

Oral tolerance induction to *Dermatophagoides pteronyssinus* and *Blomia tropicalis* in sensitized mice: occurrence of natural autoantibodies to immunoglobulin E

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Summary

Background The dust mites *Dermatophagoides pteronyssinus* (Dp) and *Blomia tropicalis* (Bt) are important sources of indoor allergens in tropical and subtropical countries. Murine models allow the analysis of the immune response and regulation of IgE production to Dp and Bt allergens. Oral tolerance induces unresponsiveness in naive animals, but its application in sensitized animals can provide useful information to improve allergy therapy.

Objective To study the profile of IgE and IgG subclasses antibody upon oral administration with Bt and Dp extract in previously sensitized mice. Further, the occurrence of autoantibodies IgG anti-IgE in the immunization and in the oral tolerance was investigated.

Methods A/Sn mice were immunized with Bt or Dp extract in alum, orally administrated with 0.25 mg of Bt or Dp extract or PBS at the 6th, 7th and 8th days after immunization and boosted twice with their respective allergens. To analyse the mice groups, specific IgE antibodies were measured by passive anaphylaxis reaction and specific IgG subclasses and anti-IgE IgG autoantibody by ELISA assay.

Results IgE levels were markedly increased in Bt-immunized mice compared with Dp-immunized mice. A distinct profile of the specific isotypes was verified in Bt-immunized mice with a preferential production of IgG3 and IgA antibodies, whereas Dp-immunized mice developed high titres of anti-Dp IgG1, IgG2a and IgG2b antibodies. The antigen feeding inhibited IgE response in both fed-mice groups but only Dp-fed mice presented decreased levels of IgG antibodies. Free anti-IgE IgG autoantibodies were detected mainly in the Dp-immunization and they correlated with the antibody isotypes found against the allergen.

Conclusions This is the first time that the murine-type I hypersensitivity is employed to study Bt-immunization, showing a marked IgE production, associated with IgG response, which is at least in part driven by T-independent antigens. The oral tolerance protocol in previously sensitized animals was able to down-modulate IgE response and points out this route as a strategy for allergy therapy.

Keywords anti-IgE IgG antibody, *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, immunoglobulin E, oral tolerance, subclasses of immunoglobulin G

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Introduction

House dust mite allergens are the most important indoor allergens associated with asthma and rhinitis. The mites *Dermatophagoides pteronyssinus* (Dp) and the *Blomia tropicalis* (Bt) are considered an important source of allergens in tropical and subtropical regions of the world [1–3]. These mites are believed to be responsible for the increasing incidence of IgE-mediated sensitization among patients, possibly due to their high indoor prevalence and phylogenetic association, leading to antigenic cross-reactivity [4]. However, there is some evidence that

Bt allergens are distinct and have relatively low to moderate cross-reactivity to *Dermatophagoides* spp. allergens [5]. Bt has one major allergen (11–13 kDa) and three other important ones of 64, 36 and 33 kDa [6], that have some peculiarities; the Blot 13 allergen, for instance, is a fatty acid-binding protein [7] and has been considered to be an independent cause of sensitization [1]. Moreover, *Dermatophagoides pteronyssinus* is composed of major allergens that are proteinases, that meet the criteria of both allergens and proallergic adjuvants [8]. So far, the majority of the immunological studies are with Dp allergens and have demonstrated that they may promote allergic immunity by inducing disturbance in the IgE network and polarization of T helper cells to a dominant Th2 phenotype [8, 9]. The peculiarities of the two mites should be considered in order to improve the immunotherapy protocols in the allergy field.

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The experimental murine models of type I hypersensitivity have been widely employed to study immunological mechanisms of IgE regulation. However, there is no evidence of animal-based experimentation using Bt allergen, and further investigation would contribute to the development of new therapeutic protocols. One natural route for treatment is the mucosae, especially the gastrointestinal system, which are composed of a complex immune system, making possible the suppression of humoral or cellular immune responses upon oral tolerance induction [10]. Oral administration of non-self antigens or autoantigens (in the case of autoimmune disease) can generate systemic regulatory mechanisms by regulatory cells and mediators [11–15]. The mechanisms of oral tolerance might not be mutually exclusive, but mediated by clonal deletion, anergy or cytokines [16–20], depending on the type and dose of the antigen, timing of oral administration, as well as the disease under study in the experimental model. Furthermore, the immune system of a naive host has considerably more possibilities for immune deviation than a primed host. However, despite the difficulty in inducing tolerance in primed mice, this condition should better mimic the clinical aspects of the disease [21–23]. Previously, we observed that Dp-sensitized mice orally administered with this allergen were able to inhibit the IgE response and the Th2 target cells [24–26].

One alternative mechanism that can occur in the immunization with those allergens is the production of natural autoantibodies IgG-anti IgE. These antibodies are occasionally present in normal donors and more frequently in a variety of allergic disorders or in other diseases with high levels of IgE response [27–30]. Some of the anti-IgE autoantibodies are non-anaphylactogenic, representing a possible protective mechanism to prevent the association of IgE with FcεRI [31, 32]. Such antibodies may represent normal physiological control and/or a regulatory role; that is why anti-IgE antibodies have been used in allergy therapy [32, 33].

In the present study we investigate the oral tolerance induction in previously sensitized mice to Bt and Dp by determining the profile of IgE and subclasses of IgG antibodies. To further understand the allergic immunological events under these conditions, the occurrence of anti-IgE IgG autoantibodies in the immunization and in the oral tolerance protocols was also investigated.

Materials and methods

Animals

Female A/Sn mice, 8 to 10 weeks old, were provided by the animal facility of the São Paulo University Medical School. Wistar Furth rats (RT1^{u,u}) both sexes, 3 to 4 months old, bred in our own animal facility were used for passive cutaneous anaphylaxis reaction (PCA).

Immunization protocol

Groups of mice were injected subcutaneously with 10 µg of *Dermatophagoides pteronyssinus* (Dp, IFIDESA-ARISTE-GUI, 112.900 UBE/mL) or *Blomia tropicalis* (Bt, kindly provided from International Pharmaceutical Immunology (IPI)-ASAC, 35.700 UBE/mL) in 6 mg of Al(OH)₃. Animals were boosted intraperitoneally on the 14th and 21st days after immunization with 10 and 50 µg of Dp or Bt, respectively,

without adjuvant. Mice were bled through the retro-orbital plexus on the 28th day after immunization and sera were stored at –20 °C until used.

Oral antigen administration protocol

Mice were fed with a total dose of 0.25 mg of Dp or Bt extract in 0.5 mL of PBS or PBS alone by gavage under light ether anaesthesia. The feeding was performed on the 6th, 7th and 8th days after immunization. All groups were boosted on the 14th and on the 21st days after immunization.

Passive cutaneous anaphylaxis (PCA)

IgE antibodies were estimated by passive cutaneous anaphylaxis reaction as described by Mota & Wong [34]. Briefly, sera dilutions from the mice were inoculated intradermally with 0.1 mL on the shaved backs of the rats. After 18 h the rats received 0.5 mg of mite extract by tail vein injection, in 1.0 mL 0.5% Evans Blue. One hour later the rats were killed and the reciprocal of the highest serum dilution that gave a spot bigger than 5 mm in diameter was taken as the PCA titre.

Determination of antibody levels

An enzyme-linked immunosorbent assay (ELISA) was performed to detect IgG isotypes and total IgE levels, as previously described by Sato et al. [25]. Briefly, to measure specific IgG antibody isotypes, 96-well microplates (Costar, Cambridge, MA, USA) were coated with 10 µg/mL of Dp or Bt extract in 0.1 M carbonate-bicarbonate buffer, pH = 9.5. The wells were blocked with PBS-0.5% bovine serum albumin (BSA) before incubation of the serum samples for 1 h at 37 °C and 4 °C overnight. Detection was performed with biotinylated anti-γ1, γ2a, γ2b, γ3, α or µ mouse chain antibodies (1 : 3000, Southern Biotechnology Association, Birmingham, AL, USA) and streptavidin-peroxidase (Sigma, St Louis, MO, USA) and TMB (3, 3', 5, 5'-tetramethyl-benzidine, Sigma). Sulphuric acid was used to stop the reaction, which was read at 450 nm in an ELISA microplate reader (Bio-rad, Hercules, CA, USA). The results were expressed as antibody titres by reference to serial dilution of a titrated serum pool from immunized adult mice with high levels of specific antibodies.

Estimates of total IgE were performed by double mAb ELISA assays, using 3 µg/mL of anti-mouse IgE (PharMingen, San Diego, CA, USA) as coating antibodies. The individual sera were diluted from 1 : 25 to 1 : 3200 and incubated as described above. After six washes a biotinylated anti-IgE antibody (1 : 1000, PharMingen) was added and plates were incubated for 1 h at 37 °C, followed by the incubation with streptavidin-peroxidase as described above. The O.D. units were correlated to the protein concentration, with standard curves derived from purified IgE (PharMingen).

Assays for free IgG anti-IgE and IgE-IgG complex

Detection of IgG anti-IgE antibody and IgE-IgG complexes was performed as previously described [24]. Briefly, direct ELISA was used for IgG anti-IgE ab determinations. Microplates were coated with 5 µg/mL purified mouse IgE-TNP (k, specific for the hapten trinitrophenol; PharMingen), then serum dilutions were added and incubated overnight at 4 °C, followed by the addition of biotinylated anti-Fcγ antibody (Sigma) or

biotinylated mouse anti- $\gamma 1$, $\gamma 2a$, $\gamma 2b$ or $\gamma 3$ (Southern Biotechnology Association). Streptavidin-peroxidase incubation and colourimetric development were performed as described above. Detection of IgE-IgG complexes was performed by IgE capture, using 5 $\mu\text{g/mL}$ monoclonal anti-IgE antibody (PharMingen) as coating. Serial dilutions of sera from three pool of each group of animals analysed were incubated, followed by mouse biotinylated anti-Fc γ (Sigma) incubation as described above.

Statistical analysis

Differences between groups were considered statistically significant when P -values were ≤ 0.05 , using paired t -test.

Results

IgE response in the murine immunization with *Blomia tropicalis* (Bt) or *Dermatophagoides pteronyssinus* (Dp)

A/Sn mice immunized with Bt or Dp extract in alum and boosted twice with the respective antigen produced high levels of specific IgE antibodies, as evaluated by passive anaphylactic cutaneous reaction (Fig. 1). IgE levels were markedly increased

in Bt-immunized mice compared with Dp-immunized mice (Fig. 1). Concomitantly to the production of specific IgE antibodies, Bt-immunized mice developed fourfold more total IgE serum levels than Dp-immunized animals (Fig. 1).

Comparison of oral tolerance induction with Dp and Bt in previously sensitized mice

In order to modulate IgE response in sensitized animals, the animals were submitted to the oral antigen administration protocol on the 6th day of immunization [24, 25]. A total of 0.25 mg of Dp or Bt extract was orally administered on three consecutive days (Fig. 2). The feeding protocol promoted a significant decrease of anti-Dp or anti-Bt IgE antibody levels when compared with their respective control groups (immunized and oral treated with PBS). Also, total IgE levels decreased in both mouse groups fed with the antigens (Fig. 2).

Despite the decrease of IgE response in the oral treatment, the IgE levels of Bt-immunized mice remained significantly high in comparison with the Dp-immunized mice.

Profile of specific antibody isotypes in the induction of oral tolerance with Bt or Dp

Distinct profiles of specific antibody isotypes were observed for Bt- and Dp-immunized mice; while in the former group IgG3

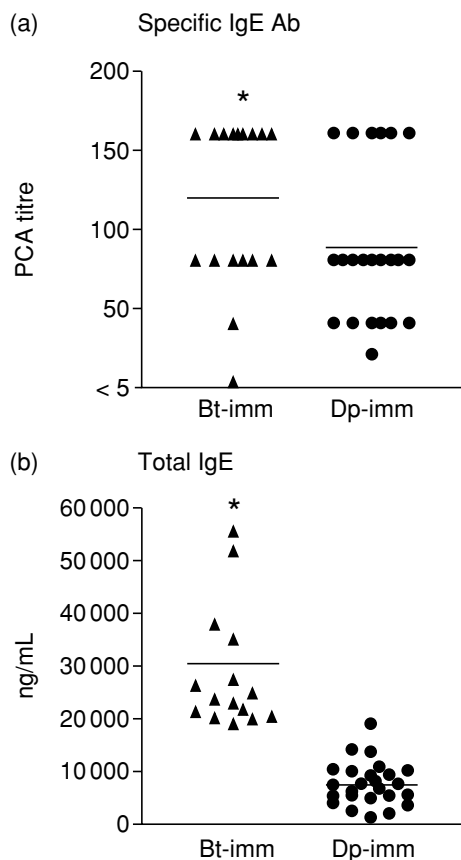


Fig. 1. IgE response to the mites *Blomia tropicalis* (Bt-imm) and *Dermatophagoides pteronyssinus* (Dp-imm) in murine immunization. A/Sn mice were immunized with 10 $\mu\text{g}/\text{Al}(\text{OH})_3$ of mite extract by the subcutaneous route and boosted intraperitoneally on the 14th and 21st days after immunization (dpi) with 10 and 50 μg of allergen, respectively, without adjuvant. The sera obtained on the 28th day were analysed for specific IgE antibodies by passive cutaneous anaphylaxis reaction (PCA) and total IgE by ELISA assays. The results are presented by mean $\pm$ SD of five different groups, each one containing three to six mice. * $P \leq 0.05$ when compared with the Dp-immunized mice group.

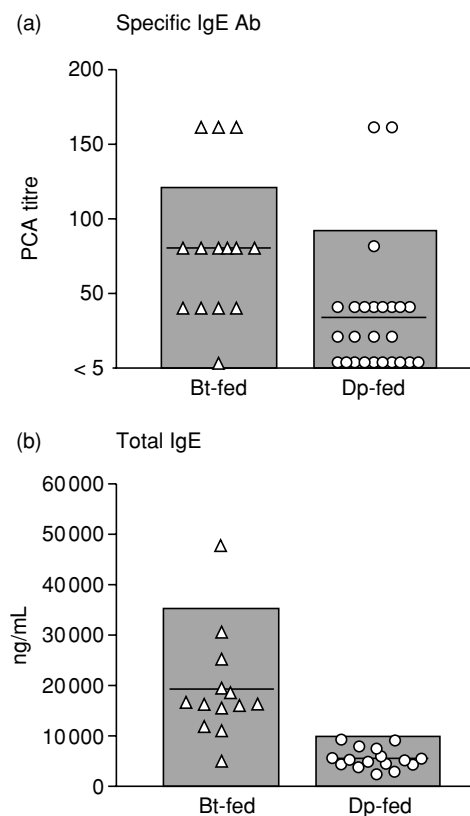


Fig. 2. IgE response in sensitized mice orally administered with Bt or Dp extract. Mite-sensitized mice were fed with 0.25 mg of Bt (Bt-fed mice) or Dp (Dp-fed mice) extract or with the vehicle (PBS) alone by gavage on the 6th, 7th and 8th days. All groups were boosted on the 14th and 21st days. Grey boxes represent the mean values for the respective groups of Bt- or Dp-immunized mice fed with PBS. The sera obtained on the 28th day were analysed for specific IgE antibodies by PCA reaction and for total IgE by ELISA assays. The results are presented by mean $\pm$ SD of five different groups, each one containing three to six mice.

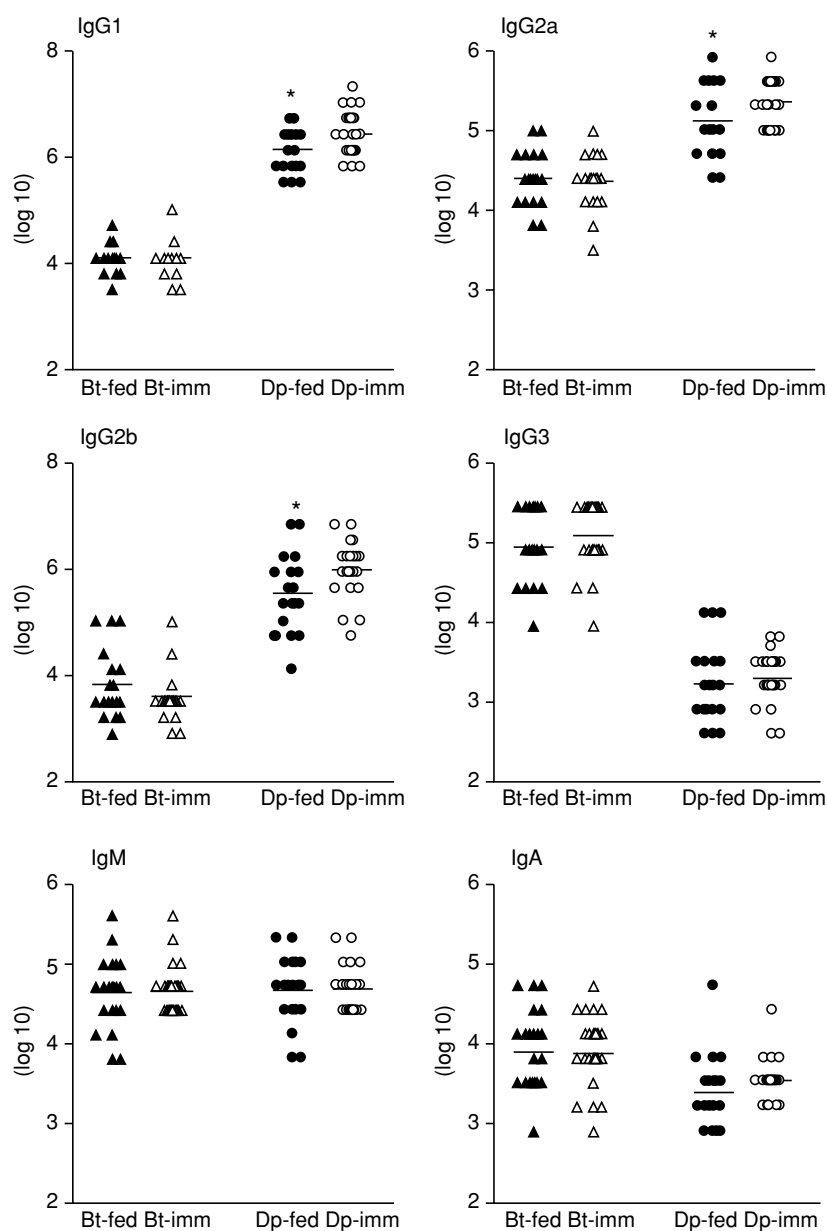


Fig. 3. Profiles of antibodies in sensitized mice orally administered with Bt or Dp extract. Mite-sensitized mice were fed with 0.25 mg of Bt (Bt-fed) or Dp (Dp-fed) extract or with PBS (Bt-imm or Dp-imm) and boosted on the 14th and 21st days. Titres of the sera obtained at the 28th day were analysed for IgG subclasses (IgG1, IgG2a, IgG2b, IgG3), IgM and IgA specific antibodies by ELISA assays. The results are presented by mean $\pm$ SD of five different groups, each one containing five to seven mice. * $P \leq 0.05$ when compared with the Dp-immunized mice group.

and IgA antibodies were predominant, the latter raised high titres of anti-Dp IgG1, IgG2a and IgG2b, rather than anti-Dp IgG3 antibodies (Fig. 3). IgM antibody levels were similar in both immunized mice groups.

In contrast to the significant decrease of IgE response in Bt-fed mice (Fig. 2), no effect was observed in the other anti-Bt IgG subclasses (Fig. 3); when gavage was performed with Dp extract, the levels of IgG1, IgG2a and IgG2b were significantly down-modulated. IgA and IgM antibody production did not alter with Bt or Dp feeding.

Induction of IgG anti-IgE autoantibodies and IgG-IgE complexes in oral tolerance to Bt and Dp

The presence of a free IgG anti-IgE autoantibody population was detected in the oral tolerance induction to both allergens, Bt

and Dp, corroborating our previous work that Dp immunization can stimulate the production of anti-IgE antibodies [24].

The production of IgG anti-IgE autoantibodies is shown in Fig. 4(a). These antibodies were significantly increased in the immunized mouse groups when compared with normal mice. The reactivity of these antibodies was higher in the Dp-immunized mice, orally treated or not, than in the Bt-immunized groups. These observations strengthen the concept that immunization *per se* can induce the production of free anti-IgE IgG autoantibodies. The investigation of IgE antibodies complexed with IgG has demonstrated a similar reactivity between groups, except for the Dp-fed mouse groups, which showed significantly lower levels compared with the Dp-immunized ones (Fig. 4b).

The evaluation of free anti-IgE IgG autoantibodies has shown that immunization with Dp promotes higher levels of IgG1 and IgG2a antibodies, and was similar for immunized and

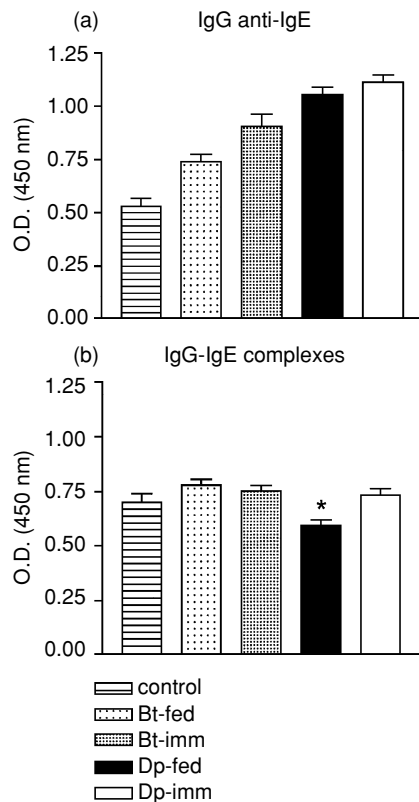


Fig. 4. Evaluation of free anti-IgE IgG autoantibody and IgE-IgG complex levels in the immunization and oral tolerance to Bt and Dp extracts. Mite-sensitized mice were fed with 0.25 mg of Bt (Bt-fed) or Dp (Dp-fed) extract or with PBS (Bt-imm or Dp-imm) and boosted on the 14th and on the 21st days. Reactivity of anti-IgE IgG autoantibody (a) and IgE-IgG complex levels (b) from three pools with sera from each mice group (28th day; 1 : 40) were evaluated by ELISA assays and represented by mean $\pm$ SD. Non-immunized and non-treated mice were control groups. * $P \leq 0.05$ when compared with the Dp-immunized mice group.

PBS-treated mice (Fig. 5). The anti-IgE IgG1 antibody levels were lower in Bt-fed mice than in the immunized mice group. The reactivity of IgG3 against IgE was barely seen among the immunized and tolerized mice groups but highly detected in mice orally treated with Bt.

Bt and Dp immunization has not induced anti-IgE antibodies of the IgG2b subclass as treated and non-treated groups showed levels similar to normal mice (Fig. 5). Overall, normal mice displayed a minimal basal reactivity of all IgG isotypes against IgE molecules.

Discussion

Oral tolerance to the dust mites *Blomia tropicalis* (Bt) and *Dermatophagoides pteronyssinus* (Dp) was studied in previously sensitized mice in order to modulate the type I hypersensitivity response. Furthermore, the occurrence of free autoantibody IgG anti-IgE was investigated in the immunization and oral tolerance protocols with these mite extracts.

In this first murine investigation of type I hypersensitivity response to the mite Bt, a mouse strain that was previously known to produce an important IgE response to the Der p extract was employed [24, 25]. Bt immunization in the A/Sn mice has induced a marked increase of anaphylactic IgE

antibody and total IgE levels, with high titres of IgG3 and IgA antibodies. This antibody profile was different from the Dp-immunized mice, which preferentially developed IgG1 antibody followed by IgG2b and IgG2a (IgG1 > IgG2b > IgG2a). The high levels of IgG1 anti-Dp antibody can be a reflex of the increase in the Th2 cytokines secretion, such as IL-4, IL-5 and IL-13, previously observed in Dp-immunized mice [24]. The IgG subclass distribution in Bt-immunized mice was mainly IgG3, followed by IgG1 and IgG2a, and lately IgG2b antibodies (IgG3 > IgG1 = IgG2a > IgG2b). The findings that Bt-immunized mice IgE antibody levels are increased and IgG1 antibody is not the main source of IgG subclasses, support the concept that IgE and IgG1 antibodies could be differentially regulated [35].

Besides the IgE response, the rising of IgA antibody levels – an isotype that depends on cytokines such as TGF- β and IL-5 [36] – suggests the induction of Th2 response in the Bt-immunized mice. On the other hand, these mice exhibited high IgG3 levels, an isotype switching that does not seem to require Th2-cytokine and is usually related to T-independent antigens. Thus, stimulation of B cells with lipopolysaccharide, a component of gram-negative bacteria [37], is sufficient on its own to induce switching to this isotype, with production of γ 3 germline transcripts [38]. Nevertheless, it is not known whether the molecules recognized by the IgG3 antibodies in Bt-immunized mice bear any allergenic property, besides their immunogenic characteristic.

In patients with atopic dermatitis associated with respiratory allergy and positive reactivity to Bt, an important frequency of Bt-specific IgG2 antibodies and also IgG4 and IgG1 was observed [39]. IgG2 is known to be produced in response to polysaccharide antigens, with a low capacity of complement activation [40]. The ability to respond to polysaccharide antigens is equivalent to the IgG3 isotype in mouse. In contrast, murine IgG3, like IgM, is an excellent complement fixing and is able to protect against bacterial infection [41]. In those patients, IgG3 subclass, an isotype usually not found at elevated levels in atopic patients, was found. This could be due to the high ability of IgG3 to activate complement cascade, and may be important in the onset of allergic symptoms that are associated with frequent infections by viruses, bacteria and fungi.

There is a close association between the cutaneous reactivity of atopic patients with both airborne-allergens, Bt and Dp [1–3]. This is probably due to the high indoor prevalence of these mites in tropical countries and/or to the relative cross-reactivity between them. *In vitro* and *in vivo* studies of IgE responses to the recombinant Blot5, a major allergen of 14 kDa obtained from a Bt cDNA library, showed 43% sequence homology to rDer p 5, demonstrating that Bt is an independent cause of sensitization [1]. Considering these evidences, it has been proposed that Bt allergens should be included in the diagnostics for evaluation of allergic diseases in the tropics [5].

Oral tolerance is a strategy to down-modulate hypersensitivity reactions, like those found in autoimmune or allergic manifestations [11–15]. Oral tolerance is usually investigated in naive animals, but we used mice sensitized to the mite extracts to better mimic an allergy disease. We have observed that the feeding with Bt or Dp negatively modulated the IgE response in sensitized mice. In relation to IgG subclasses production, they were not affected by oral administration with Bt, in

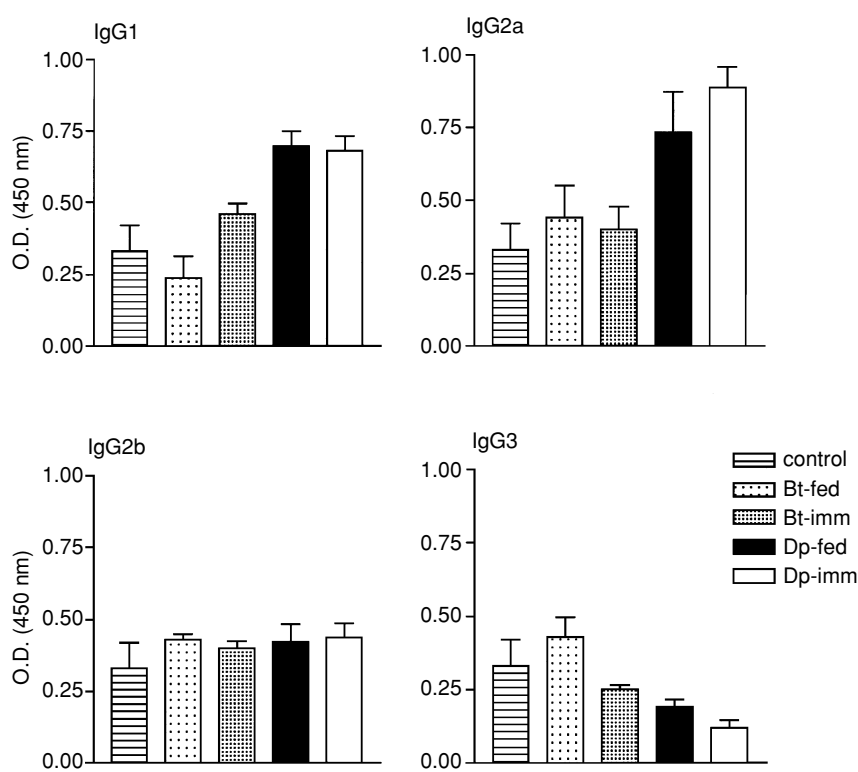


Fig. 5. Isotypes of IgG anti-IgE autoantibodies in the oral tolerance to Bt and Dp extract. Mite-sensitized mice were fed with 0.25 mg of Bt (Bt-fed) or Dp (Dp-fed) extract or with PBS (Bt-imm or Dp-imm) and boosted on the 14th and on the 21st days. Reactivity of anti-IgE IgG1, IgG2a, IgG2b and IgG3 autoantibodies, from three pools with sera from each mice group (28th day; 1:40) were evaluated by ELISA assays and represented by mean $\pm$ SD. Non-immunized and non-treated mice were control groups.

contrast to Dp-feeding, which caused significant decrease in the anti Dp IgG1, IgG2a and IgG2b antibody levels. It is possible that the antibody population directed to the T-independent antigen produced by Bt-fed mice may escape the regulatory mechanisms induced by oral tolerance. Failure of Bt-feeding to modulate other T-dependent IgG subclasses poses a question on the necessity to increase the antigen dose in oral treatment. However, it requires careful evaluation, as high-dose feeding could exacerbate pre-existing immune responses in sensitized mice [25].

One of the mechanisms of oral tolerance acting in sensitized mice fed with Dp, that was previously observed, seems to be mediated by regulatory cytokines such as TGF- β . These animals presented an increased TGF- β production, and inhibition of Th2 cytokines secretion without a shift to Th1 response [26, 42]. Presently, a significant decrease in the IgG2a levels (an isotype related to IFN- γ) was detected in Dp-fed mice. Together, these results suggest that oral tolerance to Dp promotes down-modulation of Th2 cells, not eliciting Th1 cytokines production. Induction of oral tolerance in sensitized mice is more difficult to establish due to the more complex immunological status of the primed host requiring multiple regulatory mechanisms.

Additionally, the IgG autoantibody that recognizes the IgE molecule could act as a regulatory mechanism in allergic response. Free anti-IgE IgG antibody reactivity was mainly detected with Dp-immunization rather than Bt-immunization. These antibodies are induced by the allergen immunization, but they are also found at low levels in normal mice sera. This emphasizes their possible physiological role in normal animals to prevent/block IgE deleterious function.

To further verify whether some IgE antibody can be complexed with IgG, which would prevent its detection, we have

evaluated the IgE-IgG complex in the animals' sera. The levels of IgG-IgE complex were similar in all analysed groups, but lower in Dp-fed mice. The main isotypes of anti-IgE IgG antibody were IgG1 and IgG2a in Dp-immunized mice, treated or not, correlating with the isotypes of the antibody anti-Der p. IgG3 was the main isotype of the anti-IgE IgG antibody found in Bt-immunized mice. Whether this anti-IgE autoantibody population includes non-anaphylactogenic antibodies, and plays a role in the control of IgE response in tolerized mice, is yet to be clarified. Besides, a study on autoantibody IgG anti-IgE has shown that epitope specificity provides the molecular basis for the rational design of vaccines that elicit long-term protective anti-IgE in patients [43].

In addition, non-anaphylactic antihuman IgE antibodies are now under clinical evaluation as therapeutic agents against rhinitis and asthma, alternatively to drug treatment that is insufficient to control symptoms and causes side-effects [32]. It has been reported that anti-IgE treatment decreases IgE, prevents the development of anaphylaxis, yet fails to affect T cell function, eosinophil airway infiltration and airway hyper-responsiveness in sensitized and challenged mice, differently from anti-IL-5 treatment [44]. For these reasons, the potential usefulness of combining anti-IgE therapy as an adjuvant to other forms of existing therapies, such as oral tolerance, needs to be assessed.

Altogether, the results pointed out that oral administration with Bt or Dp is able to negatively modulate the IgE antibody response in sensitized mice. The murine model of type I hypersensitivity to Bt and Dp revealed a distinct pattern of immune response, and reinforces that the mites' peculiarities have to be considered when undertaking an allergy therapy strategy.

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References

- Arruda LK, Vailes LD, Platts-Mills TA et al. Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blot5. *Am J Resp Crit Care Med* 1997; 155:343–50.
- Rizzo MC, Arruda LK, Chapman MD et al. IgG and IgE responses to dust mite allergens among children with asthma in Brazil. *Ann Allergy* 1993; 71:152–8.
- Fernandes-Caldas E, Puerta L, Mercado D, Lockey RF. Mite fauna, Der p. I, Der f I and *Blomia tropicalis* allergen levels in a tropical environment. *Clin Exp Allergy* 1993; 23:292–7.
- Arlan LG, Vyszynski-Moher DL, Fernandes-Caldas E. Allergenecity of the mite, *Blomia tropicalis*. *J Allergy Clin Immunol* 1993; 91:1042–50.
- Chew FT, Yi FC, Chua KY et al. Allergenic differences between the domestic mites *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1999; 29:9298–88.
- Caraballo L, Puerta L, Martinez B, Moreno L. Identification of allergens from the mite *Blomia tropicalis*. *Clin Exp Allergy* 1994; 24:1056–60.
- Puerta L, Kennedy MW, Jim Nez S, Caraballo L. Structural and ligand binding analysis of recombinant blo t 13 allergen from *Blomia tropicalis* mite, a fatty acid binding proteins. *Int Arch Allergy Immunol* 1999; 119:181–4.
- Hewitt CRA, Foster S, Phillips C et al. Mite allergens: significance of enzymatic activity. *Allergy* 1998; 53:60–3.
- Shakib F, Schulz O, Sewell. A mite subversive: cleavage of CD23 and CD25 by Der p. 1 enhances allergenicity. *Immunol Today* 1998; 19:313–6.
- Mowat AM. The regulation of immune responses to dietary protein antigens. *Immunol Today* 1987; 8:93–8.
- Weiner HL, Friedman A, Miller A et al. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu Rev Immunol* 1994; 12:809–37.
- Khouri SJ, Hancoc W, Weiner HL. Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with down-regulation of inflammatory cytokines and differential up-regulation of transforming growth factor- β , interleukin-4 and prostaglandin E expression in the brain. *J Exp Med* 1992; 176:1355–64.
- Holt PG, Batty JE, Turner KJ. Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. *Immunology* 1981; 42:409–17.
- Hoyne GF, Jarnicki AG, Thomas WR, Lamb JR. Characterization of the specificity and duration of T-cell tolerance to intranasally administered peptides in mice: a role for intramolecular epitope suppression. *Int Immunol* 1997; 9:1165–73.
- Hoyne GF, Thomas WR. T-cell responses to orally administered antigens. Study of the kinetics of lymphokine production after single and multiple feeding. *Immunology* 1995; 84:304–9.
- Melamed D, Fishman-Lovell J, Uni Z, Weiner HI, Friedman A. Peripheral tolerance of Th2 lymphocytes induced by continuous feeding of ovalbumin. *Int Immunol* 1996; 8:717–24.
- Friedman A, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc Natl Acad Sci* 1994; 91:6688–92.
- Chen Y, Kuchroo VK, Inobe JI, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 1994; 265:1237–40.
- Chen Y, Inobe I, Marks R, Gonella P, Kuchroo VK, Weiner HL. Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* 1995; 376:177–80.
- Miller A, Lider O, Roberts AB, Sporn MB, Weiner HL. Suppressor T cells generated by oral tolerization to myelin basic protein suppress both in vitro and in vivo immune responses by the release of transforming growth factor- β after antigen-specific triggering. *Proc Natl Acad Sci USA* 1992; 89:421–5.
- Chung Y, Chang S-Y, Kang C-Y. Kinetic analysis of oral tolerance: memory lymphocytes are refractory to oral tolerance. *J Immunol* 1999; 163:3692–8.
- Torseth JW, Gregerson DS. Oral tolerance in experimental autoimmune uveoretinitis: feeding after disease induction is less protective than prefeeding. *Clin Immunol Immunopath* 1998; 88:297–304.
- Genain CP, Abel K, Belamir N et al. Late complications of immune deviation therapy in a non-human primate. *Science* 1996; 274:2054–7.
- Sato MN, Carvalho AF, Silva AO, Maciel M, Fusaro AE, Duarte AJS. Oral tolerance induced to house dust mite extract in naive and sensitized mice: evaluation of immunoglobulin E and IgG-IgE complexes. *Immunology* 1998; 95:193–9.
- Sato MN, Carvalho AF, Silva AO, Maciel M, Fusaro AE, Duarte AJS. Low dose orally administered antigen down-regulates the T helper type 2-response in a murine model of dust mite hypersensitivity. *Immunology* 1999; 98:338–44.
- Maciel M, Fusaro AE, Duarte AJS, Sato MN. Modulation of IgE response and cytokine production in Peyer's patches and draining lymph nodes in sensitized mice made tolerant by oral dust mite administration. *J Interferon Cytokines Res* 2000; 20:1057–63.
- Magnusson CG. Major differences in specificity among naturally occurring human IgG-subclass anti-IgE autoantibodies. *J Allergy Clin Immunol* 1995; 95:1011–9.
- Shakib F, Smith SJ, Pritchard DI. Do autoantibodies to IgE play a role in IgE-mediated events. *Immunol Cell Biol* 1995; 73:109–12.
- Yu Y, de Week AL, Stadler BM, Muller U. Anti-IgE autoantibodies and bee-sting allergy. *Allergy* 1995; 50:119–25.
- Koike T, Nawata Y, Tsutsumi A, Tomioka H. Anti-IgE auto-antibody in allergic disorders. *Allergy Today* 1987; 2:4–6.
- Pawankar R. Anti-IgE treatment in allergic disease. *ACI Int* 2001; 13:4–20.
- Marone G, Spadaro G, Palumbo C, Condorelli G. The anti-IgE/anti-Fc ϵ RI α autoantibody network in allergic and autoimmune diseases. *Clin Exp Allergy* 1999; 29:17–27.
- Corne J, Djukanovic R, Thomas L et al. The effect of intravenous administration of a chimeric anti-IgE antibody on serum IgE levels in atopic subjects: efficacy, safety, and pharmacokinetics. *J Clin Invest* 1997; 99:879–87.
- Mota I, Wong D. Homologous passive cutaneous anaphylaxis in the mouse. *Life Sci* 1969; 8:813–20.
- Jung S, Siebenkotten G, Radbruch A. Frequency of immunoglobulin E class switching is autonomously determined and independent of prior switching to other classes. *J Exp Med* 1994; 179:2023–6.
- Sonoda E, Matsumoto R, Hitoshi Y et al. Transforming growth factor β induces IgA production and acts additively with interleukin-5 for IgA production. *J Exp Med* 1989; 170:1415–20.
- Lutz CT, Bartholow TL, Greenspan NS et al. Molecular dissection of the murine antibody response to streptococcal group carbohydrate. *J Exp Med* 1987; 165:531–45.
- Rothman P, Lutzker S, Gorham B, Stewart V, Coffman R, Alt FW. Structure and expression of germline immunoglobulin γ 3 heavy chain transcripts: implications for mitogen- and lymphokine-directed class-switching. *Int Immunol* 1990; 2:261–7.

- 39 Mori JC, Pires MC, Galvão CES, Ferreira de Mello JF, Golcher FM, Montealegre F. Determination of *Blomia tropicalis*-specific IgE and IgG subclasses in atopic dermatitis patients. *Allergy* 2001; 56:180–4.
- 40 Feinstein A, Richardson N, Taussing MJ. Immunoglobulin flexibility in complement activation. *Immunol Today* 1986; 7:169–74.
- 41 Briles DE, Claflin JL, Schroer K, Forman C. Mouse IgG3 antibodies are highly protective against infection with *Streptococcus pneumoniae*. *Nature* 1981; 294:88–90.
- 42 Sato MN, Fusaro AE, Victor JR et al. Oral tolerance induction in *Dermatophagoides pteronyssinus*-sensitized mice induces inhibition of IgE-response and up-regulation of TGF- β secretion. *J Interferon Cytokines Research* 2001; 21:827–33.
- 43 Shakib F, Hooi SW, Smith SJ, Furmonaviciene R, Sewell HF. Identification of peptide motifs recognized by a human IgG autoanti-IgE antibody using a phage display library. *Clin Exp Allergy* 2000; 30:1041–6.
- 44 Hamelmann E, Cieslewicz G, Schwarze J et al. Anti-interleukin 5 but not anti-IgE prevents airway inflammation and airway hyperresponsiveness. *Am J Resp Crit Care Med* 1999; 160:934–4.