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Role of IL-4 in aversion induced by food allergy in mice

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ABSTRACT

To ascertain the role of IL-4 in aversion to antigen induced by food allergy, wild type and IL-4 deficient BALB/c mice were sensitized with ovalbumin and challenged orally with egg white. Sensitized wild type mice had increased production of IL-4 by spleen and mesenteric lymph node cells in vitro, higher levels of serum anti-ovalbumin IgE and IgG1, aversion to ingestion of the antigen and loss of body weight after continuous oral challenge. Intestinal changes in wild type sensitized mice included eosinophil infiltration and increased mucus production. The IL-4 deficiency impaired the development of food allergy and the aversion to antigen, suggesting the involvement of the antigen specific antibodies. When IL-4 deficient mice received serum from sensitized wild type donors, the aversion was restored. These results indicate that production of IL-4 and specific IgE/IgG1 antibodies correlate with aversion to antigen induced by food allergy in mice.

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1. Introduction

Physiological penetration of dietary macromolecules into the organism through the gut mucosa has several recognized immunological consequences. Intestinal IgA and mucus secretion create selective filters to the penetration of such macromolecules and may play a decisive role in the operation of the immune system [1].

Food allergy is defined as an adverse immunological response (hypersensitivity) to food proteins [1]. Food allergy with acute onset of symptoms after ingestion is usually mediated by IgE. IgE-mediated response to an allergen results from a series of molecular and cellular interactions. Antigen presenting cells (APCs) present small peptide fragments in conjunction with MHC class II molecules to CD4⁺ T cells. This interaction leads to T cell proliferation and cytokine secretion, which promotes a Th2 type response. In turn, Th2 cells and their products (IL-4 and IL-13) interact with antigen-specific B cells, leading to isotype switching and the gener-

ation of antigen-specific IgE, which then binds to surface receptors on mast cells, resulting in a state termed sensitization. Upon re-exposure, food sensitizing proteins bind to IgE specific molecules and trigger the release of mediators such as histamine from mast cells, which cause allergy symptoms [2].

Epidemiological studies suggest a significant increase in allergic disease prevalence during the past two or three decades [3]. Food allergy is particularly frequent among children and may affect their final height and weight [4].

Mouse models of food allergy allow in vivo studies of the immunological components involved in the different features of the disease. We have developed an experimental food allergy model in which ovalbumin (Ova)-sensitized mice are given egg white solution (EWS) as the only liquid source. Ingestion of this solution by the sensitized mice leads to increased anti-Ova IgE and IgG1 production, intestinal edema and eosinophil infiltration in the jejunum. This model reproduces the clinical and pathological changes associated with chronic food allergy and is useful for mechanistic studies of this common clinical condition [5].

Previous studies from our group have demonstrated that given the option of drinking water or a sweetened egg white solution,

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ovalbumin (Ova)-sensitized mice displayed an aversion to ingestion of egg white, in contrast to control animals. Moreover, the aversive behavior can be acquired by control non-sensitized mice after transfer of spleen cells from sensitized animals. These results led us to conclude that immunological components can influence diet selection [6,7]. Aversion was later shown to be directly related to IgE production [8].

Many cytokines are involved in the development of allergy. Increased IL-4 production has already been demonstrated in atopic diseases. In a murine model of food allergy induced to shrimp tropomyosin, IL-4 and IL-13 production were induced in the spleen and in the jejunum of sensitized mice [9]. Allergen-specific T cells can be isolated from blood, skin, and mucosal sites in patients with food allergy and, characteristically, they express a Th2 cell phenotype releasing IL-4, IL-5, and IL-13. As mentioned above, IL-4 induces IgE production by B cells. This cytokine is also involved in other allergy-associated events such as development of Th2 cells and eosinophil infiltration of the gut mucosa [2].

The aim of this present study was to evaluate whether the production of IL-4 and its correlation with the secretion of specific antibodies can contribute to aversion to antigen ingestion and to the development of chronic ovalbumin-induced food allergy in mice.

2. Methods

2.1. Mice

Female (6 weeks old) wild type and IL-4 deficient (IL-4^{-/-}) BALB/c mice were used. Wild type mice were obtained from our animal facility at ICB/UFMG. Deficient mice were bred at the Laboratory of Gnotobiology and Immunology, Departamento de Bioquímica e Imunologia, ICB, UFMG. All mice received standard (Purina) mouse chow throughout the experiment. The investigations were consistent with the Ethical Principles in Animal Experimentation, adopted by the Ethics Committee in Animal Experimentation of our Institution (CETEA/UFMG).

2.2. Mice sensitization

On day 0, the mice received a subcutaneous (sc) injection of 0.2 ml saline containing 10 µg Ova (five times crystallized hen's egg albumin; Sigma, St. Louis, MO, USA) plus 1 mg Al(OH)₃ as adjuvant. A second sc injection of 10 µg soluble Ova was given 14 days later (day 14). Seven days after the secondary sensitization (day 21), the bottle containing tap water was replaced with 20% EWS (egg white solution) for a period of 1 or 2 weeks (from day 21 to day 28 or 35). This solution contained about 10 mg Ova/ml. The non-sensitized group received only the adjuvant diluted in saline on day 0 and saline on day 14. Donors of serum were wild type BALB/c mice sensitized with a subcutaneous injection of 0.2 ml saline containing 20 µg Ova (five times crystallized hen's egg albumin; Sigma, St. Louis, MO, USA) plus 4.5 mg Al(OH)₃ as adjuvant on day 0. A second sc injection of 20 µg Ova was given on day 14.

2.3. Serum antibody evaluation

Serum samples for measuring anti-Ova IgG1 and IgE were obtained from groups culled after 14 days of oral antigen exposure. ELISA for IgG1 was carried out using plates coated with Ova and 100 µl of 1:8000 diluted mouse sera, with goat anti-mouse IgG1 (Southern Biotechnology Associates, Birmingham, AL, USA) and rabbit anti-goat labeled with peroxidase (Southern Biotechnology Associates, Birmingham, AL, USA). The plates were developed with *o*-phenylenediamine and H₂O₂ and were read at 492 nm on an automated ELISA reader (EL800, Bio-Tek Instruments, Inc., Winooski, VT, USA).

Anti-Ova IgE was measured by capture-ELISA using plates coated with rat anti-mouse IgE, 50 µl total serum and biotinylated Ova, as previously described [10]. The results for both antibodies are reported in arbitrary units using a positive reference serum (1000 U).

2.4. Culture assay and cytokine measurement

After 7 days of oral challenge, the wild type BALB/c mice were sacrificed by cervical dislocation. Mesenteric lymph nodes and spleen cells were harvested in a RPMI 1640 (SIGMA, USA) medium containing 10% fetal bovine serum (Gibco, USA), 2 mM L-glutamine, 50 mM 2-mercaptoethanol, 10,000 U of penicillin, 10 mg streptomycin (Gibco, USA) per ml. Red blood cells were removed by lysis in a 0.144 M NH₄Cl buffer. The cells (1 × 10⁶ cells/well) were then incubated in 24-well plates for 72 h either with 5 mg/ml of ovalbumin (OVA) (five times crystallized hen's egg albumin; Sigma, St. Louis, MO, USA) or 0.5 µg/ml of concanavalin A (con-A) (Sigma, St. Louis, MO, USA) in an atmosphere of 5% CO₂ at 37 °C. After 72 h, the supernatant was removed and stored at -20 °C until further analysis. The concentrations of IL-4 was measured by ELISA in supernatants using commercially available antibodies and according to the procedures supplied by the manufacturer (R&D Systems, Minneapolis, MN, USA).

2.5. Body weight evaluation

Body weight was determined each week throughout the experiment. The final body weight of each group was obtained from the means of the individual values and expressed in grams (g).

2.6. Histological analysis

After 14 days of oral challenge, the mice were sacrificed by cervical dislocation. The proximal jejunum was taken for histological analysis. It was fixed in 10% formalin in PBS, embedded in paraffin and cut into 5 µm thick sections. The sections were stained with periodic acid Schiff (PAS) for mucus analysis or with hematoxylin-eosin to evaluate eosinophil infiltration. Ten fields from hematoxylin-eosin stained sections were randomly chosen at 40× (53.333 µm²/field) in order to count the number of eosinophils and the data are reported as number of eosinophils/field. For mucus analysis, three sections of the jejunum stained with periodic acid Schiff was submitted to morphometric analysis using an image analysis program running on an IBM computer. Images were obtained at 40× (53.333 µm²/field) with a JVC TK-1270/RGB microcamera and analyzed with the KS300 software built in a Kontron Elektronick/Carl Zeiss image analyzer. For the determination of goblet cell volume, all pixels with green hues were selected for the creation of a binary image and subsequent calculation of the total area, and data were reported as µm² PAS/field.

2.7. Aversion test

Mice were housed in individual cages for 3 days after day 21. During this period, they received two small drinking bottles. One bottle contained sweetened (0.5% saccharin) egg white solution (20%) and the other contained tap water. The positions of the bottles were alternated daily to control side-preferences. The amount of liquid ingested from each bottle was determined by weighing them daily during the aversion test. The mean consumption over the 3 days was expressed as mg of solution ingested per gram body weight (mg g⁻¹).

2.8. Serum transfer

Serum from the donors were collected 7 days after the secondary sensitization. The serum transfer to wild type and IL-4^{-/-}

BALB/c mice was performed by intravenous (iv) injection of 0.4 ml of non-sensitized or hyperimmune serum. The aversion test was carried out 3 h after the serum transfer.

2.9. Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA – Tukey) or by the Student *t*-test when appropriate. The level of significance was set at $P < 0.05$. The data shown in the figures are reported as means $\pm$ SEM for 5 animals/group.

3. Results

3.1. Specific serum antibody and IL-4 production in a murine model of food allergy

Ovalbumin-stimulated spleen and mesenteric lymph node cells showed increased production of IL-4 in sensitized wild type BALB/c animals after 7 days of continuous EWS challenge (Fig. 1A).

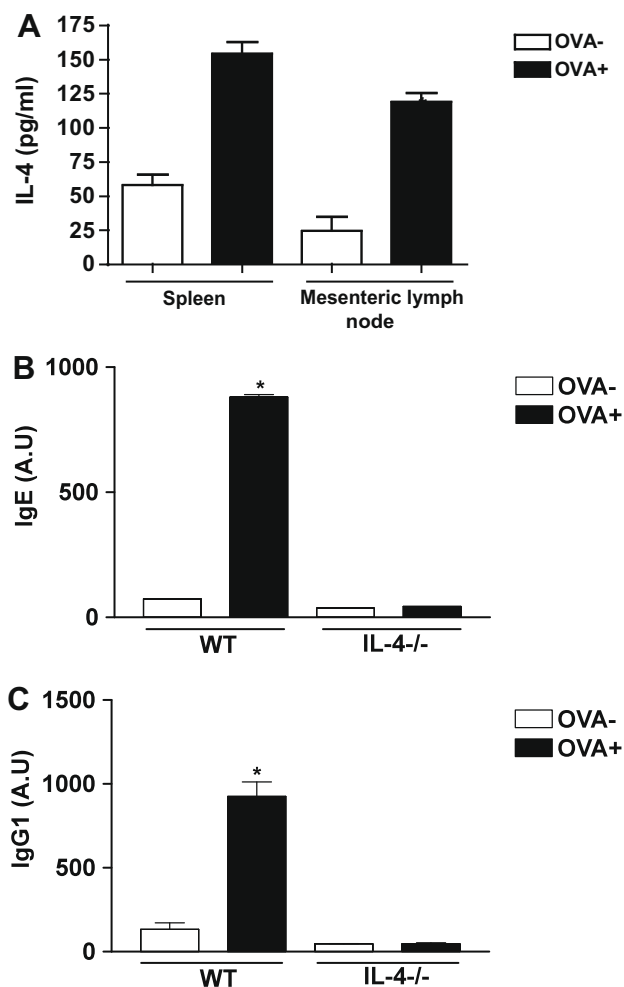


Fig. 1. Sensitization and continuous ingestion of EWS increased the production of IL-4 by spleen and mesenteric lymph node cells in vitro and induced the increase in serum concentrations of anti-Ova IgE and IgG1 in wild type BALB/c sensitized mice. Wild type and IL-4^{-/-} BALB/c mice were sensitized (OVA+) with 10 μ g Ova plus Al(OH)₃ on day 0 and with 10 μ g Ova on day 14. The non-sensitized group received only the adjuvant diluted in saline on day 0 and saline on day 14. Seven days after the secondary sensitization, all mice received EWS (20%) as the only liquid source. After 7 days, the mice were sacrificed. The spleen and mesenteric lymph nodes were removed for measurement of IL-4 and the serum was collected for measurement of anti-OVA IgE and IgG1 by ELISA. Data are reported as means $\pm$ SEM for 5 animals/group. * $P < 0.05$ compared to non-sensitized group (OVA–). (ANOVA – Tukey).

The sensitization procedure alone induced the production of both anti-Ova IgG1 and IgE in wild type but not in the IL-4 deficient BALB/c mice (data not shown). Continuous ingestion of EWS boosted the production of these specific antibodies up to the end of the experiment in sensitized wild type but not in IL-4 deficient BALB/c mice (Fig. 1B and C).

3.2. Weight loss following allergen ingestion was dependent on IL-4 production

After oral challenge with EWS, sensitized but not non-sensitized wild type BALB/c mice lost about 15% of their body weight (Fig. 2A). However, IL-4-deficient mice showed no weight loss, even when sensitized and exposed to the antigen solution by oral route (Fig. 2B).

3.3. Eosinophil infiltration and mucus production in the gut mucosa of allergic mice were dependent on IL-4 production

Prolonged EWS ingestion induced a threefold increase in eosinophil infiltration in the proximal jejunum of sensitized wild type BALB/c mice compared to non-sensitized animals. However, only few eosinophils were detected in the gut of IL-4-deficient mice, even after sensitization and oral challenge with ovalbumin (Fig. 3).

Continuous exposure to the antigen induced a significant increase in mucus production by goblet cells in the proximal jejunum of sensitized wild type BALB/c mice when compared to non-sensitized animals. On the other hand, antigen ingestion induced no increase in mucus secretion in the jejunum of IL-4-deficient mice, even when these animals were subjected to the sensitization protocol (Fig. 4).

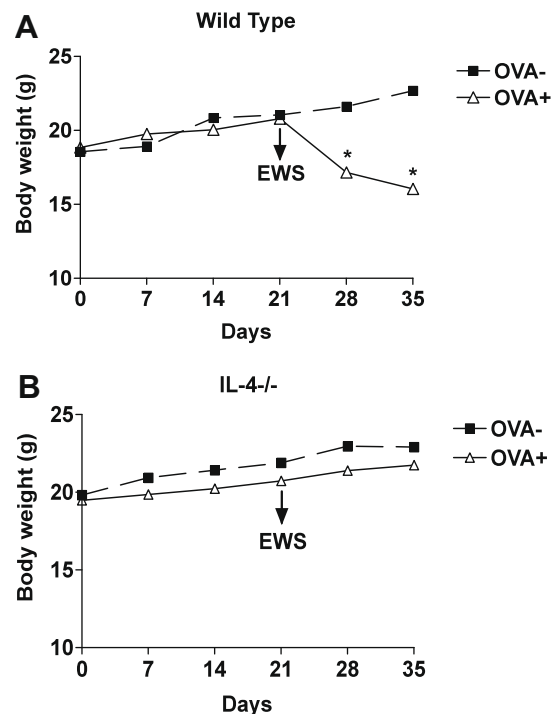


Fig. 2. Weight loss following allergen ingestion was dependent on IL-4 production. Wild type and IL-4^{-/-} BALB/c mice were sensitized (OVA+) with 10 μ g Ova plus Al(OH)₃ on day 0 and with 10 μ g Ova on day 14. The non-sensitized group received only the adjuvant diluted in saline on day 0 and saline on day 14. Seven days after the secondary sensitization, all mice received egg white solution (20%) as the only liquid source. The mice were weighed weekly throughout the experiment. Data are reported as means $\pm$ SEM for 5 mice in each group. * $P < 0.05$ compared to non-sensitized group (OVA–). (Student *t*-test).

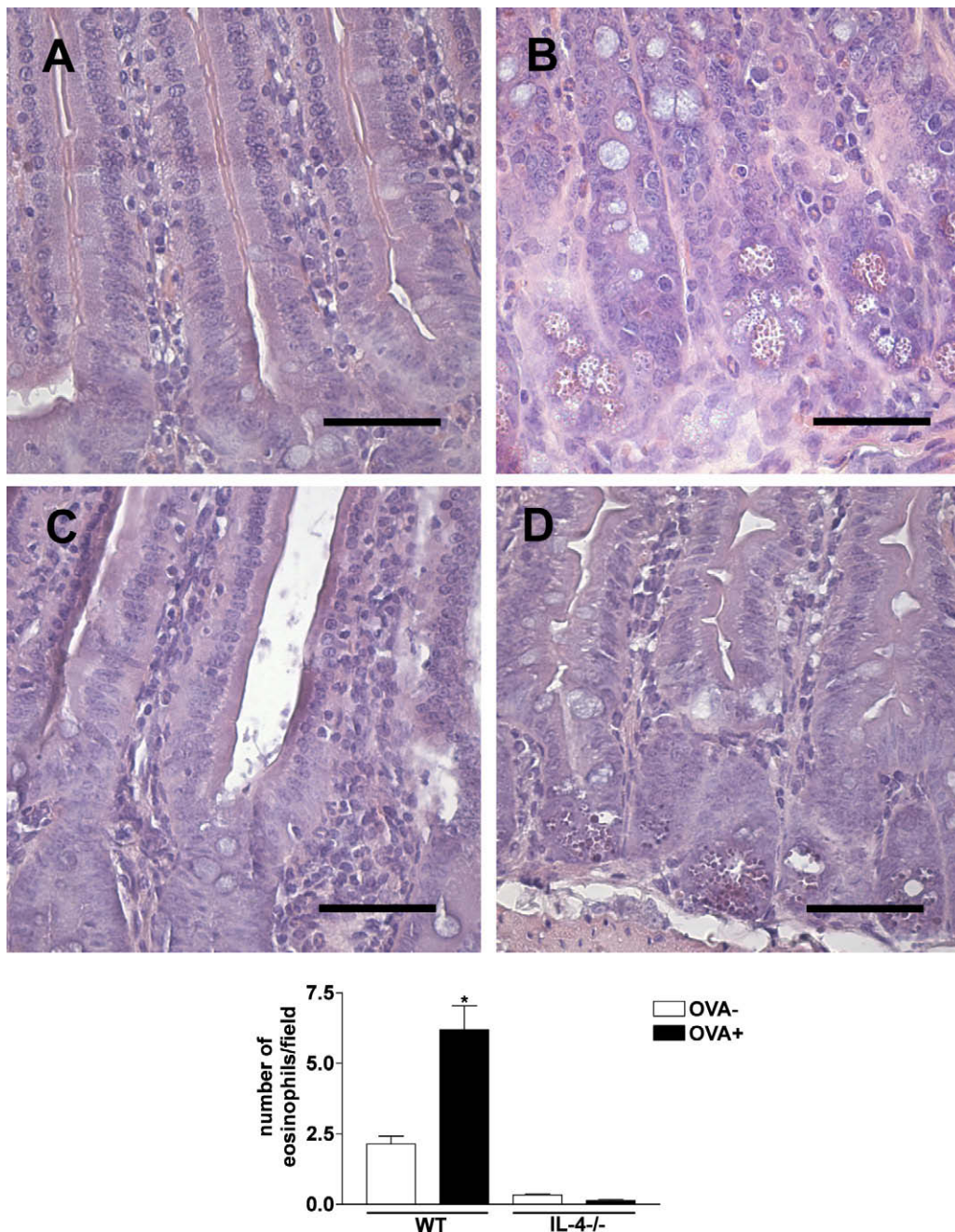


Fig. 3. Eosinophil infiltration in the gut mucosa of allergic mice was dependent on IL-4 production. Wild type and IL-4^{-/-} BALB/c mice were sensitized (OVA+) with 10 μ g Ova plus Al(OH)₃ on day 0 and with 10 μ g Ova on day 14. The non-sensitized group received only the adjuvant diluted in saline on day 0 and saline on day 14. Seven days after the secondary sensitization, all mice received EWS (20%) as the only liquid source. After 14 days, the mice were sacrificed and the proximal jejunum was collected. The number of eosinophils was evaluated in 10 fields of the jejunum. (A) non-sensitized wild type BALB/c mice; (B) sensitized wild type BALB/c mice; (C) Non-sensitized IL-4^{-/-} mice; (D) sensitized IL-4^{-/-} mice. Bar = 50 μ m. Data are reported as means $\pm$ SEM of number of eosinophils for 5 animals in each group. * P < 0.05 compared to non-sensitized group (OVA-). (ANOVA – Tukey).

3.4. Aversion to antigen ingestion was dependent on IL-4 production

When a choice of water or sweetened egg white solution (EWS) was offered, only ovalbumin-sensitized wild type BALB/c mice displayed aversion to the ingestion of the antigen solution. The volume of sweetened EWS ingested by these animals was four times lower than the volume ingested by non-sensitized group. In contrast, sensitized IL-4 deficient animals did not avoid ingesting the antigen solution (Fig. 5). Ingestion of water did not differ significantly among groups when non-sensitized and sensitized animals were compared (data not shown).

3.5. Aversion to antigen ingestion was dependent on specific antibody production

To investigate whether the effect of IL-4 in aversion was a direct effect or whether it depended of specific antibody production associated with IL-4, IL-4-deficient mice were transferred with serum from sensitized BALB/c mice. The serum transferred showed IgE levels similar to sensitized mice that were submitted to aversion test, even though the donors were not challenged orally with the antigen. When 0.4 ml of serum from sensitized donors was transferred to wild type or IL-4^{-/-} BALB/c mice, a significant aversion

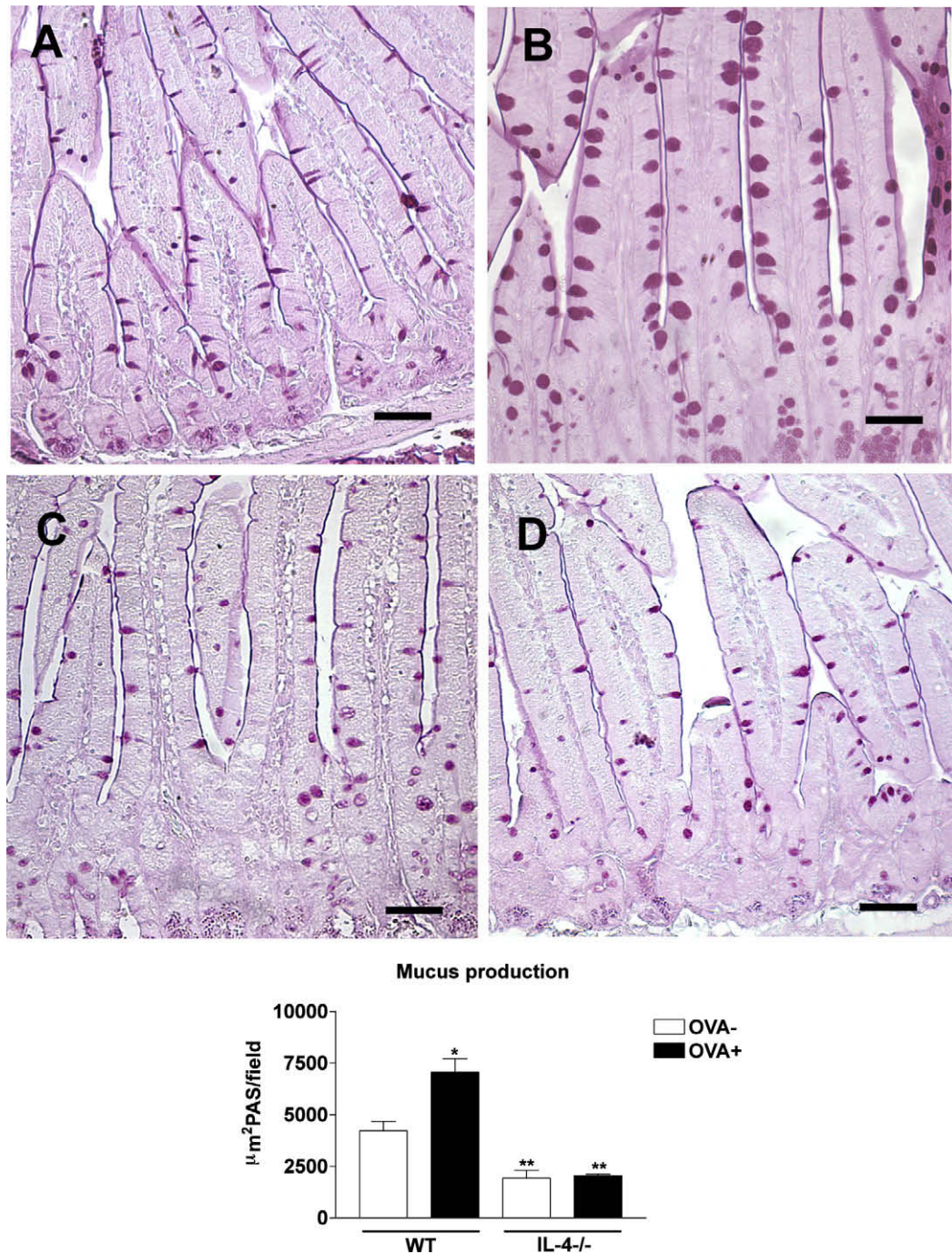


Fig. 4. Mucus production in the gut mucosa of allergic mice was dependent on IL-4 production. Wild type and IL-4^{-/-} BALB/c mice were sensitized (OVA+) with 10 µg Ova plus Al(OH)₃ on day 0 and with 10 µg Ova on day 14. The non-sensitized group received only the adjuvant diluted in saline on day 0 and saline on day 14. Seven days after the secondary sensitization, all mice received EWS (20%) as the only liquid source. After 14 days, the mice were sacrificed and the proximal jejunum was collected. The tissue was stained with P.A.S and the mucus production by goblet cells was evaluated in three fields of the jejunum. (A) non-sensitized wild type BALB/c mice; (B) sensitized wild type BALB/c mice; (C) non-sensitized IL-4^{-/-} mice; (D) sensitized IL-4^{-/-} mice. Bar = 50 µm. Data are reported as means ± SEM of mucus production for 5 mice in each group **P* < 0.05 compared to non-sensitized group (OVA-). ***P* < 0.05 compared to wild type (WT) groups. (ANOVA – Tukey).

to the ingestion of sweetened EWS was observed in both groups (Fig. 6). In contrast, the transfer of serum from non-sensitized donors did not induce the aversive behavior in any of the groups (data not shown).

4. Discussion

The gut has the greatest body surface area exposed to the outside environment, including food antigens. Normally, dietary pro-

teins induce oral tolerance, which is the suppression of immune responses to antigens that have been administered previously by the oral route. Abrogation of oral tolerance or failure to induce oral tolerance may result in the development of food allergy, characterized by the occurrence of IgE-producing B cells in response to activated T-helper type 2 (Th2) effector lymphocytes and cytokines like IL-4 [2]. We demonstrated that the cytokine IL-4 and its correlation with specific IgE and IgG1 antibodies are important for the development of almost all the characteristics of animal models of

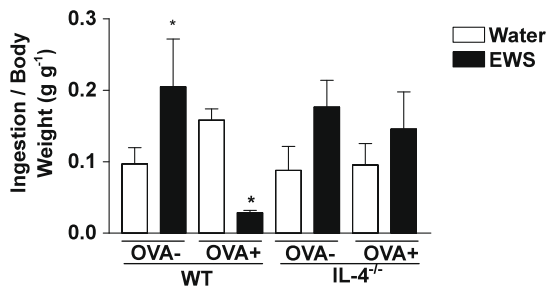


Fig. 5. Aversion to antigen ingestion was dependent on IL-4 production. Wild type and IL-4^{-/-} BALB/c mice were sensitized (OVA+) with 10 µg Ova plus Al(OH)₃ on day 0 and with 10 µg Ova on day 14. The non-sensitized group received only the adjuvant diluted in saline on day 0 and saline on day 14. After 7 days, the mice were housed in individual cages for 3 days with the option of drinking sweetened EWS or water. Data are represented as means ± SEM and expressed as mg of sweetened EWS ingestion per gram of body weight (mg g⁻¹). **P* < 0.05 compared to water ingestion (ANOVA – Tukey).

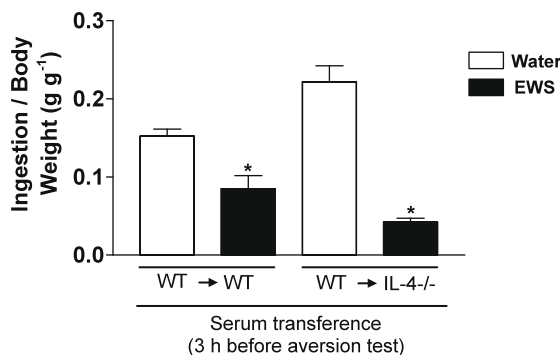


Fig. 6. Aversion to antigen ingestion was dependent on specific antibody production. The donors mice were sensitized (OVA+) with 20 µg plus Al(OH)₃ on day 0 and with 20 µg Ova on day 14. After 7 days, the serum of the donors was transferred to wild type or IL-4^{-/-} BALB/c mice. The recipient mice were housed in individual cages for 3 days with the option of drinking sweetened EWS or water. Data are represented as means ± SEM and expressed as mg of sweetened EWS ingestion per gram of body weight (mg g⁻¹). **P* < 0.05 compared to water ingestion (ANOVA – Tukey).

food allergy such as aversion to antigen ingestion, weight loss, hypersecretion of mucus and eosinophilic tissue infiltrate.

In this study, IL-4 production was increased after the stimulation with ovalbumin in spleen and mesenteric lymph nodes cultures of sensitized animals after 7 days of oral challenge. This high expression of IL-4 corroborates the hypothesis that the production of Th2 cytokines is increased in animal models of food allergy [11–13].

When ovalbumin-sensitized wild type BALB/c mice are given EWS as the only liquid option, they develop several local signs of food allergy such as mucus hypersecretion, eosinophil infiltration, edema and an increased number of mast cells in the intestinal mucosa. In addition, increased serum anti-ovalbumin IgG1 and IgE and dramatic weight loss are observed [5]. Previous studies by our research group have demonstrated that this weight loss is not associated with decreased liquid or food consumption (unpublished data). Rather, this alteration may be associated with immunological factors related to Th2 cytokines and antibodies, since IL-4^{-/-} BALB/c animals did not lose body weight when subjected to the protocol for inducing food allergy. As IL-4^{-/-} mice produced very low IgE and IgG1 levels, it is plausible that increased antibody secretion is necessary for the weight loss triggered by the allergic process.

Despite the enhancement of weight loss and antibody secretion, the intestinal alterations due to chronic antigen ingestion were lar-

gely regulated in our food allergy model. The mucosal immune system uses a number of mechanisms to avoid an injurious immune response to food antigens, including the presence of regulatory T cells in the lymphoid tissue of the gut and a physical filter represented by the mucus that limits antigen absorption [1]. Even in the presence of the immunosuppressive mechanisms in the intestinal mucosa, we were able to sensitize the animals, except the IL-4 deficient ones, by parenteral antigen administration, and the oral challenge induced mucus hypersecretion and increased eosinophil infiltration in the intestinal mucosa of the wild type sensitized group. Mucus hypersecretion is a common characteristic of allergic inflammation and has been shown to be IL-4 and IL-13 dependent [14]. The importance of IL-4 was confirmed in our study, since mucus production was impaired in IL-4^{-/-} animals and even the parenteral antigen administration and challenge were not able to induce the increase in mucus secretion.

In chronic allergic diseases, eosinophils are primed by IL-5 and attracted by chemokines, infiltrating the tissue, during continuous antigen exposure. These cells are responsible for the late phase of the allergic reaction, producing a basic protein that is toxic to the epithelium [15]. In addition to reduction in mucus secretion, IL-4^{-/-} BALB/c mice showed a diminished eosinophil infiltration in the jejunum than wild type animals, and the sensitization procedure and prolonged EWS ingestion did not change this profile. Previous studies have shown that, in a murine model of allergic asthma, IL-4 deficient mice displayed substantially fewer eosinophils in the lung tissue and bronchoalveolar lavage (BAL). These observations indicate that IL-4 is a central mediator in allergic airway inflammation, interfering with antigen-induced eosinophil recruitment [16–18].

When ovalbumin-sensitized wild type BALB/c mice were offered both water and EWS as liquid options, they displayed aversion to allergen ingestion [6]. We suggest that the flavor-aversive behavior observed in sensitized animals is an additional mechanism to protect against antigen entry by the oral route, contributing to the integrity of the intestinal mucosa. However, in this present study we have shown that when IL-4 is absent, these mice did not avoid ingesting the sweetened EWS even when subjected to the sensitization protocol with Ova. It is important to note that wild type BALB/c mice produced high levels of anti-Ova IgE and IgG1 after sensitization and that these levels were even higher after antigen ingestion; but IL-4 deficient mice did not show an accentuated humoral response, since this cytokine is crucial for IgE and IgG1 synthesis [19,20]. It has already been demonstrated that IgE production is essential for the development of food aversion: anti-IgE antibody treatment before oral challenge prevented the aversion to ingesting EWS in Ova-sensitized mice [8]. Our data confirm these previous observations and describe an upstream step in the net of factors triggering aversion to allergen ingestion in allergic animals: production of the cytokine IL-4.

Evidence suggests that food allergy influences behavioral changes and alters brain function. It has been demonstrated, for instance, that the aversion to antigen ingestion by sensitized animals is associated with elevated anxiety levels and to increased neuronal activation in emotionality-related brain areas. The development of food allergy and the resulting brain activation seem to be induced by mast cell degranulation, since these events are not observed in animals treated with anti-IgE antibodies [8,21]. A surprising number of mast cells in human and rat intestines are in contact with peptidergic nerves [22]. Hence, it is likely that IgE-induced mast cell degranulation is the major mechanism of food allergy signaling to the brain [8].

In this present study, we have shown that the same animals that demonstrated aversion to antigen ingestion when given a choice of liquid sources developed impressive food allergy signs when restricted to continuous ingestion of antigen solution. Hence,

we may infer that the same mechanism that triggers the aversive behavior in sensitized animals also leads to the development of food allergy when no choice of liquid source is available. We suggest that the development of the aversive behavior and the induction of the food allergy depends on the secretion of specific antibodies, since the IL-4^{−/−} mice did not demonstrated these alterations and showed very low levels of anti-Ova IgE and IgG1 in the serum.

To address if the cytokine IL-4 influences the aversion to antigen only by inducing the specific antibodies production, we transferred serum from sensitized animals to non-sensitized wild type and IL-4 deficient BALB/c mice. We were able to demonstrate that IL-4 is important to the development of food aversion only because it induces the production of specific antibodies, since when the IL-4 deficient animals were reconstituted with serum from sensitized donors, they demonstrated the aversive behavior even in the absence of this cytokine.

Taken together, these results support the hypothesis that production of the cytokine IL-4 and consequently specific IgE/IgG1 antibodies directly correlate with development of aversion to antigen ingestion in the ovalbumin-induced model of food allergy.

Acknowledgments

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References

- [1] S.H. Sicherer, H.A. Sampson, Food allergy: recent advances in pathophysiology and treatment, *Annu. Rev. Med.* 60 (2009) 261–277.
- [2] S. Bischoff, S.E. Crowe, Gastrointestinal food allergy: new insights into pathophysiology and clinical perspectives, *Gastroenterology* 128 (2005) 1089–1113.
- [3] M. Yazdanbakhsh, P.G. Kremsner, R. van Ree, Allergy, parasites, and the hygiene hypothesis, *Science* 296 (2002) 490–494.
- [4] L. Christie, R.J. Hine, J.G. Parker, W. Burks, Food allergies in children affect nutrient intake and growth, *J. Am. Diet. Assoc.* 102 (2002) 1648–1651.
- [5] J.C. Saldanha, D.L. Gargiulo, S.S. Silva, F.H. Carmo-Pinto, M.C. Andrade, J.I. Alvarez-Leite, M.M. Teixeira, D.C. Cara, A model of chronic IgE-mediated food allergy in ovalbumin-sensitized mice, *Braz. J. Med. Biol. Res.* 37 (2004) 809–816.
- [6] D.C. Cara, A.A. Conde, N.M. Vaz, Immunological induction of flavor aversion in mice, *Braz. J. Med. Biol. Res.* 27 (1994) 1331–1341.
- [7] D.C. Cara, A.A. Conde, N.M. Vaz, Immunological induction of flavour aversion in mice. II. Passive/adoptive transfer and pharmacological inhibition, *Scand. J. Immunol.* 45 (1997) 16–20.
- [8] A.S. Basso, F.A. Pinto, M. Russo, L.R. Britto, L.C. de Sa-Rocha, J. Palermo Neto, Neural correlates of IgE-mediated food allergy, *J. Neuroimmunol.* 140 (2003) 69–77.
- [9] F. Capobianco, C. Butteroni, B. Barletta, S. Corinti, C. Afferni, R. Tinghino, M. Boirivant, G. Di Felice, Oral sensitization with shrimp tropomyosin induces in mice allergen-specific IgE, T cell response and systemic anaphylactic reactions, *Int. Immunol.* 20 (2008) 1077–1086.
- [10] M. Russo, M.A. Nahori, J. Lefort, E. Gomes, A. de Castro Keller, D. Rodriguez, O.G. Ribeiro, S. Adriouch, V. Gallois, A.M. de Faria, B.B. Vargaftig, Suppression of asthma-like responses in different mouse strains by oral tolerance, *Am. J. Respir. Cell Mol. Biol.* 24 (2001) 518–526.
- [11] P.S. Leung, Y.S. Lee, C.Y. Tang, W.Y. Kung, Y.H. Chuang, B.L. Chiang, M.C. Fung, K.H. Chu, Induction of shrimp tropomyosin-specific hypersensitivity in mice, *Int. Arch. Allergy Immunol.* 147 (2008) 305–314.
- [12] C.R. Cardoso, G. Teixeira, P.R. Provinciatto, D.F. Godoi, B.R. Ferreira, C.M. Milanezi, D.B. Ferraz, M.A. Rossi, F.Q. Cunha, J.S. Silva, Modulation of mucosal immunity in a murine model of food-induced intestinal inflammation, *Clin. Exp. Allergy* 38 (2008) 338–349.
- [13] H. Nakajima-Adachi, A. Ebihara, A. Kikuchi, T. Ishida, K. Sasaki, K. Hirano, H. Watanabe, K. Asai, Y. Takahashi, Y. Kanamori, N. Shimojo, H. Matsuda, Y. Kohno, S. Hachimura, S. Kaminogawa, Food antigen causes TH2-dependent enteropathy followed by tissue repair in T-cell receptor transgenic mice, *J. Allergy Clin. Immunol.* 117 (2006) 1125–1132.
- [14] C. Blanchard, S. Durual, M. Estienne, K. Bouzakri, M.H. Heim, N. Blin, J.C. Cuber, IL-4 and IL-13 up-regulate intestinal trefoil factor expression: requirement for STAT6 and de novo protein synthesis, *J. Immunol.* 172 (2004) 3775–3783.
- [15] S.P. Hogan, H.F. Rosenberg, R. Moqbel, S. Phipps, P.S. Foster, P. Lacy, A.B. Kay, M.E. Rothenberg, Eosinophils: biological properties and role in health and disease, *Clin. Exp. Allergy* 38 (2008) 709–750.
- [16] G.G. Brusselle, J.C. Kips, J.H. Tavernier, J.G. van der Heyden, C.A. Cuvelier, R.A. Pauwels, H. Bluethmann, Attenuation of allergic airway inflammation in IL-4 deficient mice, *Clin. Exp. Allergy* 24 (1994) 73–80.
- [17] N.W. Lukacs, R.M. Strieter, S.W. Chensue, S.L. Kunkel, Interleukin-4-dependent pulmonary eosinophil infiltration in a murine model of asthma, *Am. J. Respir. Cell Mol. Biol.* 10 (1994) 526–532.
- [18] R. Fischer, D. Tome, J.R. McGhee, P.N. Boyaka, Th1 and Th2 cells are required for both eosinophil- and neutrophil-associated airway inflammatory responses in mice, *Biochem. Biophys. Res. Commun.* 357 (2007) 44–49.
- [19] L.B. Bacharier, R.S. Geha, Molecular mechanisms of IgE regulation, *J. Allergy Clin. Immunol.* 105 (2000) S547–S558.
- [20] E.L. Faquim-Mauro, R.L. Coffman, I.A. Abrahamson, M.S. Macedo, Cutting edge: mouse IgG1 antibodies comprise two functionally distinct types that are differentially regulated by IL-4 and IL-12, *J. Immunol.* 163 (1999) 3572–3576.
- [21] A.S. Basso, F.A. Costa-Pinto, L.R. Britto, L.C. de Sa-Rocha, J. Palermo-Neto, Neural pathways involved in food allergy signaling in the mouse brain: role of capsaicin-sensitive afferents, *Brain Res.* 1009 (2004) 181–188.
- [22] R.H. Stead, M. Tomioka, G. Quinonez, G.T. Simon, S.Y. Felten, J. Bienenstock, Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves, *Proc. Natl. Acad. Sci. USA* 84 (1987) 2975–2979.