

Short Communication

Food aversion: A critical balance between allergen-specific IgE levels and taste preference

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ABSTRACT

Animals sensitized to allergens change their feeding behavior and avoid drinking the otherwise preferred sweetened solutions containing the allergens. This phenomenon, known as food aversion, appears to be mediated by allergen-specific IgE antibodies. Here we investigated food aversion in BALB/c and C57BL/6 mice, which differ in their allergic responses to the allergen ovalbumin as well as in their preference for sweet taste. BALB/c mice present higher levels of IgE and a natural lower preference for sweet flavors when compared to C57BL/6 mice. Specifically, we studied a conflicting situation in which animals simultaneously experienced the aversive contact with the allergen and the attractive sweet taste of increasing concentrations of sucrose. We found that BALB/c mice were more prone to develop food aversion than C57BL/6 mice and that this aversive behavior could be abolished in both strains by increasing the palatability of the solution containing the allergen. In both strains food aversion was positively correlated with the levels of allergen-specific IgE antibodies and inversely correlated with their preference for sucrose sweetened solutions.

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1. Introduction

Accumulating evidence indicates a robust bi-directional interactions between the immune and nervous systems (Ader and Cohen, 1975; Hahm et al., 2004; Pacheco-Lopez et al., 2005; Wrona and Trojanar, 2003). Food allergy and other allergic diseases have emerged as major public health problems due to their dramatic increase over the past two to three decades. Food allergy affects 6% of young children and 4% of adults (Sicherer and Sampson, 2009). We and others have reported an association between food allergy and behavioral changes (Basso et al., 2003; Crayton, 1986). Specifically, using mouse models and OVA as allergen to induce food or airway allergy we found that soon after allergen challenge sensitized mice show higher levels of anxiety, increased Fos expression in emotionality-related brain areas, and aversion to solutions containing OVA (Basso et al., 2003; Costa-Pinto et al., 2005).

The gastrointestinal tract seems to be a suitable milieu for neuro-immune interactions due to the wide neural network (Clerc and Furness, 2004) and the extensive collection of gut associated lymphoid tissue. The pioneer work by Cara et al. (1994) showed

that BALB/c mice sensitized to OVA developed an aversion to the ingestion of saccharin-sweetened solutions containing the allergen. These authors also showed that food aversion can be passively transferred to naïve mice by injecting hyper-immune serum from allergic mice (Cara et al., 1994, 1997). Additionally, Basso et al. showed that OVA given by gavage to sensitized mice leads to strong activation of emotionally-related brain areas such as paraventricular nucleus of the hypothalamus (PVN) and central nucleus of amygdala (CeA) (Basso et al., 2003). Behavioral changes (food aversion and increased anxiety) as well as brain activation appear to be dependent on IgE antibodies because the administration of non-anaphylactic anti-IgE antibodies abrogated food aversion (Basso et al., 2003).

The model of food aversion used in these previous studies, known as two-bottle preference test, gives the mouse the options of drinking water and an artificially sweetened solution containing OVA. Non-allergic animals prefer to drink the saccharin-sweetened solution instead of water while allergic mice avoid drinking the sweetened solution containing the allergen. A possible drawback in using artificial sweeteners (e.g. saccharin) is that when in high concentrations they provoke a repulsive bitter flavor. Consequently, a conflicting choice between the attractive sweet taste (palatability) and the neuro-immune consequences of ingesting the allergen may not be fully appreciated. To circumvent this

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limitation, we used increasing concentrations of a natural sweetener (sucrose) to achieve more palatable solutions.

Furthermore, another variable investigated in the present study was the genetic background, since allergic responses are known to differ among mouse strains. Here we used BALB/c and C57BL/6 mice, which differ in their allergic responses to OVA. In BALB/c allergic responses are more dominated by IL-4 than IL-5 comparing to C57BL/6 mice (Drazen et al., 1996; Hogan et al., 1997). Moreover, it was shown that these strains also differ in their sweet taste. BALB/c mice present a reduced preference for sweet substances when compared with C57BL/6 mice (Lush, 1989).

We demonstrated that BALB/c mice developed higher OVA-specific IgE responses than C57BL/6 upon immunization. Consecutively, we showed that both strains presented food aversion when the allergen-solution was sweetened with saccharin, being this behavior more evident in BALB/c than in C57BL/6 mice. When sucrose was used instead of saccharin, BALB/c mice presented food aversion even at high sucrose-containing OVA-solution while C57BL/6 mice lost the aversive behavior when the allergen-solution was sweetened with higher concentrations of sucrose. By manipulating IgE levels in both strains through OVA-specific IgE transfer or through diet, it was confirmed that both IgE levels and taste preference influence food aversion behavior. We conclude that development of food aversion is critically dependent on the levels of OVA-specific IgE antibodies as well as on the taste preference for sweet solutions. Altogether our work indicates that the behavioral adaptive response, namely food aversion, involves finely refined mechanisms.

2. Material and methods

2.1. Animals

Six- to eight-week-old male BALB/c and C57BL/6 mice were obtained from our colony at the Department of Pathology, School of Veterinary Medicine-USP, São Paulo, Brazil. The animals were housed accordingly, in a controlled temperature (22–26 °C) and artificially lighted room on a 12 h-light–12 h-dark cycle (lights on at 7:00 a.m.) with free access to rodent chow and water. Animals were housed and used in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo; these guidelines are similar to those of the National Research Council, USA.

2.2. Sensitizations

2.2.1. Active OVA sensitization

Six- to eight-week-old mice were sensitized on days 0 and 14 by an intra-peritoneal injection of 4 µg Grade V chicken egg OVA (Sigma, St. Louis, MO) adsorbed to 1.6 mg of aluminum hydroxide gel (OVA/alum), suspended in PBS to a total volume of 0.4 mL. Control animals received 0.4 mL of PBS intra-peritoneally on days 0 and 14.

2.2.2. Passive sensitization by hyper-immune serum transfer

To obtain hyper-immune serum BALB/c or C57BL/6 mice were sensitized by OVA/alum given on days 0 and 7 and challenged weekly with i.p. injections of 4 µg of OVA. BALB/c mice received two OVA challenges (days 14 and 21) and C57BL/6 received four OVA challenges (days 14, 21, 28 and 35). Trunkblood was collected 5 days after the last OVA challenge and the serum from each strain was pooled for determination of OVA-specific IgE titers. The titer obtained for both strains was approximately 1.400 AU/mL. For the passive sensitization, naïve animals received 200 µL of pooled

hyper-immune serum, via ocular sinus. Thus, each recipient animal received a total dose of 280 AU of anti-OVA IgE (immune transferred group). Control animals (control transferred group) received 200 µL of serum from naïve animals.

2.3. Two-bottle preference test

Control and sensitized animals were housed individually 3 days before the preference test. Each individual cage was supplied with two identical bottles, both filled with water. On the day of the test, one bottle was filled with a sweetened solution containing 20% of egg-white. Natural and artificial sweeteners were used in different concentrations; 0.02% of saccharin and 1%, 4%, 8% or 16% of sucrose. The second bottle remained filled with water. Both bottles were offered to the animals during 24 h for the preference test. The bottles' position was switched every 4 h during the first 12 h of the test to avoid place preference. The total solution intake was measured in grams, by the difference in bottles weight before and after the test. The results are expressed using the quotient of OVA-solution intake (in grams) divided by the animal weight (in grams) in order to minimize the effects of body weight on OVA-solution consumption. The two-bottle preference test was performed on day 21 for actively sensitized mice and 24 h after serum transfer for passively sensitized mice. Sera from all animals were collected via ocular sinus just after the preference test for IgE measurements.

2.4. Determination of OVA-specific IgE

Serum OVA-specific IgE was assayed by sandwich ELISA as previously described (Russo et al., 2001). Briefly, plates were coated with anti-IgE (SouthernBiotech) and serum was added in 1/10 dilution in PBS. Subsequently, biotin-labeled OVA was added to the wells. The bound OVA-biotin was revealed by extravidin-peroxidase (Sigma). OVA-specific IgE levels of samples were deduced from an internal standard obtained from pooled sera of hyperimmunized mice that was arbitrarily assigned as 1000 U.

2.5. Statistical analyses

Statistical significances within each experiment were determined using Student *t*-test, comparing control and sensitized groups. Welch correction was used when necessary. Differences were considered significant when *p* was <0.05 ($\alpha = 0.05$). Results were expressed as means $\pm$ SD.

3. Results

3.1. Food aversion is more pronounced in BALB/c than in C57BL/6 mice

Previous studies showed that allergic BALB/c mice avoid the consumption of otherwise preferred saccharin-sweetened solution containing the allergen (Basso et al., 2003). Using a similar model, we first compared the feeding behavior of C57BL/6 and BALB/c mice. OVA-sensitized mice from both strains faced the choice of drinking water or OVA-containing solution with 0.02% saccharin. Allergic C57BL/6 as well as BALB/c mice showed food aversion since they consumed significantly less sweetened allergen-solution than the corresponding control groups (Fig. 1). However, food aversion was more pronounced in BALB/c mice. While allergic C57BL/6 mice consumed roughly 3-fold less allergen-solution than the control group (0.08 versus 0.22, $p < 0.05$) allergic BALB/c mice strongly avoided the allergen-solution (0.03 $\pm$ 0.01), consuming 5-fold less than the control group (0.15 $\pm$ 0.04). It is also important to note that non-sensitized C57BL/6 mice showed a slight higher

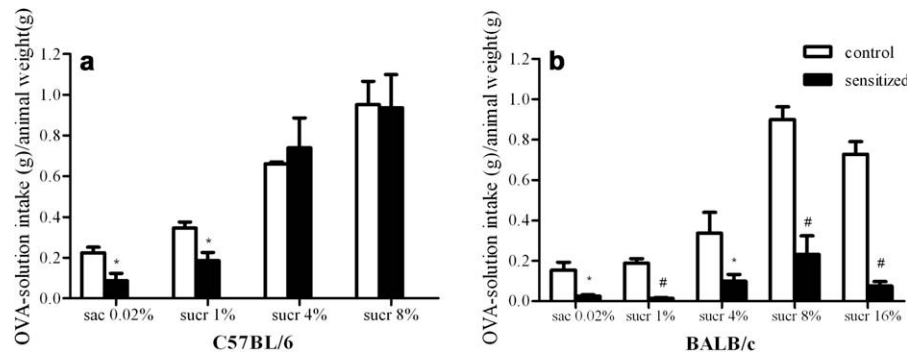


Fig. 1. Two-bottle preference test of control and sensitized C57BL/6 or BALB/c mice using different sweetened OVA-solutions. Mice were sensitized twice with OVA/alum, on days 0 and 14. Control animals received only PBS. On day 21, animals were submitted to the preference test. (a) Control and sensitized C57BL/6 mice were allowed to drink water and OVA-solution containing saccharin (sac.) 0.02%, sucrose (sucr.) 1%, 4% or 8%. $n = 5$. (b) Control and sensitized BALB/c mice were allowed to drink water and OVA-solution containing saccharin 0.02% sucrose 1%, 4%, 8% or 16%. $n = 5$. T-test significant difference between immune and control group: * $p < 0.05$ and # $p < 0.001$. Values represent OVA-solution intake (measured in grams) divided by individual animal weight (in grams). Numbers represent means $\pm$ SD. "n" Refers to the number of animals per group.

preference for sweet flavor as they consumed significantly more saccharin solution than BALB/c control group (0.22 versus 0.15, respectively; $p < 0.05$). The number of animals used per group was five ($n = 5$).

After establishing the protocol of food aversion in C57BL/6 mice to OVA-solutions containing saccharin, the same protocol was performed substituting saccharin by a natural sweetener (sucrose) in different concentrations, 1%, 4%, 8% or 16%. The concentration of saccharin could not be increased due to the increasing bitterness that is associated with higher concentrations of artificial sweeteners.

Fig. 1 shows that at 1% of sucrose, food aversion was observed in allergic mice from both strains. This behavior was, once again, stronger in BALB/c than in C57BL/6. Allergic BALB/c mice showed a 15-fold decrease in the consumption of the sweetened OVA-solution compared to the control group (0.01 versus 0.19) (Fig. 1b). Differently, the ingestion of allergen-solution observed in allergic C57BL/6 mice was less than 1-fold lower than control group, 0.35 and 0.19, respectively (Fig. 1a). Once more, it is important to notice that C57BL/6 strain presented a significant higher preference for sweet solutions compared to BALB/c mice. Basal consumption of the sweetened solution for control C57BL/6 mice was 0.35, compared to 0.19 for control BALB/c animals ($p < 0.001$). The number of animals used per group was five ($n = 5$).

The sucrose concentration was then increased in order to improve the palatability of the allergen-solution offered to the animals. This improvement in palatability was demonstrated by the increased consumption of sweetened solution by control animals from both strains (Fig. 1a and b). At 4% sucrose, food aversion was observed in allergic BALB/c mice but not in allergic C57BL/6 mice. At 8% and 16% sucrose solution, BALB/c mice still exhibited an intense food aversion. The BALB/c control group's intake of 8% sucrose egg-white solution was 0.90 ± 0.06 , whereas allergic mice ingested only 0.23 ± 0.09 (Fig. 1b). Similar values were found when animals faced a 16% sucrose OVA-solution (control animals consumed 0.73 and sensitized animals consumed 0.08). It is important to notice that at 8% sucrose the difference between strains' taste preference for the sweet flavors was no longer observed. Control BALB/c mice consumed as much as control C57BL/6 animals (0.90 and 0.95, respectively). The number of animals used per group was five ($n = 5$).

Altogether, these results clearly showed that food aversion in BALB/c mice is maintained despite increasing the palatability of the allergen-solution whereas in C57BL/6 mice this behavior is lost. Hence, we can conclude, that food aversion in both strains is a bal-

ance between intensity of avoidance towards the antigen-containing solution and the preference for sweet flavor taste.

3.2. Levels of IgE are critically involved in food aversion behavior

Immunological food aversion appears to be triggered by IgE (Basso et al., 2003). We found that OVA-specific IgE antibodies were roughly 7-fold higher in allergic BALB/c (693 ± 282 AU, $n = 10$) than in allergic C57BL/6 mice (104 ± 49 AU, $n = 10$) (Fig. 2a). Non-sensitized animals did not present detectable titers of OVA-specific IgE ($n = 5$). Therefore, one explanation for the difference in food aversion displayed by BALB/c and C57BL/6 could be the levels of OVA-specific IgE antibodies. In order to test this hypothesis we investigated food aversion in animals where the immune system was dietetically manipulated.

We have previously shown that a protein-free amino acid-based diet (Aa diet) increases IgE levels (Menezes et al., 2003; Mucida et al., 2004). The Aa diet contains the equivalent amounts of amino acids present in a 15% casein diet and its detailed composition has been previously published (Reeves et al., 1993). Mice were kept on Aa diet from weaning (3 weeks of age) and they were submitted to experimental procedures when reached the adult age (6–8 weeks).

Thus, we performed the same allergic protocol and consequent two-bottle preference test in C57BL/6 mice fed on a Aa diet. Indeed, the levels of OVA-specific IgE in allergic animals were significantly increased comparing to IgE titers present in sensitized animals fed on a standard chow (Fig. 2b). Allergic C57BL/6 mice fed on an amino acid-based diet presented 450 ± 211 AU ($n = 7$), 4-fold higher than animals fed on standard diet (104 ± 49 AU, $n = 10$). Non-sensitized animals did not present detectable titers of OVA-specific IgE.

Food aversion was observed when performing the two-bottle preference test using 1% sucrose in animals fed on amino acid-based diet. Allergic C57BL/6 mice ingested 0.11 ± 0.04 and control mice consumed 0.29 ± 0.03 (Fig. 3a, $n = 5$). However, food aversion in C57BL/6 fed on protein-free was more intense than in animals fed on standard chow (Fig. 1b). Allergic C57BL/6 mice fed on amino acid-based diet consumed 3-fold less than control group whereas allergic mice fed on standard chow solution ingested only 1-fold less than control mice. Surprisingly, at both 4% and 8% sucrose solution, allergic mice still ingested significantly less than control mice ($n = 5$). Therefore, differently from C57BL/6 fed on a standard chow, C57BL/6 fed on a protein-free diet maintained food aversion behavior when facing high sucrose allergen-solutions. We conclude that in the presence of higher

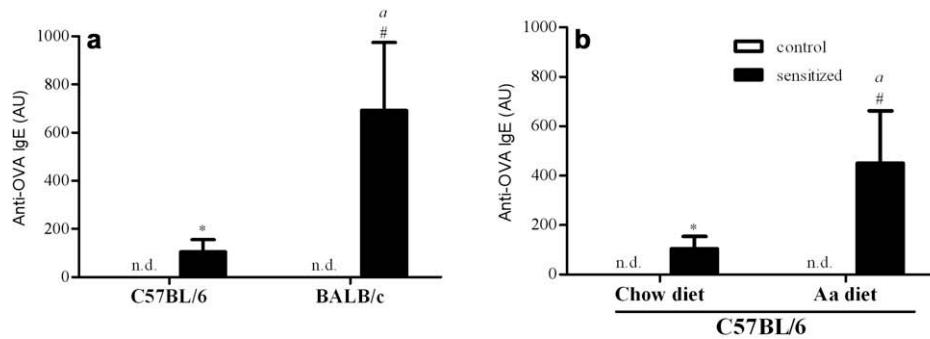


Fig. 2. Serum levels of anti-OVA IgE. Mice were sensitized twice with OVA/alum, on days 0 and 14. Control animals received only PBS. On day 21, animals were submitted to the 24 h two-bottle preference test and bled soon after the test for anti-OVA IgE measurements. IgE levels were measured by ELISA, according to the protocol described in Section 2. (a) Anti-OVA IgE levels of control and sensitized mice from C57BL/6 and BALB/c strains. Control groups, $n = 5$. Immune groups, $n = 10$. (b) Anti-OVA IgE levels of control and sensitized C57BL/6 fed on a chow or on a protein-free amino acid-based (Aa) diet. Immune group fed chow diet: $n = 10$. Immune group fed Aa diet: $n = 7$. Control groups: $n = 5$. Control animals showed non-detectable (n.d.) levels of anti-OVA IgE. Significant difference between control and sensitized animals: * $p < 0.05$ and # $p < 0.001$. Values represent antibodies titers in arbitrary units (AU), represented by means $\pm$ SD. "n" Refers to the number of animals per group.

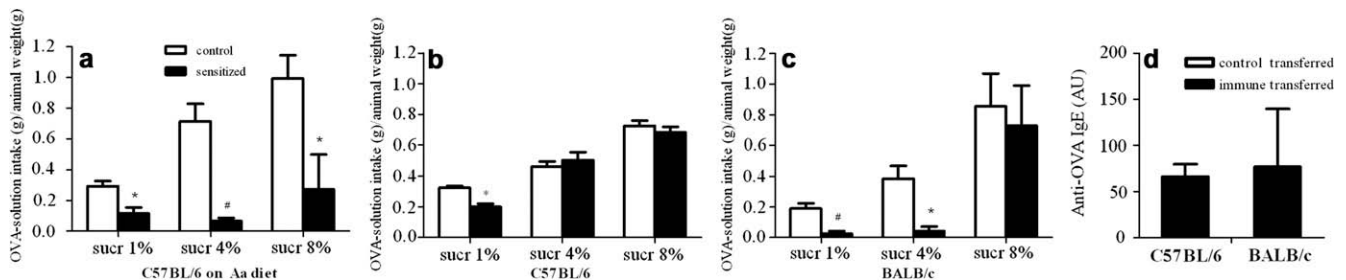


Fig. 3. Manipulation of IgE levels by protein-free amino acid-based diet (Aa diet) or by hyper-immune serum transfer. (a) C57BL/6 mice were fed on a protein-free amino acid-based diet from weaning. Thirty days after weaning, animals were sensitized twice with OVA/alum on days 0 and 14; control animals received only PBS. On day 21, animals were allowed to drink water or OVA-solution containing 1%, 4% or 8% of sucrose. $n = 5$. (b) Na C57BL/6 mice received immune serum (immune transferred) or control serum (control transferred), intra-venously. Twenty-four hours later animals were allowed to drink water or OVA-solution containing 1%, 4% or 8% of sucrose. $n = 4$. (c) Na BALB/c mice received immune serum (immune transferred) or control serum (control transferred), intra-venously. Twenty-four hours later animals were allowed to drink water or OVA-solution containing 1%, 4% or 8% of sucrose. $n = 5$. (d) Serum levels of anti-OVA IgE of immune transferred C57BL/6 and BALB/c mice soon after the two-bottle preference test. IgE levels were measured by ELISA, according to the protocol described in Section 2. $n = 5$. Value represent OVA-solution intake (measured in grams) divided by individual animals weight (in grams). Numbers represent means $\pm$ SD. Significant difference between immune and control groups. * $p < 0.05$ and # $p < 0.001$. "n" Refers to the number of animals per group.

levels of specific IgE, food aversion behavior is enhanced in C57BL/6 mice.

3.3. Taste preference modulate food aversion behavior

In order to directly investigate the relation between food aversion, OVA-specific IgE levels and taste preference, experiments with passive transfer of IgE antibodies and different concentrations of sucrose were performed. The dose of OVA-specific IgE selected to be transferred was around 280 AU, which is higher than the titers presented by actively sensitized C57BL/6 (104 ± 49 AU, Fig. 2a) and lower than the titers presented by actively sensitized BALB/c mice (693 ± 282 AU, Fig. 2a).

Immune serum was transferred to naïve C57BL/6 and BALB/c mice (immune transferred animals). Control animals received serum from naïve mice. The two-bottle preference test was performed 24 h after the transfer. The titers of anti-OVA IgE were measured in immune transferred animals after the preference test (i.e., 48 h after the transfer). C57BL/6 mice presented 66.5 ± 13.3 AU and BALB/c mice presented 77 ± 62.5 . Ten days later no anti-OVA IgE was detected in immune transferred animals (data not shown).

At 1% of sucrose, we found that BALB/c mice that received immune serum consumed six to seven-times less sweetened OVA-solution than the control group (0.19 versus 0.03, $n = 4$) (Fig. 3c). The food aversion behavior was also observed in C57BL/

6. Animals transferred with immune serum consumed 1.5-fold less than the control group (Fig. 3b, $n = 4$). By increasing the sucrose concentration to 4%, passively transferred BALB/c mice maintained food aversion and ingested 10-times less sweetened OVA-solution than control animals (Fig. 3c, $n = 4$). In contrast, C57BL/6 mice at 4% sucrose lost food aversion (Fig. 3b, $n = 4$). Interestingly, at 8% sucrose allergic BALB/c mice consumed as much allergen-solution as control mice. Therefore, food aversion behavior was for the first time abolished in BALB/c mice (Fig. 3c, $n = 4$). These results strongly suggest that by establishing similar levels of OVA-specific IgE antibodies, food aversion is critically dependent on the preference for the solution containing the allergen, which varies with the genetic background and thus the animal strain.

4. Discussion

Allergies are on the rise in westernized countries. Experimental animal models permitted a tremendous understanding of the bi-directional interactions between the immune and nervous systems. An emerging picture of the interactions of allergy and brain is that allergens can affect profoundly the levels of anxiety, increased Fos expression in emotionality-related brain areas, and induce food aversion to sweetened solution containing the allergen (Basso et al., 2003). Also, we have previously shown using a model of experimental asthma that OVA-sensitized mice, differently from

control animals, hesitated entering a dark chamber in which the allergen had been previously nebulized (Costa-Pinto et al., 2005). In both models, the dark–light exploration and the food preference, non-allergic mice chose the naturally preferable options, i.e. the dark environment or the sweet solution. In contrast, sensitized animals faced a conflicting situation where the positive stimulus was associated with the allergen. The decision of avoiding the positive stimulus, because this would implicate the contact with the allergen, is the behavioral adaptive response resultant of a complex and finely controlled process involving immune, endocrine, genetic, individual and environmental factors.

In this context, the present work aimed to study whether food aversion could be influenced by the genetic background and taste preference. We used BALB/c and C57BL/6 mice because these strains have been well characterized in OVA allergic models and they show different preferences for sweet flavors. Our results suggest that food aversion can be modulated and the decision of avoiding the contact with the allergen depends on the reward associated with it.

We first found that food aversion was induced in OVA-sensitized BALB/c and C57BL/6 mice when applying two-bottle preference test using saccharin as artificial sweetener. However, food aversion was more pronounced in BALB/c than in C57BL/6 mice, since the difference between control and sensitized groups was higher in BALB/c than in C57BL/6 mice.

When we tested food aversion using different sucrose concentrations, we found that BALB/c mice showed a more sustained food aversion than C57BL/6 animals, which lost food aversion at 4% sucrose. Because food aversion was more intense in BALB/c mice, it is possible that this behavior was due to their lower preference for sweet taste, the positive stimulus used in the protocol. This fact could facilitate animals' decision of avoiding the contact with the allergen. At 8% sucrose, the difference in solution intake between controls C57BL/6 and BALB/c mice was no longer observed. It has been reported that differences in sweet preference among strains are less evident at high concentrations of sweeteners, including sucrose (Capeless and Whitney, 1995; Dotson and Spector, 2004).

Therefore, it is possible that IgE levels, that play a key role in food allergy, are responsible for the difference observed in these mouse strains. Indeed, the levels of OVA-specific IgE antibodies were 7-fold higher in BALB/c when compared with C57BL/6 mice. To circumvent this aspect we passively transferred fixed levels of anti-OVA IgE to recipients of both strains. We chose a dose of OVA-specific IgE (280 AU) that is lower than that reached in the serum of allergic BALB/c animals (693 AU), but still higher than obtained in allergic C57BL/6 mice (104 AU). In these experiments BALB/c mice again displayed more aversion to allergen-containing solution than C57BL/6 mice, but food aversion was lost when we increased the palatability of OVA-containing sucrose solution. We conclude by these findings that circulating IgE levels also play a critical role in establishing food aversion.

The difference in sweet taste avidity is apparently related with T1R3 receptor protein, encoded by *Tas1r3* gene, involved in transduction of sweet taste (Max et al., 2001). It has been shown that the effects of *Tas1r3* genotype on behavioral responses to many sweeteners tend to be largest at low and intermediate concentrations but diminish as sweetener concentration increases (Inoue et al., 2007). Natural preference for sweet taste (and possibly for umami taste) in mammals seem to be determined by polymorphisms in the *Tas1r3* gene, which widely varies among strains. In this regard, C57BL/6 has been referred to as a taster genotype, whereas BALB/c mice are classified as a non-taster genotype (Ninomiya and Imoto, 1995). The critical 'to drink or not to drink' scenario during immune-mediated food aversion could be even more complicated since, at least in humans, T1Rs assemble in

heteromeric G-coupled receptors that transduce either sweet (T1R2+3) or umami (T1R1+3) taste. Since it is not entirely clear what are the sensors for the bitter taste in artificial sweeteners, or whether this heteromeric receptors are crucial in mice senses, other taste receptor combinations cannot be ruled out yet in our paradigm.

It is important to emphasize that animals facing solutions with high concentrations of sucrose ingested a large amount of liquid, roughly 5-fold more than their normal daily intake (average of 5 mL per day).

Altogether, even though we focus on the role of two different driving forces, namely natural preference for a sweet taste, in intensity and robustness of immune-induced aversion for an antigen-laced solution, in determining animal behavior, a physiological crosstalk and possible modulation of senses must also be taken into account. Adequate motivation for a specific stimulus can modify several internal parameters (collectively described by Cabanac as the '*milieu interieur*') changing the setpoints of physiological systems, including the senses. Thus, the word alliesthesia was proposed to describe the phenomenon of internal signals (that could be represented here by immune-mediated food aversion) modifying the sensations aroused by peripheral receptors (Cabanac, 1971). Therefore, the experimental design described here may have not only shown a critical balance between two equally important biological responses, but also evidenced a more complex crosstalk, in which the very sensorial response triggered by a sweet taste can be modulated by an immune response.

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