



Role of mast cell degranulation in the neural correlates of the immediate allergic reaction in a murine model of asthma

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Abstract

Experimental airway allergy in mice leads to increased activity in specific hypothalamic and amygdaloid nuclei, and behavioral changes. The experiments described here were designed to determine the role of anaphylactic antibodies, mast cell degranulation, and lung inflammation in the neural and behavioral correlates of an experimental murine asthma-like response. Animals were sensitized intraperitoneally with ovalbumin adsorbed to alum, and challenged by intranasal ovalbumin instillation or aerosol. To induce immunological tolerance, animals were fed ovalbumin in the drinking water for 5 consecutive days, along with primary sensitization. Depletion of IgE was also accomplished with a non-anaphylactic anti-IgE antibody. Mast cell degranulation was inhibited by cromolyn. In addition to BALB/c animals, C3H/HeJ mice were used for their relative resistance to lung allergic inflammation. We confirmed that ovalbumin challenge in allergic mice leads to increased activity in the paraventricular nucleus of the hypothalamus and central nucleus of the amygdala, and avoidance behavior towards an allergen-associated compartment. Moreover, these responses were precluded by oral tolerance or anti-IgE treatment, even in the presence of IgG1. Cromolyn abrogates both responses in the presence of anaphylactic antibodies. Finally, although sensitized C3H/HeJ mice did not develop airway inflammation, they exhibited brain and behavioral changes similar to BALB/c animals. The repercussions of murine allergic asthma on brain and behavior are IgE-dependent, mediated by mast cell degranulation, and do not require a pulmonary inflammatory infiltrate, suggesting that the early phase of this immediate allergic response suffices for the brain activation associated with avoidance behavior towards exposure to the allergen.

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1. Introduction

Asthma is a chronic respiratory disease characterized by intermittent airway obstruction, eosinophilic inflammation, airway hyperreactivity, and high levels of IgE. Its underlying mechanisms are complex and may vary, but appear to be altogether caused by an intrapulmonary allergen-driven T helper type 2 (Th2) response characterized by increased

secretion of cytokines (IL-4, IL-5, IL-13, and others) by CD4⁺ T lymphocytes (Sousa Mucida et al., 2003).

In asthmatic patients, the allergic reaction progresses in two distinct phases representing segments of the same phenomenon: an early response mediated by IgE and mast cells, and a late phase, during which Th2 lymphocytes, cytokines, and other cells build an inflammatory milieu that play a major role in the disease (Maddox and Schwartz, 2002).

Although the pathophysiology of asthma has been well characterized in humans and experimental models, little is known about its influence on brain activity and behavior. Descriptions of asthma triggered by artificial roses in allergic individuals, the ‘rose effect’, date to the 19 century

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(Mackenzie, 1886), and suggest an important role for immunoneuroendocrine interactions in asthma. Changes in emotional status and increased levels of anxiety are commonly associated with asthma crises (Lawrence, 2002; Lehrer et al., 1993); on the other hand, the distress associated with a recurrent disease can be responsible for generating pathological anxiety in patients suffering from long-term asthma (Dahlen and Janson, 2002; Rietveld et al., 2000).

Peripheral inflammation has long been implicated with changes in brain activity and behavior, thus influencing the outcome of several biological phenomena (Steinman, 2004). We previously reported some neural and behavioral correlates of allergic reactions using murine models of ovalbumin (OVA)-induced food allergy (Basso et al., 2003) and asthma (Costa-Pinto et al., 2005). Mice allergic to OVA avoided drinking an antigen-containing solution or entering a box compartment previously associated with OVA nebulization. Furthermore, OVA-sensitized animals displayed an increase in the activity of the paraventricular nucleus of the hypothalamus (PVN) and of the central nucleus of the amygdala (CeA) shortly after the OVA challenge (Basso et al., 2003; Costa-Pinto et al., 2005), represented by neurons stained for the Fos protein, a product of the immediate early gene and transcription factor *c-fos* (Sagar et al., 1988). These areas, the major collections of corticotropin-releasing factor (CRF)-expressing neurons in the central nervous system (CNS) (Gray, 1993), are involved in affective and emotional responses compatible to those of anxiety and avoidance in rodents (Menzaghi et al., 1993, 1994).

The neural and behavioral responses associated with food allergy are IgE-dependent, since abrogation of anti-OVA IgE antibodies prevented the development of food aversion and the increase in the activity of the PVN and CeA previously observed in this model (Basso et al., 2003).

In an attempt to further dissect the pathways associated to the crosstalk between allergic reactions and the CNS, we employed neonatal capsaicin destruction of C-sensitive fibers and were able to show that these neurons are partially involved in signaling food allergy to the mouse brain (Basso et al., 2001). Indeed, activation of the PVN by the allergic response was totally blunted by capsaicin, unlike that of the CeA. We also found a partial inhibition of Fos-staining in the nucleus of the solitary tract (NTS), an important relay station conveying visceral sensory information to the brain (Basso et al., 2004). The suggestion that C-sensitive fibers play a role in transmitting signals derived from allergic responses within the gastrointestinal tract or the airways to the brain is strengthened by the already reported intimate anatomical contact between mast cells and the nerve endings of those afferents in mucosal surfaces (Bienenstock et al., 1988a, 1991; Greene et al., 1988; Kieran, 1990; Stead et al., 1989; Williams et al., 1997).

The relevance of both IgE-dependent mechanisms and C-sensitive fibers for signaling allergic responses triggered in the gastrointestinal tract, with consequent behavioral changes and brain activation, prompted us to ask whether

asthma signaling to the mouse brain relies on IgE antibodies. Moreover, we focused on whether mast cell degranulation was crucial or not to these phenomena.

In this study we evaluated the activation of the PVN and CeA by Fos-staining (Sagar et al., 1988) and the development of aversion directed at the allergen, employing an adapted passive avoidance paradigm (Costa-Pinto et al., 2005), and asked whether asthma signaling to the mouse brain depends on IgE, employing a protocol of immunological tolerance that specifically blunts the IgE response (Russo et al., 2001), or depletion by a rat anti-IgE antibody (Haak-Frendscho et al., 1998). We also used an inhibitor of mast cell degranulation (Shishikura et al., 2006) to assess their relevance in these phenomena. Finally, using a mouse comparatively resistant to allergic airway inflammation (Brewer et al., 1999; Keane-Myers et al., 1998) we evaluated the role of pulmonary inflammatory cell infiltration on the neural and behavioral correlates of murine asthma.

2. Methods

2.1. Animals

Six- to eight-week-old male BALB/c or C3H/HeJ mice from our breeding unit at the Department of Pathology, School of Veterinary Medicine, University of São Paulo, Brazil, were housed accordingly in controlled temperature (22–26 °C) and artificially lighted rooms on a 12 h light–12 h dark cycle (lights on at 7:00 am) with free access to rodent chow and water. Experiments were carried out in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, based on the “Guide for the Care and Use of Laboratory Animals”, by the National Research Council, USA, always in the morning to minimize variation due to circadian rhythmicity.

2.2. Sensitization and airway challenge with OVA

We used two different protocols of sensitization and challenge (Fig. 1), as described before (Costa-Pinto et al., 2005). Protocol A consists of sensitization by two i.p. injections on days 0 and 7 with 4 µg OVA (grade V) adsorbed to 1.6 mg of aluminum hydroxide gel (alum) in 0.4 mL PBS (Sigma) followed by a single challenge on day 14 by instillation of 10 µg OVA in 50 µl saline delivered with the aid of a micropipette into the nostrils under light halothane anesthesia. Protocol B consists of a single i.p. sensitization with 4 µg OVA/1.6 mg of alum on day 0, followed by aerosol challenge on days 12, 13, and 14 by means of two 30-min nebulizations with 2.5% OVA in saline per day, 30 min apart, in

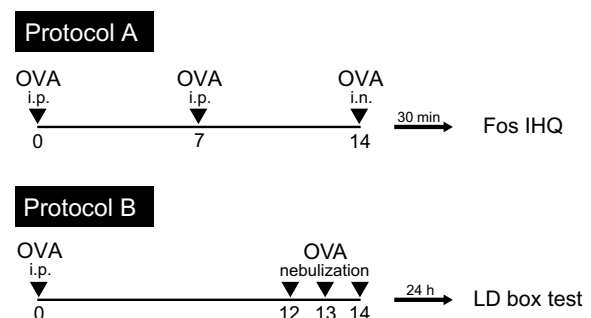


Fig. 1. Diagram illustrating protocols A and B used for sensitization and challenge.

their home cages. These protocols were used to fit particular requirements of each experiment. Protocol A ensures more accuracy in the route and dose of allergen delivery, and allows determining the immediate effects of a single challenge on brain activity. Protocol B resembles natural exposure to airborne allergens and ensures a manipulation-free challenge, minimizing the influence of stressful operator-induced changes in behavior. Blood samples for antibody quantification were collected 24 h after the single or last OVA challenge.

2.3. Antibody quantification by ELISA

OVA-specific serum IgG1 antibodies were assayed by ELISA as previously described by Russo et al. (2001). Nunc-Immuno Plates Maxi-Sorb (Inter-Med, Brazil) were coated overnight at 4 °C with 2 µg OVA per well, rinsed with (PBS)-Tween 0.05%, and blocked with 0.25% casein in PBS. Serum samples were added and incubated for 1 h at room temperature. Bound antibodies were detected by goat anti-mouse IgG1, followed by peroxidase-conjugate rabbit anti-goat IgG (H+L) antibodies (Southern Biotechnology, USA). The reaction was developed in 0.5 mg/mL OPD (Sigma) and 0.0015% H₂O₂ in pH 5.0, 0.1 M citric acid/sodium citrate buffer (Merck, Brazil), stopped with 4 M H₂SO₄, and read at 490 nm using a Multiskan EX (Thermo Labsystems, Finland). The concentration of antibodies was compared to IgG1 standards run in parallel. OVA-specific serum IgE was measured in plates coated overnight at 4 °C with 2 µg/mL of goat anti-mouse IgE antibody (Southern Biotechnology). Serum samples were added to the plate, followed by biotinylated OVA. Bound OVA was detected by extrAvidin peroxidase (Sigma) followed by 100 µl/well of the substrate described above. OVA-specific IgE levels of samples were compared to pooled sera of hyperimmunized BALB/c mice, arbitrarily assigned to 1000 U.

2.4. Fos immunohistochemistry

Animals sensitized and challenged according to protocol A were used for Fos immunohistochemistry in all experiments. Following the second sensitization, mice were gradually habituated to the anesthetic procedure required for nasal instillation: each animal was daily removed from its home cage, transferred into a similar box without bedding, and restrained by a transparent glass container for 1 min. Immunohistochemistry was performed on samples collected 90 min after nasal OVA challenge (Costa-Pinto et al., 2005). Mice were deeply anesthetized with xylazine and ketamine and immediately perfused intracardially with 25 mL of 0.1 M phosphate-buffered saline (PBS) followed by 100 mL of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Their brains were removed, post-fixed for 5 h, and cryoprotected overnight in 30% sucrose in PB. Frozen 30-µm coronal sections were cut and collected in PB. Floating sections were incubated overnight with an anti-Fos antibody (Ab-5, Calbiochem, USA) 1:1000 with 5% normal goat serum in 0.3% Triton X-100 PB. The sections were rinsed and incubated for 90 min with a goat anti-rabbit biotinylated antibody (Jackson, USA) 1:200 in 0.3% Triton X-100 PB, incubated in avidin–biotin (Vector, USA), and developed using diaminobenzidine and hydrogen peroxide. Positive neurons were counted by image analysis (ImageJ, NIH, USA).

2.5. Passive avoidance test

Animals sensitized and challenged according to protocol B were used in all behavioral studies. We used an adapted passive avoidance test (Costa-Pinto et al., 2005) based on the discrimination of a compartment that had been previously paired with ovalbumin challenge for the evaluation of the behavioral impact of antigen exposure in sensitized mice. On day 15, mice were given one training session, during which they were individually placed in the lit part of our test apparatus (the light-dark box, or LD) that consists of an acrylic box of dimensions 45 × 20 × 20 cm (*l* × *b* × *h*) divided in two compartments: one illuminated (by a 60 W bare bulb) comprising one third of its total area, and the other dark, constituting the remaining two thirds (Bourin and Hascoet, 2003). These two cham-

bers are connected by a sliding door (5 × 5 cm). The latency to the first entry in the dark compartment was recorded (cut-off 1 min). Upon passage to the dark compartment, the connecting door was closed and the animal received a 10-min nebulization with 2.5% OVA. On day 16, mice were exposed to the test session: they were reintroduced into the lit compartment and the latency for the passage to the dark chamber recorded (cut-off 5 min). Non-sensitized animals were sham-injected on day 0 and handled as sensitized mice for all other procedures.

2.6. Immunological tolerance to OVA

Oral tolerance to OVA was induced according to Russo et al. (2001), by offering the mice a 1% OVA solution in water (Sigma, grade II) for 5 consecutive days, prepared daily, from day 0 to 4. Average daily consumption of this solution reached 30–50 mg of OVA per animal. This protocol of oral tolerance has been shown to selectively inhibit the production of antigen-specific IgE, while maintaining levels of IgG1 similar to those found in non-tolerant, immunized mice (Russo et al., 2001).

2.7. Depletion of IgE antibodies

IgE antibodies were depleted by a single intravenous administration (200 µg/mouse, in a volume of 400 µl) of a non-anaphylactic anti-IgE antibody (rat anti-mouse, R95-32, Pharmingen, USA) 1 h before the second ovalbumin sensitization (day 7) for protocol A, or the first challenge (day 12) for protocol B. This antibody binds IgE in both soluble and bound to the FcεRI forms, but does not induce histamine release by IgE-sensitized rat basophilic leukemia cells (Haak-Frendscho et al., 1998). Control animals received isotype control rat IgG.

2.8. Inhibition of mast cell degranulation

Mice were treated with disodium cromoglycate (cromolyn) according to a protocol modified from Cieslewicz et al. (1999) by daily nebulization (20 mg/mL in saline) for 10 min, during 4 consecutive days. Mice submitted to protocol A were nebulized with cromolyn on days 11, 12, and 13, and 1 h before intranasal challenge of OVA (day 14). Mice submitted to protocol B were nebulized with cromolyn 1 h prior to each challenge (days 12, 13, and 14) and 1 h before the training session of the avoidance test (day 15).

2.9. Evans blue dye extravasation

The efficacy of cromolyn in inhibiting mast cell degranulation was indirectly evaluated by airway vascular permeability, measured with Evans blue (EB) as proposed by Sirois et al. (1988). Mice under light halothane anesthesia received Evans blue in saline intravenously (25 mg/mL in 0.9% NaCl; 10 µl per gram of body weight), along with 10 mg OVA in 50 µl of saline intranasally. After 10 min the animals were anesthetized with ketamine and xylazine, injected with 0.1 mL of sodium heparin, and perfused intracardially with 10 mL PBS. The airways were removed, rinsed in PBS, equally divided in two parts, and subsequently weighed in an analytical scale; one half-sample was dried for 24 h, at 37 °C, for determination of dry weight, and the other half was chopped and dipped in formamide (SIGMA, 4 mL/gram of tissue) for dye extraction, for 24 h at 37 °C. Extracted samples were read in triplicates at 620 nm (Titertek® Multiskan MCC340, ICN) and concentrations compared to standards of Evans blue in formamide. Vascular permeability was expressed as microgram Evans blue/g dry tissue.

2.10. Statistical analyses

All data were analyzed by *t* test or ANOVA followed by the Tukey post hoc test. Data were transformed in decimal logarithms when required. *p* < .05 was considered significant for all comparisons. Data from all experiments are represented as means ± standard deviation. SigmaStat version 3.0 for Windows® was used throughout the study.

3. Results

3.1. Effects of oral tolerance on brain activity and on avoidance behavior

Mice were divided in two groups: sensitized animals challenged with OVA (I+O), and tolerant animals, sensitized and challenged with OVA (T+O). Animals sensitized and challenged with OVA had high levels of anti-OVA IgE while tolerant mice failed to mount an IgE response (152.6 ± 73.9 and 11.4 ± 17.3 A.U., respectively; $t(25) = 6.958$, $p < .05$) (Fig. 2a). Nevertheless, non-tolerant animals presented levels of anti-OVA IgG1 similar to those obtained in tolerant mice (112.8 ± 19.6 , and 104.0 ± 29.5 $\mu\text{g/mL}$, respectively).

Sensitized animals (I+O) had a marked increase in Fos immunostaining in the PVN (152.5 ± 11.4 Fos-positive cells; $F(3,12) = 216.7$, $p < .05$) following challenge (Fig. 2b), while tolerant mice (T+O) displayed a pattern of activation (9 ± 1 Fos-positive cells) similar to that found with intranasal saline administered to tolerant or sensitized mice ($T+S = 6.4 \pm 2.2$, and $I+S = 6.9 \pm 1.3$ Fos-positive cells, respectively). Similar findings were observed for the CeA ($I+O = 106.4 \pm 11.3$; $T+O = 8.5 \pm 1.6$; $T+S = 12.1 \pm 5.6$; and $I+S = 7.2 \pm 0.9$ Fos-positive cells; $F(3,12) = 111.97$, $p < .05$) (Fig. 2c).

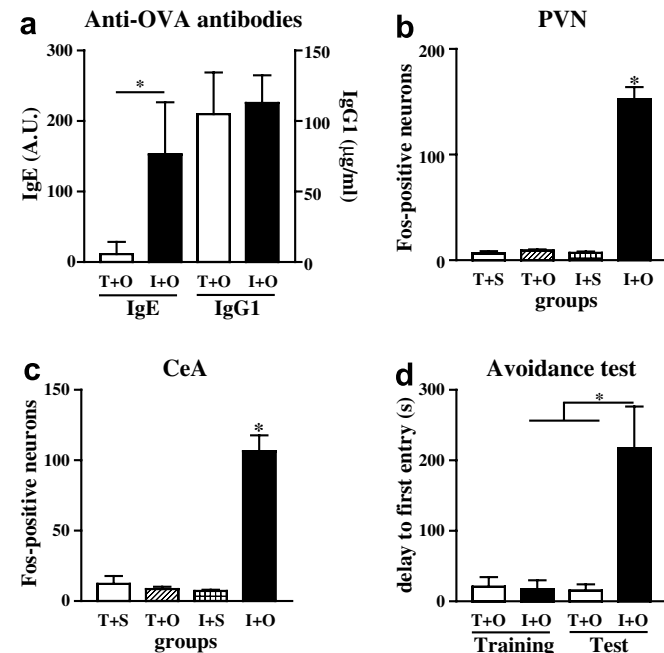


Fig. 2. Tolerance to ovalbumin. Tolerant mice challenged with ovalbumin (T+O) do not mount the IgE response to OVA, maintaining normal levels of anti-OVA IgG1 antibodies, compared to non-tolerant animals, sensitized and challenged with OVA (I+O) (a). Precluding the IgE response to OVA prevents the increase in activity of the PVN (b) and CeA (c), and the development of aversion to the OVA-associated compartment of the LD box (d). (a) $*p < .05$ for I+O compared to T+O, in regard to IgE levels (results combined from mice sensitized by protocols A and B; $n = 13$ –14 per combined group); (b) and (c) $*p < .05$ for I+O compared to all others ($n = 4$ per group); (d) $*p < .05$ for I+O on the test day compared to itself on the training day, or to T+O on the test day ($n = 9$ –10 per group).

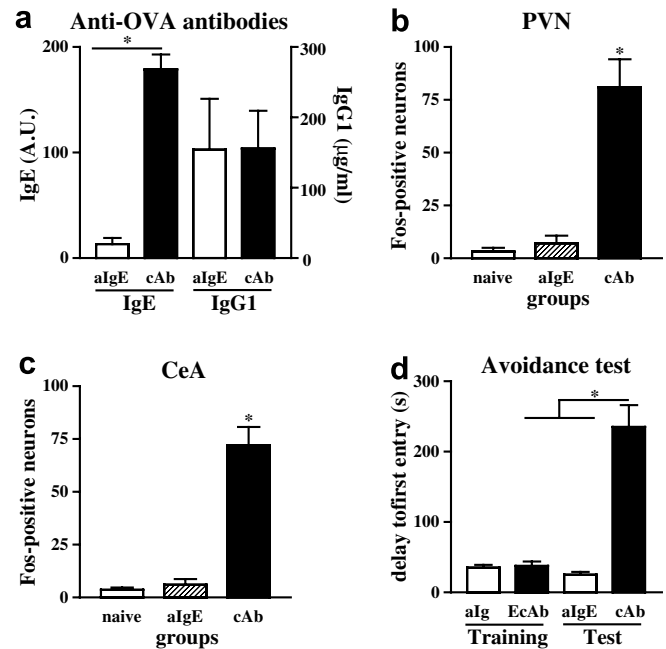


Fig. 3. Depletion of IgE. Prior treatment with a rat anti-mouse IgE antibody (aIgE) does not interfere with the IgG1 response to ovalbumin (a), but prevents the activation of the PVN (b) and CeA (c) in sensitized and challenged mice, and precludes the development of aversion to the OVA-associated compartment of the LD box (d). (a) $*p < .05$ for aIgE compared to cAb (mice treated with isotype control rat IgG), in regard to IgE levels (results combined from mice sensitized by protocols A and B; $n = 8$ –9 per combined group); (b) and (c) $*p < .05$ for cAb compared to naive and aIgE animals ($n = 5$ per group); (d) $*p < .05$ for cAb on the test day compared to itself on the training day, or to aIgE on the test day ($n = 4$ per group).

Accordingly, T+O mice behaved like I+O animals in the training session (20.6 ± 13.8 , and 17.4 ± 12.4 s, respectively) of the LD, but T+O mice did not avoid the dark compartment on the test session (15 ± 9.2 s), while I+O animals showed this avoidance response (217.1 ± 59.2 s) compared to themselves on the training session ($I+O$ on the training session = 17.4 ± 12.4 s, $t(18) = 10.448$, $p < .05$) or to T+O animals on the test session ($t(18) = 10.676$, $p < .05$) (Fig. 2d).

3.2. Effects of IgE depletion on brain activity and on avoidance behavior

Mice were divided in two groups: aIgE (sensitized with ovalbumin and treated with rat anti-IgE antibody) and cAb (sensitized with ovalbumin, and injected with isotype control rat IgG). Anti-IgE caused a marked reduction in circulating levels of anti-OVA IgE antibodies (aIgE = 13.3 ± 5.8 , and cAb = 178.7 ± 14.2 A.U.; $t(15) = 26.290$, $p < .05$) (Fig. 3a). Depletion of IgE completely blunted the increase in activity of the PVN (aIgE = 7 ± 3.6 , and cAb = 81 ± 13.2 Fos-positive cells; $F(2,12) = 65.073$, $p < .05$) and CeA (aIgE = 6 ± 2.7 , and cAb = 72 ± 8.7 Fos-positive cells; $F(2,12) = 81.439$, $p < .05$) (depicted in Fig. 3b and c, respectively) observed in allergic animals following challenge. Moreover, while cAb mice avoided the dark compartment of the LD on the test session (235 ± 31 s), aIgE animals did

not develop aversion to the OVA-associated place (25 ± 4 s) (Fig. 3d). Thus, cAb group on the test session differs from itself on the training session (235 ± 31 , and 37.5 ± 6.4 s, respectively; $t(6) = 12.439$, $p < .05$) and from the aIgE group on the test session (25 ± 4 s; $t(6) = 13.394$, $p < .05$).

3.3. Role of mast cells on brain activity and behavior of allergic mice

Cromolyn was used in order to assess the role of antigen-induced mast cell degranulation on the neural and behavioral correlates of an allergic response in the airways. Animals were sensitized (I) or not (NI) with OVA, and treated (Cro) or not (O) with cromolyn; all animals were challenged with OVA. Cromolyn had no influence on anti-OVA IgE or IgG1 antibody levels (data not shown).

Treatment with cromolyn completely inhibited the increase in Fos staining in the PVN (I+Cro = 3.6 ± 1.8 , and I+O = 120.9 ± 23.8 Fos-positive cells; $F(3,13) = 102.11$, $p < .05$) (Fig. 4a) and CeA (I+Cro = 2.3 ± 1.8 , and I+O = 87.2 ± 28.1 Fos-positive cells; $F(3,13) = 58.654$, $p < .05$) (Fig. 4b) of animals from group I+O, compared to I+O; cromolyn alone had no influence on this parameter

since mice from groups NI+Cro and NI+O displayed similar patterns of staining.

Cromolyn also prevented the development of avoidance in the LD box. While mice from all groups quickly moved to the dark compartment on the training session, only I+O mice avoided it on the test session (259.5 ± 64.3 s). Prior treatment with cromolyn (I+Cro) precluded the development of aversion to the OVA-associated compartment (Fig. 4c). Thus, I+O on the test session differs from itself on the training session (259.5 ± 64.3 , and 10.8 ± 8.8 s, respectively; $t(10) = 9.380$, $p < .05$) and from the I+Cro group on the test session (22.3 ± 13.5 s; $t(11) = 9.579$, $p < .05$).

The efficacy of cromolyn inhibition of mast cell degranulation was assessed by EB extravasation. Cromolyn inhibited from 43.2% to 46.3% (depending of the protocol used for sensitization and challenge) of the increase in pulmonary vascular permeability following OVA challenge in sensitized mice consistent with previous findings (Fig. 4d). It is noteworthy that cromolyn prevented roughly half of the increase in vascular permeability (I+Cro = 124 ± 27 , and I+O = 231 ± 50.9 μ g EB/g dry tissue, using Protocol A; $F(2,12) = 77.931$, $p < .05$; I+Cro = 172.1 ± 23.4 , and I+O = 303.3 ± 18 μ g EB/g dry tissue using Protocol B; $F(2,14) = 49.038$, $p < .05$), even though I+Cro mice still had higher EB extravasation than naïve animals (naïve = 38.6 ± 9.1 μ g EB/g dry tissue using Protocol A, and 30.3 ± 6.9 μ g EB/g dry tissue using Protocol B).

3.4. Involvement of inflammatory cells on the neural and behavioral correlates of allergy

We used C3H/HeJ mice in order to analyze the role of allergic inflammatory cell infiltration because these animals develop a very mild lung inflammatory response (Brewer et al., 1999). Mice were sensitized (I) or not (NI) with ovalbumin, and challenged with saline (S) or ovalbumin (O). Interestingly, the levels of anti-OVA IgE (I+O = 165 ± 73 , and NI+O = 2.5 ± 2.3 A.U.; $t(11) = 4.369$, $p < .05$) and IgG1 (I+O = 102.3 ± 11.4 , and NI+O = 6 ± 2.6 μ g/mL; $t(15) = 16.413$, $p < .05$) were similar to those found in BALB/c animals (Fig. 5a), but they did not display any allergic infiltration of inflammatory cells to their lungs (Fig. 6).

Regarding brain activity and behavior, C3H/HeJ mice behaved similarly to BALB/c animals following OVA challenge. Allergic mice displayed more Fos-positive neurons in the PVN (I+O = 115.8 ± 7.8 , NI+O = 3.8 ± 0.9 , and I+S = 3.4 ± 1.1 Fos-positive cells; $F(2,9) = 253.87$, $p < .05$) and in the CeA (I+O = 76.2 ± 10.5 , NI+O = 3.6 ± 2.4 , and I+S = 4 ± 1.6 Fos-positive cells; $F(2,9) = 49.038$, $p < .05$) (Fig. 5b and c, respectively), and developed aversion (I+O = 270.2 ± 45.8 s) to the compartment previously associated with OVA nebulization (Fig. 5d) compared to themselves on the training session (12.6 ± 9.5 s; $t(8) = 12.303$, $p < .05$) or to non-sensitized animals challenged with OVA on the test session (NI+O = 8.6 ± 8.5 ; $t(8) = 12.549$, $p < .05$). These responses are thus observed in the presence of anti-OVA IgE levels comparable to those found in sensitized

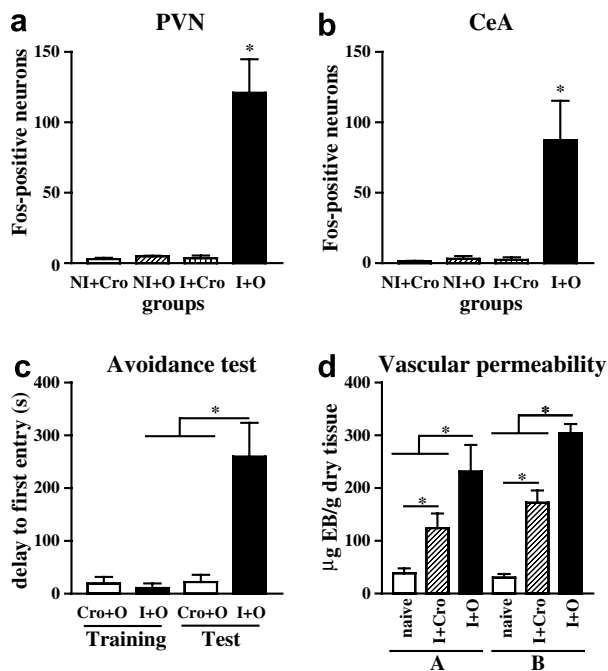


Fig. 4. Inhibition of mast cell degranulation. Sensitized mice treated with cromolyn (I+Cro) displayed no increase in activity of the PVN (a) or CeA (b), as seen in untreated sensitized animals (I+O), nor developed aversion to the OVA-associated compartment (c); cromolyn in protocols A or B partially prevents the increase in vascular permeability in the airways of allergic animals (d). (a) and (b) $p < .05$ for I+O compared to all others ($n = 4-5$ per group); (c) $p < .05$ for I+O on the test day compared to itself on the training day, or compared to I+Cro on the test day ($n = 6-7$ per group); (d) $p < .05$ for I+O compared to I+Cro and to naïve animals, and $p < .05$ for I+Cro compared to naïve animals, using either protocol of sensitization and challenge (a or b; $n = 5$ per group, or $n = 4-7$ per group, respectively).

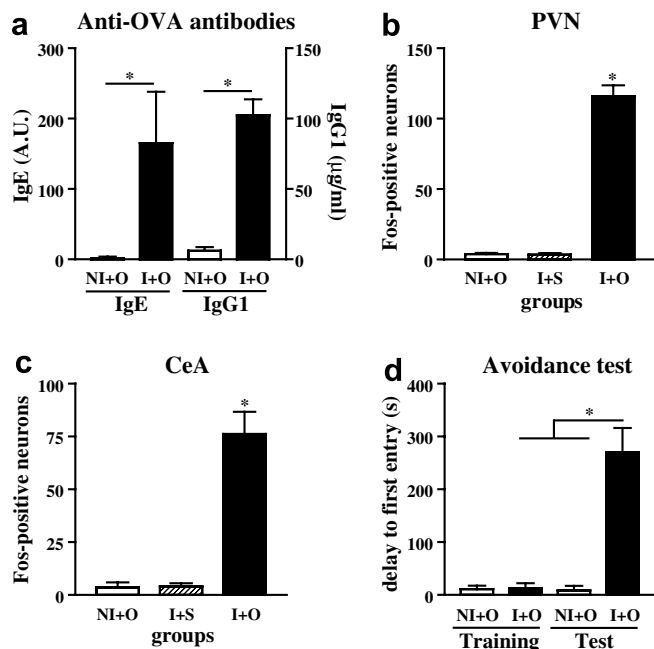


Fig. 5. Role of inflammatory cell infiltrate. Sensitized and challenged C3H/HeJ mice (I+O) mount IgE and IgG1 antibodies to ovalbumin (a), and display an increase in the activity of the PVN (b) and CeA (c) compared to non-sensitized animals challenged with ovalbumin (NI+O), similar to that seen in BALB/c mice. Allergic C3H/HeJ mice also develop aversion to the OVA-associated compartment of the LD box (d). (a) $*p < .05$ for I+O compared to NI+O (results combined from mice sensitized by protocols A and B; $n = 4-9$ per combined group, or $n = 4-13$ per combined group, respectively); (b) and (c) $*p < .05$ for I+O compared to NI+O and immunized animals receiving intranasal saline—I+S ($n = 4$ per group); (d) $*p < .05$ for I+O on the test day compared to itself on the training day, or to NI+O on the test day ($n = 5$ per group).

BALB/c animals, but in the absence of a pulmonary inflammatory infiltrate.

4. Discussion

We previously described the neural and behavioral correlates of murine asthma and food allergy, and also showed that food allergy signaling to the mouse brain is dependent on IgE (Basso et al., 2003) and partially relies on C-sensitive fibers (Basso et al., 2001), employing the nucleus of the solitary tract as a relay station (Basso et al., 2004). This allergic reaction leads to increased activity of brain areas associated with emotional and affective responses that are compatible with anxiety and aversion to antigen-containing solutions.

Here we focused on whether the responses observed following airway challenge in allergic mice depend solely on the early events in allergy, more specifically antigen-induced IgE-dependent mast cell degranulation. We found that IgE-mediated mast cell degranulation plays a pivotal role in signaling an asthma-like response to the mouse brain. Surprisingly, a lung allergic response such as eosinophilic inflammation is neither required nor sufficient to influence brain activity or behavior.

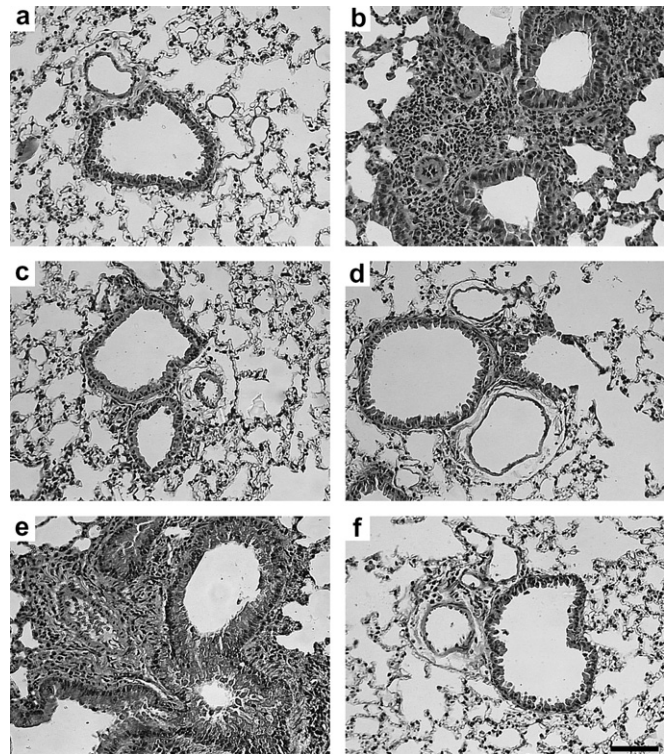


Fig. 6. Representative sections of mouse lung following multiple challenges with ovalbumin, according to protocol B described in Section 2. Non-sensitized (a) or sensitized (b) BALB/c mouse. (c) Tolerant BALB/c mouse. (d) BALB/c mouse treated with anti-IgE antibody. (e) BALB/c mouse treated with cromolyn. (f) Sensitized C3H/HeJ mouse. Hematoxylin and eosin staining; scale bar = 100 μm.

Emotional and behavioral changes have long been associated with allergy, probably ever since the description of the “rose effect” (Mackenzie, 1886). Nonetheless, a solid neuroimmune basis for these interactions had been controversial. The early phase of allergic reactions, for its promptness, has been chosen as a working hypothesis for signaling the brain during the elaboration of neural and behavioral responses in allergy (Basso et al., 2003; Costa-Pinto et al., 2005).

The early phenomena in allergic reactions rely on IgE-mediated, allergen-driven cellular release of mediators (Dombrowicz et al., 1993). Nonetheless, murine mast cells can also be activated by IgG1 antibodies (via low affinity FcγRIII receptors) (Hazenbos et al., 1996). Thus, mast cell degranulation can be triggered by antigen binding to anti-OVA IgE or IgG1 (Miyajima et al., 1997; Oshiba et al., 1996). The neural and behavioral responses described here are exclusively IgE-dependent since they were precluded by anti-IgE treatment or an oral tolerance protocol known to prevent the IgE response without inhibiting the production of specific IgG1 (Russo et al., 2001).

Mast cells are key players during the early phase of allergies since degranulation by allergen cross-linking of FcεRI (high-affinity IgE receptors) culminates in a massive release of mediators: preformed amines, eicosanoids, and others, with potent and rapid effects on blood vessels and broncho-

pulmonary smooth muscle cells. This explains the majority of symptoms experienced by asthmatic patients during the early phase (Maddox and Schwartz, 2002).

Mast cells have been found in close contact with nerve fibers (Heine and Forster, 1975; Jabonero, 1959; Stead et al., 1989), supporting an important interaction between these structures in many tissues (Bienenstock et al., 1988a,b; Greene et al., 1988; Kiernan, 1990; Williams et al., 1997). This communication is bi-directional: neurons change their firing rate following mast cell degranulation (Greene et al., 1988), and activation of mast cells may follow electrical stimulation and depolarization of nerves (Kiernan, 1990). These findings suggest a feedback role for mast cells and nerves, particularly capsaicin-sensitive afferents (nonmyelinated sensory C-fibers) in the modulation of immunoneuroendocrine crosstalk (Theoharides, 1990).

C-fibers, representing the majority of vagal afferents innervating the airways (Lee et al., 2003), have been involved in vascular and bronchial changes during allergy (Saria, 1988), and inhalation of chemical irritants or pollutants (Wang et al., 1996). Several aspects of human asthma are mimicked by vagal stimulation, administration of substance P or neurokinin A. Peptides released from tracheo-bronchial C-fibers participate in anaphylactic responses, and in other inflammatory airway conditions (Lundberg and Saria, 1983; McDonald, 1987; Saria, 1988). Moreover, vagal electrical stimulation concomitant to antigenic challenge increases the release of histamine by mast cells into the airway circulation (Leff et al., 1986), and potentiates allergic reactions in dogs.

Changes in short-circuit electrical current triggered by antigenic challenge in sensitized rat tracheal mucosa are completely blocked by doxantrole (inhibitor of mast cell degranulation), and decreased 50% by neonatal capsaicin, implicating mast cells and C-sensitive fibers on local regulation of nerve firing and mast cell activity (Bienenstock et al., 1988b). Mast cells in the trachea and bronchi degranulate following vagal stimulation; capsaicin or transection of this nerve rostral to the nodose ganglion prevent this response (Kiernan, 1990) suggesting a role for central reflexes involving substance P-containing neurons in bronchoconstriction.

Peripheral inflammation is commonly associated with changes in brain activity and behavior (Kent et al., 1992). Because behavioral data employing the LD box was acquired from animals that received multiple OVA challenges, we had to consider the possibility of an ongoing lung inflammatory infiltrate that could underlie the described changes in behavior. Using C3H/HeJ mice we showed that site-specific infiltration of immune cells is not required for the responses described here. Moreover, cromolyn-treated mice develop lung inflammation, without brain or behavioral correlates of allergy, reinforcing that an inflammatory infiltrate did not suffice to signal the mouse brain.

The involvement of discrete brain regions in humans during exacerbation of asthma has been described (Rosenkranz et al., 2005). We show the relevance of areas

within the limbic system, particularly the hypothalamus and amygdala. Since our data were collected soon after the first site-specific contact with the allergen, our results could represent an existing target of immune-to-brain signaling, before conditioning, learning, and late-phase reactions take place, which could explain some differences observed.

Perception of asthma by patients with long-term disease can greatly influence their perspective regarding quality of life. Understanding modulation of emotions and behavior by immunity is relevant for the decisions on the therapeutic approach to control the disease, but also improve the support on psychological disorders associated with chronic allergy. Building a solid basis of the mechanisms that underlie immunoneuroendocrine interactions in allergy weakens the long-held concept that immune modulation of brain and behavior is merely a reminiscent of old anecdotes.

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