Bystander Effect in Synergy to Anergy in Oral Tolerance of Blomia Tropicalis/Ovalbumin Murine Co-Immunization Model

C. R. OLIVEIRA,1 E. A. F. TANIGUCHI,1 A. E. FUSARO,1 J. R. VICTOR,1 C. A. BRITO,1 A. J. S. DUARTE,1 and M. N. SATO1,2

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Oral tolerance is an important approach in allergic diseases and murine model can provide useful information to improve its understanding and therapeutic measures. To address the influence of non-related allergen sensitization in immunized mice with the mite Blomia tropicalis (Bt) or ovalbumin (OVA) or with both Bt/OVA allergens. Furthermore, we sought to verify oral tolerance effect in the Bt/OVA co-immunization model. Mice sensitized with Bt and then exposed to OVA developed an enhanced IgE response to both allergens; contrariwise, this effect was not observed when OVA-sensitization was prior to Bt-sensitization. Co-injection of Bt and OVA led to a dominant IgE response towards OVA over Bt, which was not observed when co-immunization was performed with a 240-fold less amount of OVA. Induction of oral tolerance with OVA, prior to co-immunization, suppressed IgE response to both allergens, probably as a consequence of the increased levels of IFN-γ found in these animals. The results evidenced that, depending on allergenic potential, new allergen exposure may exert an adjuvant effect to the first allergen used in the sensitization. The bystander suppression to non-related allergens through oral tolerance should be a useful mechanism to control sensitization to new allergens.

KEY WORDS: Blomia tropicalis; ovalbumin; co-immunization; IgE; cytokines; oral tolerance.

INTRODUCTION

The prevalence of atopy, allergic rhinitis, asthma and eczema has increased in recent decades. House-dust mites are the commonest cause of allergic sensitization and are considered to be one of the main factors for the increasing incidence in IgE-mediated sensitization among patients. The mite Blomia tropicalis (Bt) is the most important allergen for allergic diseases in tropical and subtropical regions (1, 2). However, despite the bulky work investigating mites, there are scarce data on type-I hypersensitivity to Bt in murine models (3, 4).

Besides genetic predisposition, exposure to multiple inhalant allergens in daily life of atopic individuals may contribute to polysensitization. It has been considered that house-dust mite sensitization and, at a lower degree, pollen sensitization, probably play a role in triggering the development of polysensitization in atopic asthmatic children (5). Currently, allergen immunotherapies that alter the natural course of disease can reduce symptoms and may prevent the evolution towards polysensitization (6, 7).

Oral tolerance is the physiologic mechanism that prevents hypersensitivity to food proteins and probably to commensal bacteria (8, 9). This ability, inherent to the mucosal immune system, has increased the interest in studying oral tolerance in autoimmune (10–12) and in allergic diseases (13–15). The phenomenon of oral tolerance, which leads to systemic immunologic unresponsiveness, involves at least three main mechanisms: anergy, clonal deletion and suppression (10, 11, 16–20). The antigen dose has been shown to be an important factor, as high oral dose leads to T cell clonal deletion or anergy and low antigen dose favors active suppression (10, 11, 16–20). The antigen dose has been shown to be an important factor, as high oral dose leads to T cell clonal deletion or anergy and low antigen dose favors active suppression (17, 21). Some regulatory T cells, involved in oral tolerance model, have been related to active suppression in vitro and in vivo through antigen-specific and effector non-specific manners (10, 21, 22). Bystander suppression occurs when an immune response to one epitope suppresses the response to another one by secreting suppressive cytokines that act unspecifically. Considering that oral tolerance could mediate bystander suppression and exert suppression to a
non-related antigen, it is an attractive strategy for therapy in allergic individuals.

In the present study, we addressed the influence of a non-related antigen exposure on the type-I hypersensitivity response to Bt or to OVA. Moreover, the effect on the IgE response when both allergens, Bt extract and OVA, were co-injected as well as oral tolerance induction to OVA in co-immunized mice were investigated.

MATERIAL AND METHODS

Animals

Female A/Sn mice 8–10 weeks old, bred in our own animal facility (São Paulo University Medical School), and Wistar Furth rats (RT1u), 3–4 months old, were used for passive cutaneous anaphylaxis (PCA) reaction.

Immunization Protocol

Groups of mice were injected subcutaneously (s.c.) with 20 µg of Blomia tropicalis extract (Bt, 37,650 units of biological equivalents/mL, kindly provided by the International Pharmaceutical Immunology-ASAC) in 6 mg Al(OH)₃. Mice were boosted intraperitoneally (i.p.) on the 14th and 21st day after immunization (d.a.i.) with 20 and 100 µg 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 use. A similar procedure was carried out for the ovalbumin immunization (OVA, Grade V, Sigma, St. Louis, MO). To assess the influence of a new allergen exposure on the ongoing sensitization to the mite Bt or to OVA, (i) groups of mice immunized with Bt extract were, 2 weeks later, subjected to the OVA-immunization protocol and (ii) groups of mice immunized with OVA were submitted to the Bt-immunization protocol 2 weeks later. Mice were bled at the end of each allergen-immunization protocol. Co-immunization was achieved through s.c. injection of Bt (20 µg) and OVA (20 µg) in 6 mg Al(OH)₃, and boosting was carried out through i.p. injection on the 14th d.a.i. with Bt (20 µg) and OVA (20 µg) in saline and, once again, on the 21st d.a.i. with 100 µg of each allergen, without adjuvant. Another group of co-immunized mice received fixed concentration of Bt extract (20 µg/0.2 mL) with decreased amounts of OVA (0.5, 0.166, or 0.08 µg) in the co-immunization. Control groups were immunized with equivalent concentrations of OVA.

Oral Antigen Administration Protocol

Mice were fed with a single 50.0 mg OVA dose in 0.5 mL of PBS or PBS alone by gavage under light anesthesia. The feeding was performed on the 5th day prior OVA-immunization or co-immunization with OVA and Bt extract. All groups were boosted on the 14th and on the 21st day after immunization.

Passive Cutaneous Anaphylaxis (PCA)

IgE antibodies were estimated by passive cutaneous anaphylaxis reaction as described by Mota and Wong (23). Briefly, 0.1 mL of serum dilutions from each mouse was inoculated intradermally on the shaved back of the rats. After 18 h, the rats received 0.5 mg of mite extract or OVA in 1.0 mL 0.5% Evans Blue by tail vein injection. One hour later, the rats were killed, and the reciprocal of the highest serum dilution that produced a spot larger than 5 mm in diameter was taken as the PCA titer.

Determination of Antibody Levels

An enzyme-linked immunosorbent assay (ELISA) was performed to detect IgG isotypes as previously described (3, 24). Briefly, 96-wells microplates (Costar, Cambridge, MA) were coated with 20 or 5 µg/mL of Bt extract or OVA, respectively, in 0.1 M carbonate–bicarbonate buffer, pH 9.5. The wells were blocked with PBS-0.5% bovine serum albumin (BSA Sigma, St. Louis, MO) before incubation of the serum samples for 1 h at 37°C and overnight at 4°C. Detection was performed with biotinylated anti-γ1 or anti-γ2a mouse chain antibodies (1:3000, Southern Biotechnology Ass, Birmingham, AL) and streptavidin-peroxidase (1:200,000, Sigma, St. Louis, MO) and TMB (3, 24). Briefly, 0.1 mL of serum dilutions from each mouse was expressed as antibody titers in reference to serial dilution of a titrated serum pool from immunized adult mice with high levels of specific antibodies.

Cytokine Measurements by ELISA

Spleens from mice were aseptically collected and pressed through a stainless steel wire mesh in RPMI 1640 culture medium (GIBCO BRL, Gaithersburg, MD), supplemented with 10% fetal calf serum (FCS, Hyclone III Lb., Inc., USA) and 10 mg/mL gentamicin. Single spleen cell suspensions were centrifuged on Ficoll–Hypaque gradient, washed and cultured in 96-wells microplate (Costar, Cambridge, MA) at 8.0 × 10⁵ cells/well in 0.2 mL of supplemented RPMI medium, Bt extract (100 µg/mL) or OVA (200 µg/mL, Sigma) for 24 and 72 h at 37°C in a humidified 5% CO₂ incubator; after which cell-free supernatants were stored at −70°C until cytokine assay. IL-2, IL-4, IL-10, IFN-γ (OPTEia™ Pharmingen, San Diego, CA) and TGF-β1 (Promega,