reported in the various cases of allergies [24].

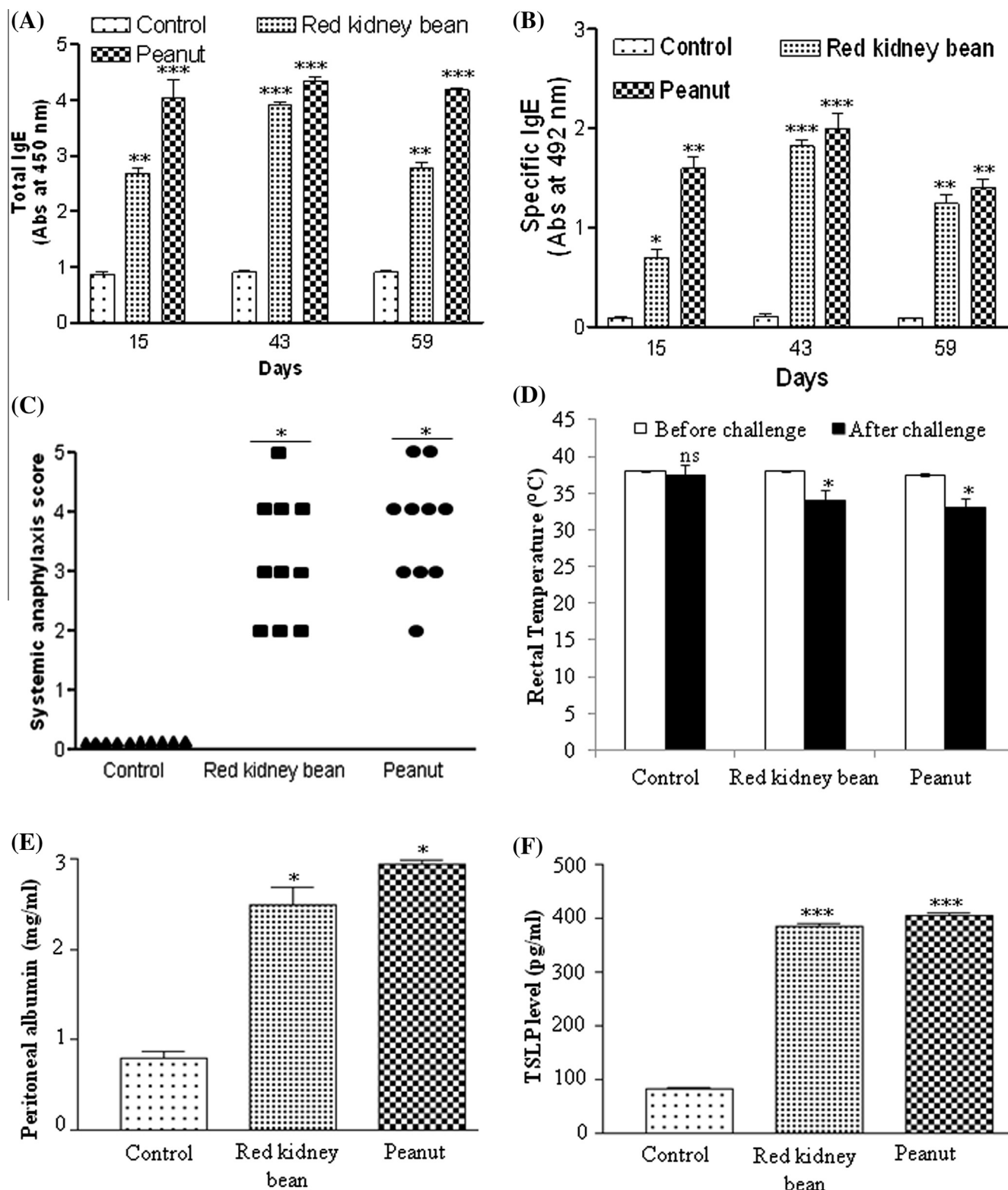


Fig. 1. Immunoglobulin levels and anaphylaxis in mice. (A) Total IgE (B) specific IgE (C) systemic anaphylaxis score (D) rectal temperature (E) peritoneal albumin and (F) TSLP levels in the red kidney bean treated and control mice (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Recent studies have reported that TSLP is involved in the initiation of Th2 differentiation as well as in allergic inflammatory responses [25]. Further, TSLP has been found to be expressed by epithelial cells at barrier surfaces, activated bronchial smooth muscle cells, and activated mast cells in allergic subjects [26]. In this study, the increase amount of TSLP in red kidney bean treated

group indicated the severity of allergy induced by red kidney bean oral exposure.

The prevalence of food allergy is limited to the susceptible individuals because of the several factors including the impaired oral tolerance. The oral tolerance has been reported to be linked with the expression of CD4 + CD25⁺ Treg cells [27]. The depletion of

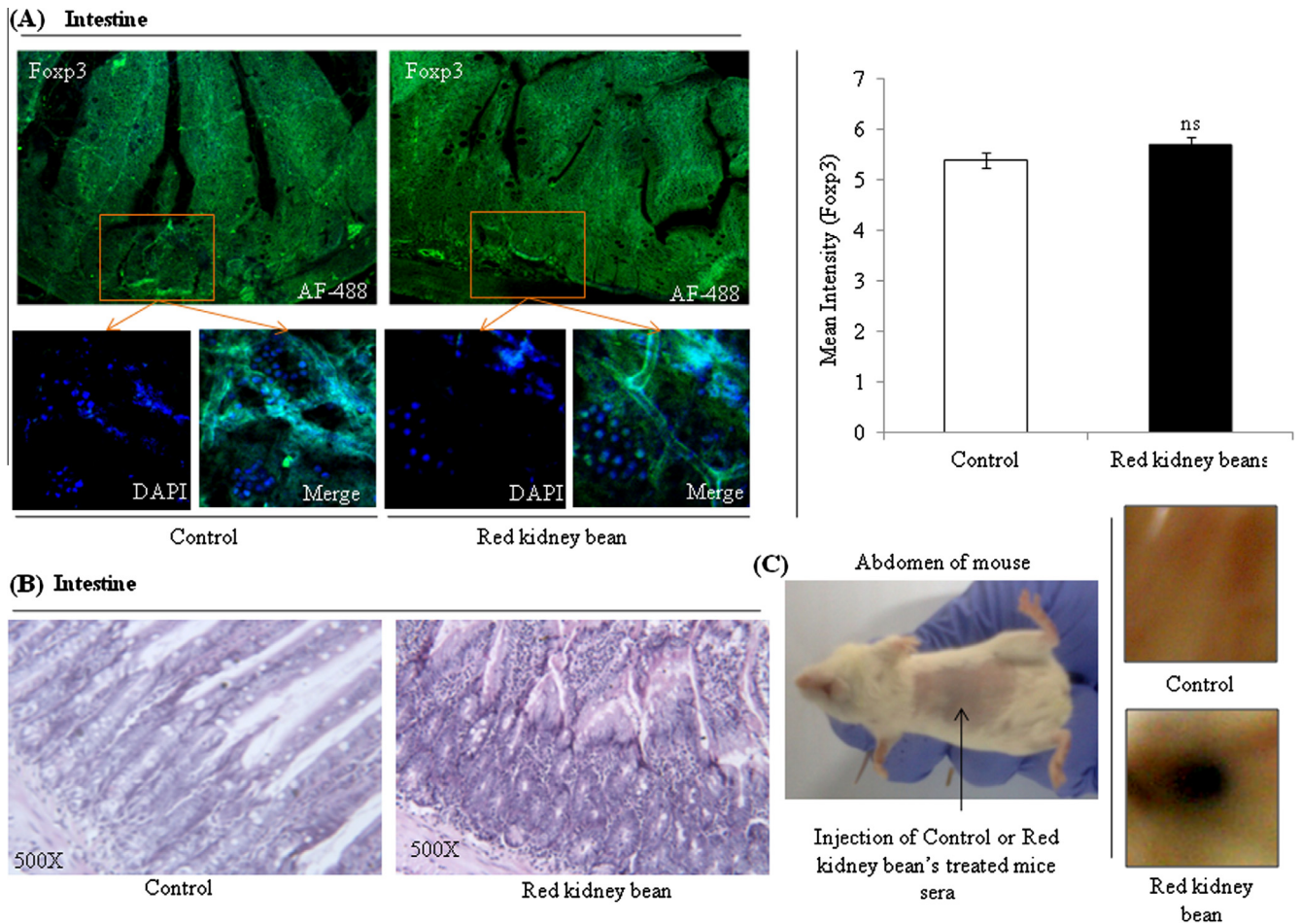


Fig. 2. Foxp3, histopathology and passive cutaneous anaphylaxis. (A) Level of intestinal Foxp3 (B) histopathology of intestine and (C) passive cutaneous anaphylaxis in red kidney bean treated and control mice (ns = nonsignificant). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

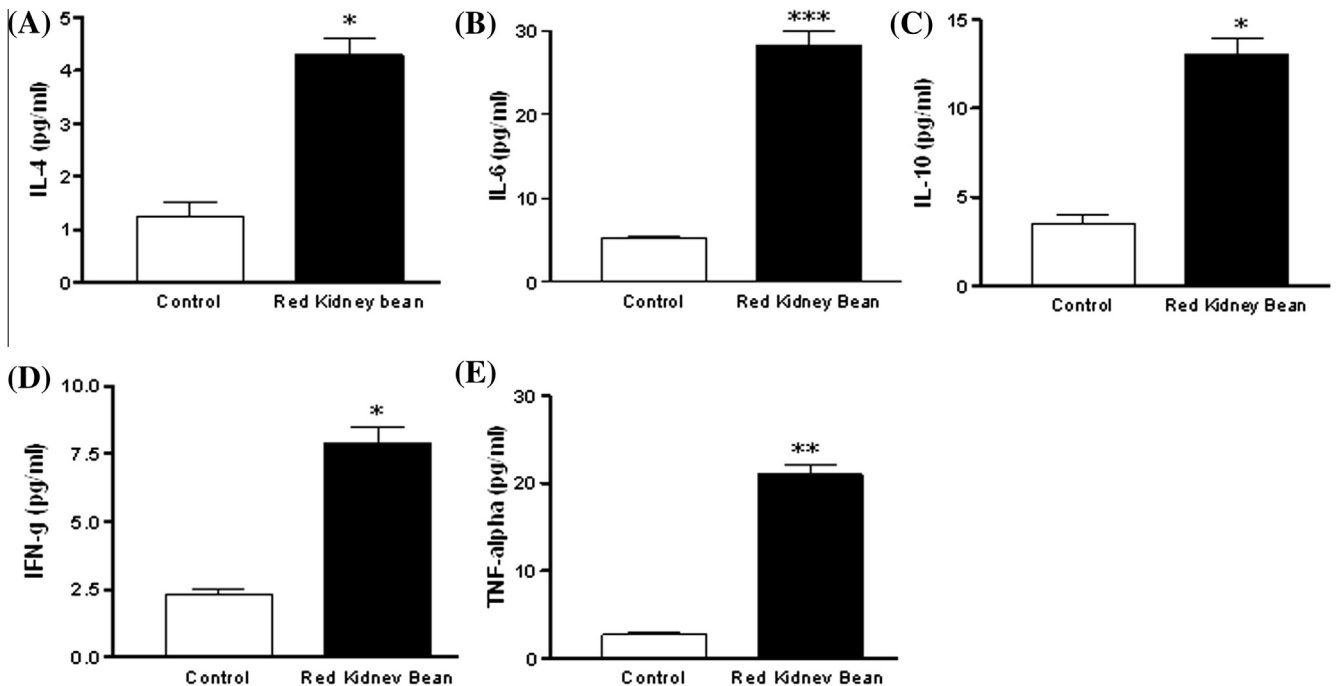


Fig. 3. Cytokines in the serum. (A) IL-4 (B) IL-6 (C) IL-10 (D) IFN-gamma and (E) TNF-alpha levels in the sera of red kidney bean treated and control mice (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

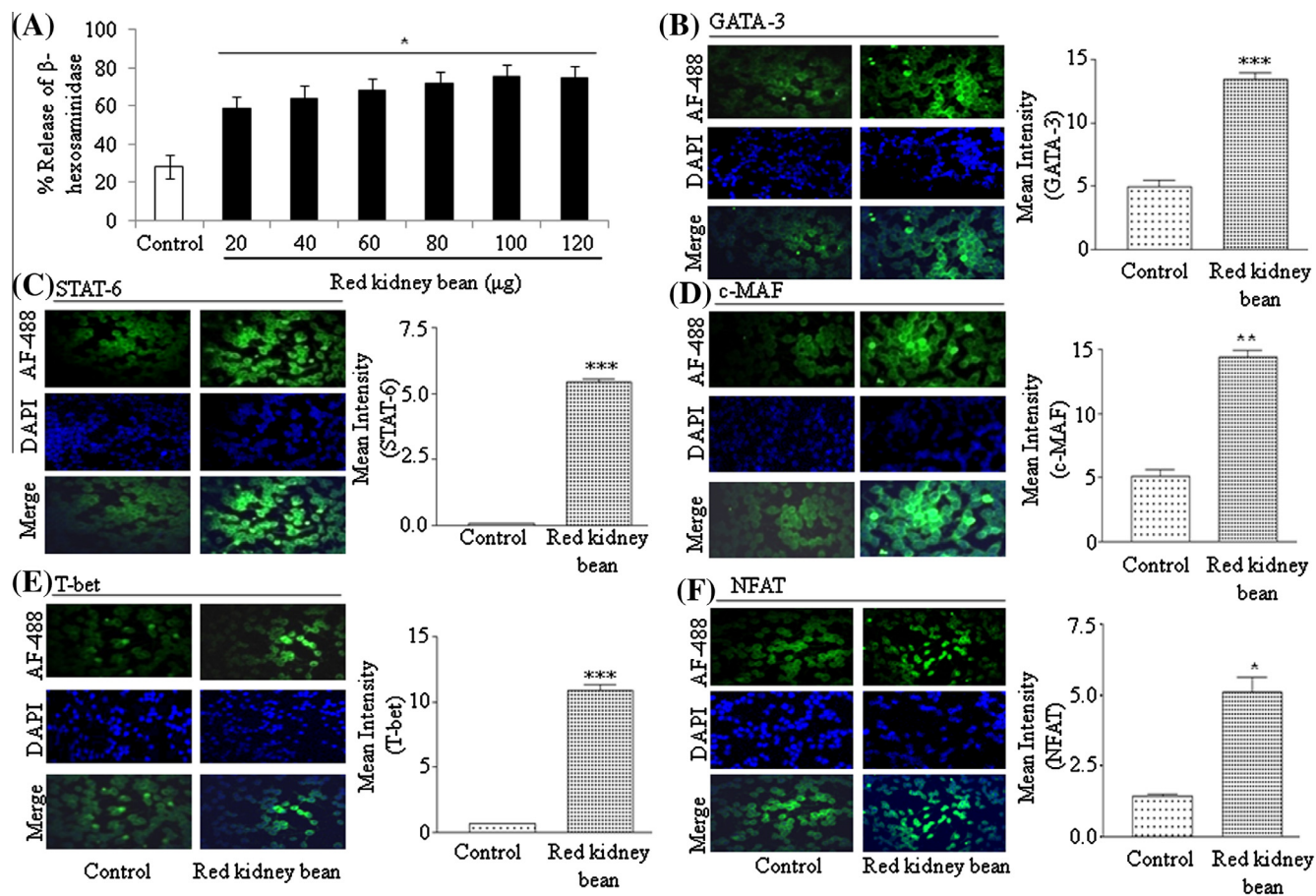


Fig. 4. Mediator release and Th1/Th2 transcription factors in RBL-2H3 cells. (A) Release of β -hexosaminidase (B) expressions of GATA-3 (C) STAT-6 (D) c-MAF (E) T-bet and (F) NFAT in control and red kidney bean treated cells, respectively. Alexa fluor-488 goat antimouse IgG (AF-488) was used as secondary antibody while DAPI was used for nuclear staining. Merged image is indicating both AF-488 and DAPI staining (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

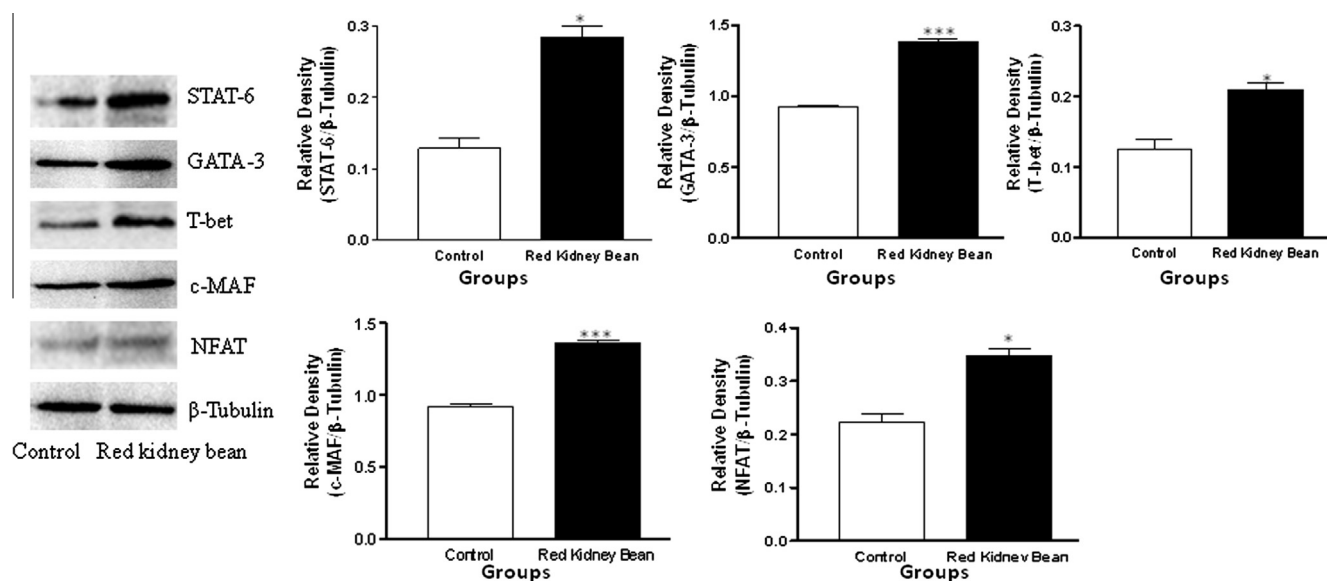


Fig. 5. Western blots for transcription factors in RBL-2H3 cells. The figure demonstrates Western blot result of STAT-6, GATA-3, T-bet, c-MAF, and NFAT along with the respective densitometry (* $p < 0.05$; *** $p < 0.001$).

CD4 + CD25+ Treg cells during sensitization to peanut increases allergen-specific antibody levels and mast cell degranulation after oral challenge with peanut in mice [27,28]. In our study, the intestine of red kidney bean treated mice showed a non significant in-

crease of Foxp3 level. This result showed the possibility of red kidney bean induced allergenicity because, the level of Foxp3 has been reported to be enhanced in partial or complete tolerated mice in case of whey [29].

PCA is used to observe the immediate type allergic reactions in several foods [30]. In this study, a significant increase was observed in the skin of naïve mice indicating the possibility of IgE mediated reactions in red kidney bean treated and sensitized mice. Similarly, in an adjuvant free mouse model of oral allergenic sensitization for rice seed protein, the PCA reaction has been found positive in the treated group [31].

Cytokines are very important molecules involved in the prevalence of allergic reactions. In this study the levels of cytokines IL-4, IL-6, and IL-10 were found elevated in the sera of red kidney bean treated mice. The enhanced levels of IL-4, IL-6, and IL-10 have been reported to induce the allergic and inflammatory responses [32]. Further, the levels of IFN- γ and TNF- α were found enhanced in the sera of red kidney bean treated mice. It has been reported that IFN- γ and TNF- α activate mast cells and induces release of allergic mediators [33].

RBL-2H3 cells are one of the pivotal tools to elaborate the mast cells/basophils degranulation potential induced by allergens [34]. When allergens come in contact with IgE primed mast cells it gets cross-linked to the two IgE. Following this, a cascade of signaling events takes place in the mast cells/basophils that ultimately leads to degranulation of the cells and release of several mediators including β -hexosaminidase [35]. In this study, passively sensitized RBL-2H3 cells following exposure of red kidney bean showed enhanced release of β -hexosaminidase. The release of β -hexosaminidase indicates the mast cells/basophils degranulation potential of red kidney bean.

A transcription factor is a protein which binds to specific DNA sequences, and regulates genetic information from DNA to mRNA [36,37]. Transcription factors like STAT-6, GATA-3, NFAT, c-MAF, and T-bet have been reported to be actively involved in Th2/Th1 balance [7]. Higher expressions of STAT-6, GATA-3, NFAT, T-bet and c-MAF in red kidney bean treated RBL-2H3 cells indicated the prevalence of mixed type of allergic reactions. It has been reported that STAT6 is a key transcription factor required for antigen-mediated production of cytokines in mast cells [38]. In one of the study carried out by Ishijima et al. (2011), it has been reported that GATA factors directly activate PLC- γ 1 gene transcription through a conserved GATA-binding motif that resides in the 5'-upstream sequence [39]. The role of PLC- γ is well studied during the mast cell degranulation [40]. The IL-4 gene transcription is critically regulated by c-MAF [41]. NFAT transcription factors have been reported to play an important role in the allergic events especially in Ca⁺⁺ signaling during the degranulation of mast cells [42]. Han et al. (2011) studied antiallergic properties of *Psidium guajava* ethyl acetate extract on IgE-mediated allergic responses in RBL-2H3 cells and found that the extract suppressed antigen-induced activation of NFAT that ultimately lead to reduced production of Th2 cytokines and Fc ϵ RI-dependent signaling events in mast cells [43]. The increased level of T-bet expression indicated toward the possibility of Th1 reactions along with Th2 induced by red kidney bean proteins. This may be because of the presence of amylase inhibitors or lectins in red kidney bean [44]. The phytohemagglutinins of red kidney bean may induce the expression of T-bet found in the study carried out in the bone marrow derived mast cells (BMMCs) [45]. Taking together, the outcomes of the different transcription factors in the red kidney bean treated RBL-2H3 cells indicated an environment positive to allergic reactions.

5. Conclusion

In summary, oral exposure of red kidney bean in BALB/c mice led to the enhanced levels of total and specific IgE, anaphylactic symptoms, TSLP, serum cytokines and albumin levels. Further, *in vitro* study using RBL-2H3 cells demonstrated an enhanced re-

lease of β -hexosaminidase along with up regulated levels of transcription factors GATA-3, STAT-6, T-bet, c-MAF and NFAT indicating mixed fate of allergic reactions. Taken together, red kidney bean may induce allergic symptoms both in the *in vivo* and *in vitro* conditions.

Conflict of interest

The authors have declared that there is no conflict of financial interest.

Acknowledgments

We are grateful to the Director of the Institute for his keen interest in this study. This work was financially supported by the Network Project InDepth (BSC 0111) of Council of Scientific and Industrial Research (CSIR), New Delhi. Thanks are due to Pradeep K Srivastva, PhD, Gorakhpur, U. P. for his editorial assistance. SK, AKV and AS are thankful to CSIR, New Delhi for their Senior and Junior Research Fellowships, respectively. AS and AKV convey their gratitude to Academy of Scientific and Innovative Research, New Delhi. This is a CSIR-IITR manuscript number #3102.

References

- [1] J. Wang, H.A. Sampson, Food allergy, *J. Clin. Invest.* 121 (2011) 827–835.
- [2] L.P. Dourado, M.L. Novello, D.M. Alvarenga, Z. Menezes, D.A. Perez, N.V. Batista, et al., Experimental food allergy leads to adipose tissue inflammation, systemic metabolic alterations and weight loss in mice, *Cell. Immunol.* 270 (2) (2011) 198–206.
- [3] A.K. Verma, S. Kumar, M. Das, P.D. Dwivedi, A Comprehensive review of legume allergy, *Clin. Rev. Allergy Immunol.* (2012), <http://dx.doi.org/10.1007/s12016-012-8310-8>.
- [4] J. Noh, G. Noh, H.S. Kim, A.R. Kim, W.S. Choi, Allergen-specific responses of CD19(+)CD5(+)Foxp3(+) regulatory B cells (Bregs) and CD4(+)Foxp3(+) regulatory T cell (Tregs) in immune tolerance of cow milk allergy of late eczematous reactions, *Cell. Immunol.* 274 (1–2) (2012) 109–114.
- [5] F. van Wijk, S. Hartgring, S.J. Koppelman, R. Pieters, L.M. Knippen, Mixed antibody and T cell responses to peanut and the peanut allergens Ara h 1, Ara h 2, Ara h 3 and Ara h 6 in an oral sensitization model, *Clin. Exp. Allergy* 34 (9) (2004 Sep) 1422–1428.
- [6] A.K. Verma, S. Kumar, A. Tripathi, B.P. Chaudhari, M. Das, P.D. Dwivedi, Chickpea (*Cicer arietinum*) proteins induce allergic responses in nasobronchial allergic patients and BALB/c mice, *Toxicol. Lett.* 210 (1) (2012 Apr 5) 24–33.
- [7] S. Kumar, A.K. Verma, M. Das, P.D. Dwivedi, Molecular mechanisms of IgE mediated food allergy, *Int. Immunopharmacol.* 13 (4) (2012 Aug) 432–439.
- [8] J. Noh, J.H. Lee, G. Noh, S.Y. Bang, H.S. Kim, W.S. Choi, et al., Characterisation of allergen-specific responses of IL-10-producing regulatory B cells (Br1) in cow milk allergy, *Cell. Immunol.* 264 (2) (2010) 143–149.
- [9] J. Noh, G. Noh, S.J. Lee, J.H. Lee, A. Kim, H.S. Kim, W.S. Choi, Tolerogenic effects of interferon-gamma with induction of allergen-specific interleukin-10-producing regulatory B cell (Br1) changes in non-IgE-mediated food allergy, *Cell. Immunol.* 273 (2) (2012 Jan) 140–149.
- [10] J.D. Astwood, J.N. Leach, R.L. Fuchs, Stability of food allergens to digestion *in vitro*, *Nat. Biotechnol.* 14 (1996) 1269–1273.
- [11] S. Kumar, A.K. Verma, A. Misra, A. Tripathi, B.P. Chaudhari, R. Prasad, et al., Allergic responses of red kidney bean (*Phaseolus vulgaris* cv chitra) polypeptides in BALB/c mice recognized by bronchial asthma and allergic rhinitis patients, *Food Res. Int.* 44 (9) (2011) 2868–2879.
- [12] X.M. Li, B.H. Schofield, C.K. Huang, G.I. Kleiner, H.A. Sampson, A murine model of IgE-mediated cow's milk hypersensitivity, *J. Allergy Clin. Immunol.* 103 (1999) 206–214.
- [13] J. Sun, K. Arias, D. Alvarez, R. Fattouh, T. Walker, S. Goncharova, et al., Impact of CD40 ligand, B cells, and mast cells in peanut-induced anaphylactic responses, *J. Immunol.* 179 (10) (2007 Nov) 6696–6703.
- [14] R.P. Kirwan, R.J. Wordinger, A.F. Clark, C.J. O'Brien, Differential global and extra-cellular matrix focused gene expression patterns between normal and glaucomatous human lamina cribrosa cells, *Mol. Vis.* 15 (2009) 76–88.
- [15] M. Husain, H.J. Boermans, N.A. Karrow, Mesenteric lymph node transcriptome profiles in BALB/c mice sensitized to three common food allergens, *BMC Genomics* 12 (12) (2011), <http://dx.doi.org/10.1186/1471-2164-12-12>.
- [16] S.C. Diesner, R. Knittelfelder, D. Krishnamurthy, I. Pali-Schöll, L. Gajdzik, E. Jensen-Jarolim, et al., Dose-dependent food allergy induction against ovalbumin under acid-suppression: a murine food allergy model, *Immunol. Lett.* 121 (1) (2008 Nov) 45–51.
- [17] F. Blanc, K. Adel-Patient, M.F. Drumare, E. Paty, J.M. Wal, H. Bernard, Capacity of purified peanut allergens to induce degranulation in a functional *in vitro*

- assay: Ara h 2 and Ara h 6 are the most efficient elicitors, *Clin. Exp. Allergy* 39 (2009) 1277–1285.
- [18] Immunofluorescence General Protocol 12/8/2012. Available from: <<http://www.cellsignal.com/support/protocols/IF.html>>.
- [19] H. Towbin, T. Staehelin, J. Gordon, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, *Proc. Natl. Acad. Sci. USA* 76 (1979) 4350–4354.
- [20] X.W. Chen, K.W. Lau, F. Yang, S.S. Sun, M.C. Fung, An adjuvant free mouse model of oral allergenic sensitization to rice seeds protein, *BMC Gastroenterol.* (2011) 11(62), doi: 10.1186/1471-230X-11-62.
- [21] L.P. Dourado, J.C. Saldanha, D.L. Gargiulo, M.L. Noviello, C.C. Brant, M.L. Reis, Role of IL-4 in aversion induced by food allergy in mice, *Cell. Immunol.* 262 (1) (2010 Jan) 62–68.
- [22] P. Rupa, Y. Mine, Oral immunotherapy with immunodominant T-cell epitope peptides alleviates allergic reactions in a Balb/c mouse model of egg allergy, *Allergy* 67 (1) (2012 Jan) 74–82.
- [23] J. Bennett, S. Maltby, E. Frohwerk, K. Jian, H. Merckens, M. Tunis, et al., Adhesion molecules in experimental peanut allergy, *Allergy Asthma Clin. Immunol.* 6 (Suppl. 3) (2010) P10, <http://dx.doi.org/10.1186/1710-1492-6-S3-P10>.
- [24] N. Schmitz, K. Dietmeier, M. Bauer, M. Maudrich, S. Utzinger, S. Muntwiler, et al., Displaying Fel d1 on virus-like particles prevents reactogenicity despite greatly enhanced immunogenicity: a novel therapy for cat allergy, *J. Exp. Med.* 206 (2009) 1941–1955.
- [25] S. Jang, S. Morris, N.W. Lukacs, TSLP promotes induction of Th2 differentiation but is not necessary during established allergen-induced pulmonary disease, *PLoS One* 8 (2013) e56433, <http://dx.doi.org/10.1371/journal.pone.0056433>.
- [26] S.F. Ziegler, D. Artis, Sensing the outside world: TSLP regulates barrier immunity, *Nat. Immunol.* 11 (2010) 289–293.
- [27] V.J. Schulz, J.J. Smit, K.J. Willemsen, D. Fiechter, I. Hassing, R. Bleumink, L. Boon, et al., Activation of the aryl hydrocarbon receptor suppresses sensitization in a mouse peanut allergy model, *Toxicol Sci.* 123 (2011) 491–500.
- [28] F. Van Wijk, E.J.M. Wehrens, S. Nierkens, L. Boon, A. Kasran, R. Pieters, et al., CD4+CD25+ T cells regulate the intensity of hypersensitivity responses to peanut, but are not decisive in the induction of oral sensitization, *Clin. Exp. Allergy* 37 (2007) 572–581.
- [29] B.C. van Esch, B. Schouten, S. de Kivit, G.A. Hofman, L.M. Knippels, L.E. Willemsen, Oral tolerance induction by partially hydrolyzed whey protein in mice is associated with enhanced numbers of Foxp3+ regulatory T-cells in the mesenteric lymph nodes, *Pediatr. Allergy Immunol.* 22 (8) (2011 Dec) 820–826.
- [30] C. Herouet-Guicheney, H. Aldemir, R. Bars, D. de Barbeyrac, P. Kennel, D. Rouquie, Inter-laboratory comparisons of assessment of the allergenic potential of proteins in mice, *J. Appl. Toxicol.* 29 (2) (2009) 141–148.
- [31] X.W. Chen, K.W. Lau, F. Yang, S.S. Sun, M.C. Fung, An adjuvant free mouse model of oral allergenic sensitization to rice seeds protein, *BMC Gastroenterol.* 11 (2011).
- [32] C. Ozdemir, M. Akdis, C.A. Akdis, T-cell response to allergens, *Chem. Immunol. Allergy* 95 (2010) 22–44.
- [33] D. Metcalf, D. Baram, Y.A. Mekori, Mast cells, *Physiol. Rev.* 77 (1997) 1033–1079.
- [34] Y. Song, C. Qu, K. Srivastava, N. Yang, P. Busse, W. Zhao, et al., Food allergy herbal formula 2 protection against peanut anaphylactic reaction is via inhibition of mast cells and basophils, *J. Allergy Clin. Immunol.* 126 (2010) 1208–1217.e3.
- [35] N. King, R. Helm, J.S. Stanley, S. Vieths, D. Lüttkopf, L. Hatahet, Allergenic characteristics of a modified peanut allergen, *Mol. Nutr. Food. Res.* 49 (10) (2005 Oct) 963–971.
- [36] D.S. Latchman, Transcription factors: an overview, *Int. J. Biochem. Cell Biol.* 29 (1997) 1305–1312.
- [37] M. Karin, Too many transcription factors: positive and negative interactions, *New Biol.* 2 (1990) 126–131.
- [38] R. Malaviya, F.M. Uckun, Role of STAT6 in IgE receptor/FcεRI-mediated late phase allergic responses of mast cells, *J. Immunol.* 168 (2002) 421–426.
- [39] Y. Ishijima, S. Ohmori, A. Uenishi, K. Ohneda, GATA transcription factors are involved in IgE-dependent mast cell degranulation by enhancing the expression of phospholipase C-γ1, *Genes Cells* 17 (2012) 285–301.
- [40] D.D. Metcalfe, R.D. Peavy, A.M. Gilfillan, Mechanisms of mast cell signaling in anaphylaxis, *J. Allergy Clin. Immunol.* 124 (2009) 639–646.
- [41] Y. Yang, J. Ochando, A. Yopp, S. Jonathan, Y.D. Bromberg, IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4 T cell activation, *J. Immunol.* 174 (2005) 2720–2729.
- [42] N. Hermann-Kleiter, G. Baier, NFAT pulls the strings during CD4+T helper cell effector functions, *Blood* 115 (2010) 2989–2997.
- [43] E.H. Han, Y.P. Hwang, H.G. Kim, J.H. Park, J.H. Choi, J.H. Im, et al., Ethyl acetate extract of *Psidium guajava* inhibits IgE-mediated allergic responses by blocking FcεRI signaling, *Food Chem. Toxicol.* 49 (2011) 100–108.
- [44] S. Kumar, A.K. Verma, M. Das, S.K. Jain, P.D. Dwivedi, Clinical complications of kidney bean (*Phaseolus vulgaris* L.) consumption, *Nutrition* 29 (2013) 821–827.
- [45] S. Kumar, A.K. Verma, A. Sharma, D. Kumar, A. Tripathi, B.P. Chaudhari, et al., Phytohemagglutinins augment red kidney bean (*Phaseolus vulgaris* L.) induced allergic manifestations, *J. Proteomics* (2013) doi:pii: S1874-3919(13)00065-1. 10.1016/j.jprot.2013.02.003.