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Sensitization and Elicitation of an Allergic Reaction to Wheat Gliadins in Mice

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We developed a mouse model of allergy to wheat flour gliadins, a protein fraction containing major wheat allergens. We compared the antibody responses (i.e., specific IgE and IgG1) and the profiles of cytokines secreted by reactivated splenocytes induced after intraperitoneal injections of gliadins in three strains of mice, namely, Balb/cJ, B10.A, and C3H/HeJ. The intensities of the allergic reactions elicited by intranasal challenge were also compared. Both the sensitization and elicitation were the highest in Balb/cJ mice, whereas weak or no reaction was observed in the others strains. Interestingly, the specificity of the mouse IgE against the different gliadins (i.e., α-, β-, γ-, ω1,2-, and ω5-gliadin) was similar to that observed in children allergic to wheat flour. Balb/cJ mice may thus provide a relevant model for the study of sensitization and elicitation by wheat gliadins and for improving our understanding of the specific role and mechanisms of action of the different classes of gliadins.

KEYWORDS: Wheat; allergy; gliadins; IgE; mouse model; Balb/cJ

INTRODUCTION

Wheat is involved in different types of adverse reactions, e.g., baker's asthma, food allergy, and celiac disease. The prevalence of food allergy to wheat (WFA) has increased during the past decade, in both children and adults (*1*−*4*). In the Isle of Wight and Berlin cohorts, wheat appeared as a frequent food allergen for children (*5, 6*). The associated clinical symptoms differ in adults and children. While atopic eczema/dermatitis syndrome (AEDS) occurs mainly in children, urticaria (U) and wheat-dependent exercise-induced anaphylaxis (WDEIA) are mostly observed in adults (*1*).

On the basis of their solubility, wheat flour proteins are divided in albumins and globulins (AG) that are soluble in water and saline buffers and in gliadins and glutenins that are insoluble. All were shown to be involved in WFA (*7*−*9*). Gliadins are divided into α- and β-, γ-, and ω-gliadins which represent 44−60%, 30−45%, and 6−20% of total gliadins, respectively (*10*). ω gliadins are composed of ω1,2- and ω5-gliadin. The gliadin fraction contains several allergens involved in WFA: ω5-gliadins represent major allergens responsible for anaphylaxis and/or urticaria (*7, 9, 11, 12*); α and β-gliadins are frequently recognized by IgE of children with AEDS; and recently, anti-γ- and -ω2-gliadin specific IgE were detected in sera from patients who developed hypersensitivity to wheat protein hydrolysates (*13*).

Relevant animal models are of interest for improving our understanding of the mechanisms of allergic sensitization and elicitation of symptoms. Because of the current knowledge of murine immunology and the availability of genetically well characterized strains and reagents, mouse has been widely used to study the induction and polarization of the immune response toward a Th1, Th2, or Th17 phenotype. Schematically, IgE production in mice is induced by IL-4 and IL-13 secreted by Th2 cells. IgE response is accompanied by IgG1 production which is also induced by IL-4. On the other hand, Th1 cells produce IFNγ that induces T-cell-mediated immunity and IgG2a production and downregulates Th2 cells (*14*). Th17 cells have been described to be involved in autoimmune diseases (*15*).

The Balb/cJ strain is a Th2-biaised high-IgE responder strain. It has been widely used for sensitization to food allergens, e.g., cow's milk proteins (including β-lactoglobulin), peanut proteins, or ovalbumin. A specific IgE response was then obtained after both intragastric and intraperitoneal (i.p.) administration (*16*−*19*). Intraperitoneal administration of ovalbumin or bovine β-lactoglobulin in Balb/cJ mice induced IgE responses specific to the same epitopes as those involved in human allergic patients (*19, 20*). This strain was also used for the study of the early and late phase of the allergic reaction after i.p. sensitization and airway challenge. A link between the structural features of the protein and the physiopathology of the allergic reaction was thus demonstrated (*17*). C3H/HeJ mice can also be sensitized to milk and peanut (*21*−*24*). This strain is considered to be less able to

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develop oral tolerance to food and is then highly susceptible to developing food allergy likely because it is deprived of "Toll-like" receptor 4 (TLR 4) (*25, 26*), although recent reports on TLR4 may suggest other mechanisms (*27*). Kozai et al. successfully sensitized B10.A female mice with wheat-fractionated proteins adsorbed on alum and showed that gliadins and glutenins were mainly involved in WDEIA (*28*).

Since relevant animal models are missing, we compared the capacity of three different strains, namely, Balb/cJ, C3H/HeJ, and B10.A, to be sensitized with gliadins and to elicit an allergic response upon challenge to allow further analysis of the role and mechanisms of action of the different gliadins in wheat allergy.

MATERIALS AND METHODS

Apparatus and Reagents. Enzyme immunoassays were performed in 96-well microtiter plates (Immunoplate Maxisorb, Nunc, Roskilde, Denmark) using specialized Titertek microtitration equipment from Labsystems (Helsinki, Finland). Unless otherwise stated, all reagents were of analytical grade and from Sigma (St. Louis, MO).

Protein Extraction and Purification. Gliadins were extracted from wheat flour (cultivar Hardi), using the sequential procedure of Osborne et al. (*29*) adapted by Nicolas et al. (*30*). Briefly, the albumin−globulin fraction of the flour was removed by several washings using saline buffer; the flour was then suspended in 70% ethanol for 1 h at room temperature to solubilize the gliadins. After centrifugation (20000*g* for 20 min at 4 °C), the supernatant, i.e., the whole gliadin extract (GE), was collected and freeze-dried. The presence of the different proteins in the GE was assessed using acid-polyacrylamide gel electrophoresis (PAGE). GE was dissolved (1 mg/mL) in 0.025 M acetic acid buffer containing 20% (v/v) glycerol and 6 M urea. Ten micrograms was loaded on an acid-PAGE gel (*31*). SDS−PAGE under nonreducing and reducing conditions was also performed to detect a possible contamination by glutenins.

The different wheat gliadins (α, β, γ, ω1,2, and ω5) were prepared using several steps of chromatography (*8*). The purity and composition were analyzed by SDS−PAGE and reversed-phase HPLC (*7*).

WFA Patients. Sera were obtained from 38 WFA patients (21 children and 17 adults from service of clinical immunology and allergology, A. D. Moneret-Vautrin, Nancy hospital) suffering from atopic dermatitis, (AD), asthma (Ast), urticaria (U), or wheat-dependant exercise-induced anaphylaxis (WDEIA). Sera were sampled from patients during prick-in-prick (PIP) tests to natural wheat flour. Wheat allergy was confirmed one month later by a positive double-blind placebo-controlled food challenge (DBPCFC) after a wheat-free diet of 3 weeks or by an evident positive effect of avoidance diet when DBPCFC could not be performed for ethical reasons. DBPCFC corresponded to the consumption every 20 min of increasing doses of wheat flour included in apple sauce or of placebo (corn starch or fecula from potatoes) (*32, 33*). Blood collection, PIP, and DBPCFC were performed with informed consent of the patients or their parents, and after ethical committee approval.

Fluorimetric Enzyme-Linked Immunosorbent Assay (F-ELISA) for Quantification of Gliadin Specific Human IgE. The quantification of human IgE specific for GE and of purified gliadins was performed as described by Bodinier et al. (*34*).

Mice Sensitization. Three-week-old female Balb/cJ mice from the Centre d'Elevage René Janvier and 4−5-week-old female B10.A and C3H/HeJ mice, both from Harlan, were fed a semisynthetic diet deprived of plant proteins. This diet was essentially composed of starch potato, casein, sucrose, mineral, and vitamin mixes and was prepared at INRA (Unité de Préparation des Aliments Expérimentaux, Jouy-en-Josas, France). The absence of gluten was verified using a specific competitive ELISA (data not shown). Mice were housed in filtered cages under classical specific pathogen-free (SPF) husbandry conditions and were acclimated for 2−3 weeks before immunizations. All experiments were performed according to the European Community rules of animal care and with permission 91-122 of the French Veterinary Services.

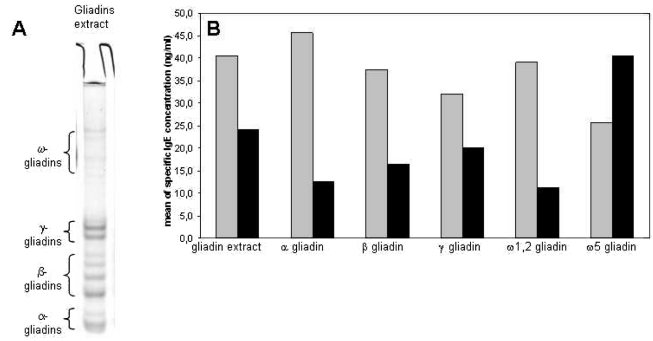

Figure 1. Analysis of the protein composition of the whole gliadin extract (GE) by electrophoresis (**A**) and of the IgE binding capacity of purified gliadins using sera of patients with WFA (**B**). Acid-PAGE was performed to analyze the composition and purity of the gliadin extract (A). A F-ELISA was performed to quantify specific IgE (nanograms per milliliter) against the five purified gliadins and the whole gliadin extract (GE) in patients with food allergy to wheat. The allergic studied population consisted of 17 adults and 21 children with atopic dermatitis (AD), asthma (Ast), urticaria (U), or wheat-dependant exercise-induced anaphylaxis (WDEIA). The means of specific IgE concentrations are represented for children (gray bars) and adults (black bars).

Before use, freeze-dried GE was solubilized in 70% ethanol at 5 mg/mL and then slowly diluted at 0.2 mg/mL in sterile phosphate-buffered saline (PBS, Gibco, Invitrogen). Intraperitoneal immunizations with 10 or 20 μg of GE adsorbed onto alum (Alhydrogel 3%, Superfos Biosector als) were performed on days 0, 10, 20, and 30 (0.2 mL per mouse). Six to eight mice per dose and per strain were used. Control mice ($n = 6-8$ per strain) received alum with PBS following the same protocol.

Assessment of Specific Anti-Gliadin IgE and IgG1. The efficiency of mice sensitization was evaluated by the concentration of anti-gliadin specific IgE and IgG1 produced. Determinations were performed in individual serum samples collected from the retro-orbital venous plexus on day 26 (after three i.p. injections) and day 39 (after four i.p. injections). Gliadins in solution in 50% ethanol (1 mg/mL) were slowly diluted in 50 mM carbonate buffer (pH 9.6) at 5 μg/mL and then used to coat 96-well microtiter plates (Maxisorp Nunc, Roskilde, Denmark). ELISA determinations were then performed as previously described (*35*).

Specific IgE against each purified wheat gliadin (i.e., α-, β-, γ-, ω1,2-, and ω5-gliadin) were assayed similarly but with pooled sera from each group using plates coated with the purified allergens instead of GE.

Elicitation of the Allergic Reaction. On day 40, all mice were challenged by intranasal administration of 50 μL of a solution of GE in PBS (0.2 mg/mL) prepared as previously described for the sensitization studies. The solution was slowly applied to one nostril under light anesthesia (Isoflurane, Baxter), with the aid of a micropipette. On day 41, mice were deeply anaesthetized by i.p. injection (200 μL/mice) of a cocktail containing Ketamine (15 mg/mL) and Xylazine (2 mg/mL) (Imalgène 500, Merial, Lyon, France; Rompum 2%, Bayer Pharma, Puteaux, France). The trachea was cannulated, and bronchoalveolar lavage fluids (BAL) were collected in sterile saline and kept in ice until determinations of cellular influx, particularly eosinophilia, and Th1/Th2 cytokines (*17*). Total cell counts were performed after Trypan Blue exclusion on Malassez cells, and differential cell counts were performed after cytocentrifugation and staining with Diff-Quick stain (LaboModerne). Aliquots of BAL were centrifuged; supernatants were collected and stored at −80 °C. Th1 and Th2 cytokines (i.e., IL-2, IL-4, IL-5, IL-10, IL-12, GM-CSF, IFNγ, and TNFα) were assayed using BioPlex technology and a mouse Th1/Th2 cytokine kit from Bio-Rad, following the manufacturer's recommendations. All BALs were analyzed individually.

Gliadin Specific Activation of Cytokine Production by Spleen Cells. After collection of BAL, anesthetized mice were sacrificed by vertebral dislocation and spleen cells were harvested. After lysis of

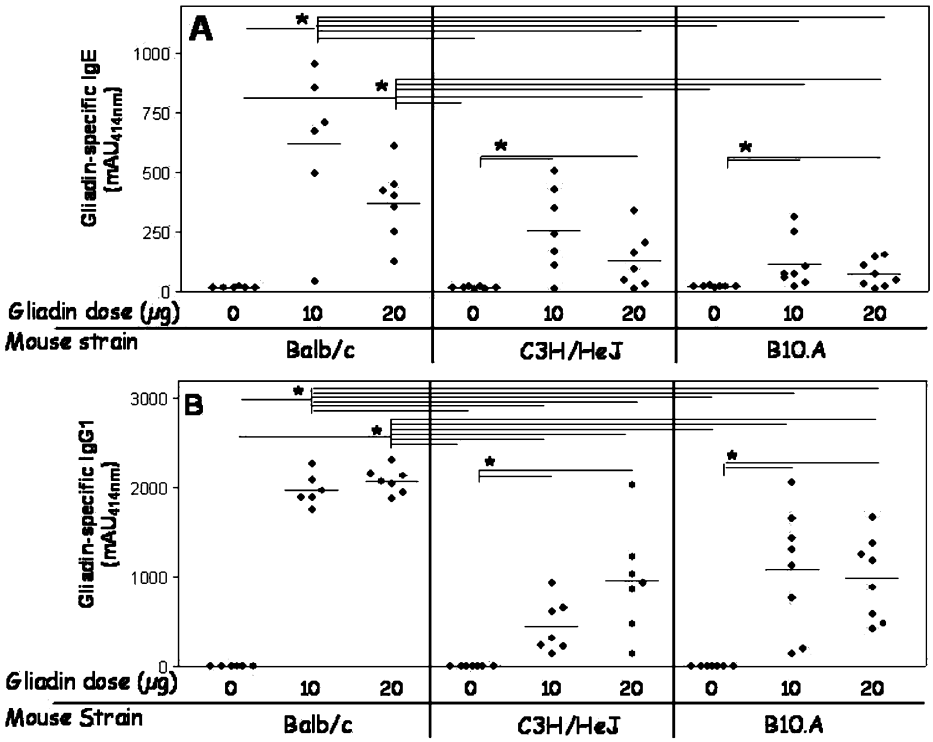

Figure 2. Anti-gliadin specific IgE (**A**) and IgG1 (**B**) induced in three different strains of mice after four i.p. administrations of the whole gliadin extract (GE) adsorbed on alum. Intraperitoneal immunizations with 10 or 20 μg of GE adsorbed onto alum were performed on days 0, 10, 20, and 30. The injected volume was 0.2 mL per mouse. Six to eight mice per dose and per strain were used. Control mice ($n = 6-8$ per strain) received alum with PBS following the same protocol (noted as 0 μg). Sera were collected on day 39, and specific antibodies were assayed on plates coated with GE as described in Materials and Methods. Results are expressed as absorbance units at 414 nm. An asterisk indicates $p < 0.05$ (Kruskal–Wallis and Mann–Whitney U test).

red blood cells (180 mM NH_4Cl and 17 mM Na_2EDTA) and several washes, pooled splenocytes were resuspended in RPMI-10 [RPMI supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (all from Gibco)]. Cells were incubated in 96-well culture plates (10^6 cells/well) in the presence of GE (20 μg/mL) for 60 h at 37 °C (5% CO_2). PBS and ovalbumin (20 μg/mL) were used as negative controls, and concanavalin A (1 μg/mL) was used as a positive control. Supernatants were then collected and stored at −80 °C until further assay. Th1/Th2 cytokines (i.e., IL-2, IL-4, IL-5, IL-10, IL-12, GM-CSF, IFNγ, and TNFα) were assayed using BioPlex technology and the mouse Th1/Th2 cytokine kit from Bio-Rad, following the manufacturer's recommendations.

Statistical Analyses. A Kruskall–Wallis nonparametric analysis followed by a Mann–Whitney U test was used to analyze the differences between IgE and IgG1 production and eosinophil influx in each group (e.g., Balb/cJ vs C3H/HeJ vs B10.A mice) for each dose (0, 10, or 20 μg) used for sensitization P values of <0.05 were considered statistically significant. An analysis of variance and Dunett's post test were used to interpret the differences in cytokine production in the BAL among three mouse strains.

RESULTS

Composition of the Gliadin Extract and Specificity of Patient IgE against Gliadins. The composition of GE was analyzed by acid-PAGE and SDS–PAGE. Acid-PAGE confirmed the presence of the four gliadins, i.e., α-, β-, γ-, and ω-gliadins (**Figure 1A**). A slight contamination by LMW glutenins was evidenced by SDS–PAGE but represented only a small percentage of the total protein amount (<5%, data not shown).

The specificity of the serum IgE of 38 patients with WFA was analyzed using GE and the five purified gliadins (i.e., α-, β-, γ-, ω1,2-, and ω5-gliadin) (**Figure 1B**). Gliadin extract was well recognized by both adults and children with WFA, confirming the previous observations (*8, 9*). However, as described by Battais et al. (*7*), IgE specificity was different in adults and children. Children demonstrated an intense IgE response against α- and β-gliadins, a moderate response against γ-gliadin, and a weak response against ω5-gliadin. Inversely, in adults the strongest IgE response was toward ω5-gliadin.

Mice Sensitizations. Sensitizations were first evaluated by assaying the concentrations of anti-gliadin specific IgE and IgG1 in serum after three (data not shown) and four (**Figure 2**) i.p. administrations of GE adsorbed on alum. Control mice received alum in PBS and are termed "0 μg" of gliadins.

Levels of anti-gliadin specific IgE (**Figure 2A**) and IgG1 (**Figure 2B**) were significantly higher in immunized mice than in strain-matched control mice. In the three strains, no significant difference ($p = 0.05$) was observed between the two doses, i.e., 10 and 20 μg, after three and four i.p. administrations. In Balb/cJ mice, specific IgE (**Figure 2A**) and IgG1 (**Figure 2B**) production was significantly higher than in the two other strains, whatever the dose ($p < 0.05$), except for IgE production in C3H/HeJ mice at the 10 μg dose ($p < 0.1$).

These results were confirmed by the pattern of cytokines produced by GE-reactivated splenocytes (**Figure 3**). In Balb/cJ mice, high concentrations of Th2 cytokines (i.e., IL-4, IL-5, IL-10, and GM-CSF) but moderate concentrations of Th1 cytokine (IFNγ) were produced when compared to those in control mice. No significant IL-12 secretion was detected whatever the dose (data not shown). At the sensitizing dose of 10 μg, specific IgE and IgG1 productions were higher but secretion of Th2 cytokines was lower than at the 20 μg dose. More than 10-fold lower levels of Th2

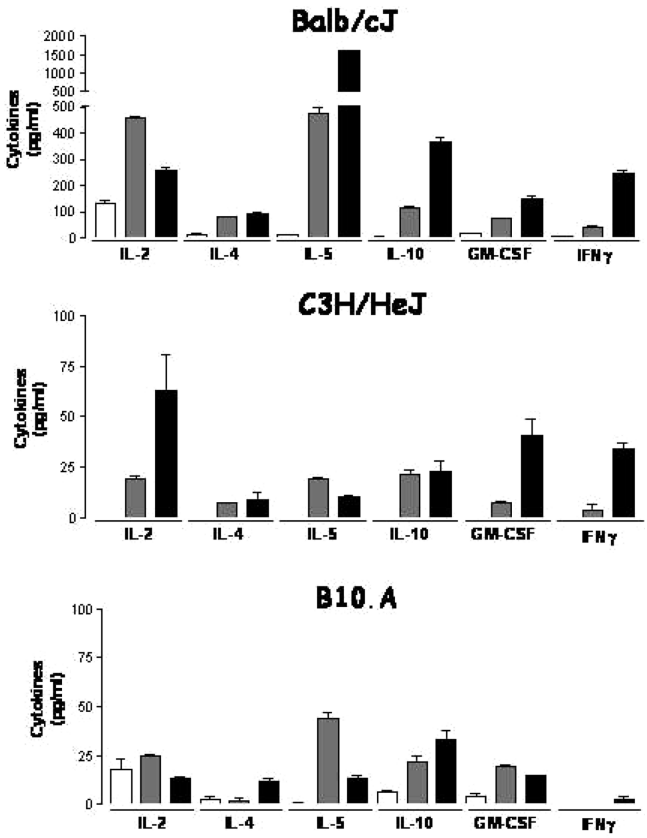

Figure 3. Gliadin specific cytokine secretion after ex vivo reactivation of spleen cells from Balb/cJ, C3H/HeJ, and B10.A mice sensitized with GE. Balb/cJ, B10.A, or C3H/HeJ mice were immunized by four successive i.p. administrations of 0 (white bars), 10 (gray bars), or 20 μg (black bars) of GE adsorbed on alum. On day 41, spleens from mice of the same group were pooled, and cells were incubated in 96-well culture plates in the presence of gliadin extract (20 μg/mL) for 60 h at 37 °C (5% CO_2). Cytokines were assayed in duplicate, using the BioPlex Th1/Th2 kit, and means ± the standard error of the mean are represented. Results obtained with PBS or ovalbumin (negative controls) and concanavalin A (positive control) were as expected (data not shown).

cytokines were secreted by reactivated splenocytes from C3H/HeJ and B10.A mice when compared to Balb/cJ mice, without any significant dose effect.

Elicitation of the Allergic Reaction. Markers of the elicitation of the allergic reaction, e.g., IL-4, IL-5, IL-10, and GM-CSF, were evidenced in BAL collected 24 h after intranasal challenge of Balb/cJ mice with GE (**Figure 4**). As for IgE levels, no difference between the two doses used for sensitization was observed. In addition, a low but significant level of production of IL-12, but not of IFNγ or TNFα, was detected (data not shown). On the other hand, Th2 (or Th1) cytokines were not detected in BAL from B10.A or C3H/HeJ mice, whatever the sensitizing dose, with the exception of a weak but significant release of IL-5 in BAL of B10.A mice sensitized with 10 μg of GE (**Figure 4**).

As shown in **Figure 5**, a significant eosinophil influx was observed after challenge in Balb/cJ mice, whatever the sensitizing dose. An eosinophil influx was also evidenced in the group of B10.A mice in which a release of IL-5 was observed. No significant eosinophil influx was observed in the other groups. Interestingly, although slight modifications were observed, total cell numbers (**Figure 5**) were not significantly different between strains and for the same strain between sensitizing doses ($p > 0.05$).

Specificity of the Mouse IgE Response against Purified Gliadins. Specific IgEs produced against purified α-, β-, γ-, ω1,2-, and ω5-gliadin were identified in pooled sera from Balb/cJ mice. Results are shown in **Figure 6** and demonstrate an intense IgE response against α-, β-, and γ-gliadins and a moderate one against ω-gliadin. Balb/cJ mice produced the significantly highest concentration of specific IgE against all gliadins, but the pattern of IgE specificity was similar in the three strains (not shown).

DISCUSSION

In this study, we developed a mouse model of allergy to wheat gliadins by testing different doses of sensitization by a whole gliadin extract and by comparing three strains of Th2-biased mice. We have found significant differences in the intensity of the sensitization between the strains. Balb/cJ mice produced the strongest IgE and IgG1 responses to the GE, which was confirmed by the pattern of Th2 cytokine produced by gliadin-reactivated splenocytes. The strength of the elicitation of the allergic reaction correlated with the sensitization levels, as demonstrated by the very intense Th2 cytokine release and the eosinophil influx in the BAL of Balb/cJ mice after intranasal challenge. On the other hand, and in line with the low levels of sensitization observed, B10.A and C3H/HeJ mice did not elicit a visible allergic reaction upon intranasal challenge.

IL-4 and IL-13 are major inducers of the Th2 response (*14, 36*). IL-5 is also a crucial mediator in the early recruitment of eosinophils in the lung after intranasal challenge (*37*). Splenocyte secretion from Balb/cJ mice was characterized by a clear-cut Th2 cytokine pattern upon allergen reactivation, in line with the high level of production of anti-gliadin specific IgE and IgG1. Upon intranasal challenge, intense secretions of IL-4, IL-5, IL-10, and GM-CSF and a significant eosinophil influx were evidenced in BALs which reflected the elicitation of an allergic reaction. On the other hand, a weak secretion of Th2 cytokines by splenocytes from C3H/HeJ and B10.A strains was observed despite significant IgE and IgG1 responses. Upon challenge, no or a low level of production of Th2 cytokines and no eosinophil influx were detected in BAL, which is very likely a consequence of a weak sensitization.

Many factors influence the propensity of humans and mice to develop an allergy, and the genetic background plays a crucial role. In mice, differences in MHC (*38*) and allelic variation in the IL-5 receptor α chain in A/J and C57/BL6 mice (*39*) have been suggested as likely reasons for strain-dependent susceptibility in the development of allergic responses. C3H/HeJ mice are considered a good model of allergy (*22*), and their deficiency in TLR4 has suggested that this might be an important factor for sensitization (*25*). TLR4 may influence the polarization of the immune response and the intensity of the allergic reaction, but this is also highly dependent on the nature of the antigen (*40*). Recently, B10.A mice have been shown to be a valuable model of allergy to egg and wheat (*22, 28, 41*). Balb/cJ mice have been largely used in studies of allergy to milk and peanut proteins because of their strong potential for IgE production (*17−19, 42*). In the context of WFA, this study shows that Balb/cJ was the strain which developed the most significant sensitization and manifestations of allergy to gliadins. Our results differ from the study of Kozai et al., who have successfully sensitized B10.A mice to wheat proteins with a significant IgE production and with the development of clinical signs after intragastric challenge. Mice also developed reduced exercise performances and mucosal damages of the small intestine (*28*). In our study, even if B10.A mice developed significant IgE and

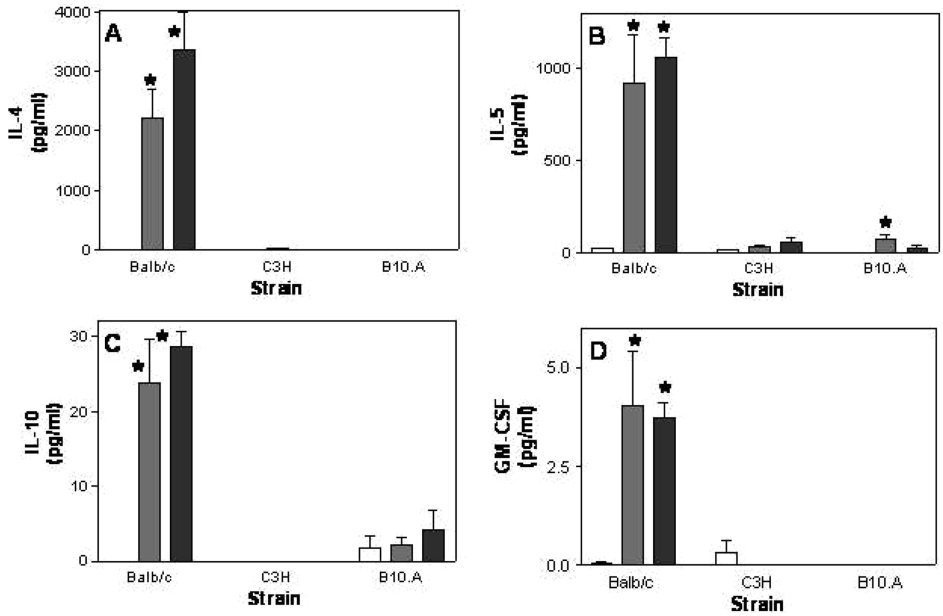

Figure 4. IL-4 (**A**), IL-5 (**B**), IL-10 (**C**), and GM-CSF (**D**) assayed in BAL collected 24 h after intranasal challenge of sensitized mice with gliadin extract (GE). Balb/cJ, B10.A, and C3H/HeJ mice were immunized by four successive i.p. administrations of 10 (gray bars) or 20 μg (black bars) of GE adsorbed on alum. Control mice received alum and PBS (noted as 0 μg, white bars). Each group of mice was then challenged by intranasal administration of 10 μg of GE, and bronchoalveolar lavages fluids (BAL) were collected 24 h later. Cytokines were assayed in BAL using BioPlex technology. Means of six to eight mice per group $\pm$ the standard deviation are represented. An asterisk indicates $p < 0.05$ using analysis of variance and Dunett's post test, with strain-matched control.

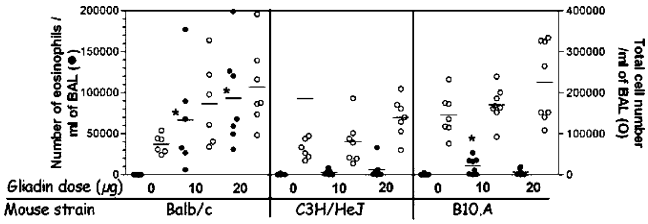

Figure 5. Eosinophil influx and total cell numbers in BALs collected 24 h after intranasal challenge of sensitized mice with gliadin extract (GE). Balb/cJ, B10.A, and C3H/HeJ mice were immunized by four successive i.p. administrations of 10 or 20 μg of GE adsorbed on alum. Control mice received alum and PBS (noted as 0 μg). Each group of mice was then challenged by intranasal administration of 20 μg of GE, and bronchoalveolar lavages fluids (BAL) were collected 24 h later. Total cell counts (white symbols) and differential cell counts (black symbols) for individual mice are shown. An asterisk indicates $p < 0.05$ (Kruskal–Wallis and Mann–Whitney U post test).

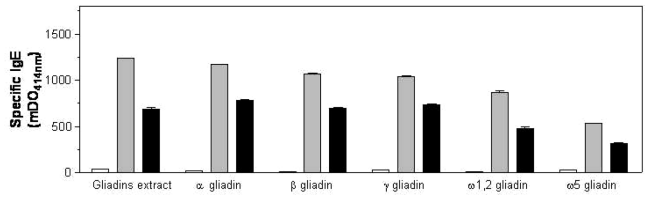

Figure 6. IgE specificity against the five purified gliadins in Balb/c mice sensitized with gliadin extract (GE). The pooled sera of Balb/c mice sensitized with 0 (white bars), 10 (gray bars), or 20 μg (black bars) of GE were tested with an ELISA for their specificity against pure α-, β-, γ-, ω1,2-, and ω5-gliadin. Data for IgE binding to GE are also shown.

IgG1 responses, correlating with a Th2-type cytokine production by reactivated splenocytes, only weak allergic reactions were elicited after intranasal challenge.

It is generally recognized that environmental factors, including exposure to microbial conditions, may interact with the genetic background to prevent or enhance the development of an allergic reaction. We did provoke and elicit peanut and cow's milk allergy in Balb/cJ mice (*18*), whereas such induction of an allergic reaction was not observed by other groups using this strain (*22*). Many factors may contribute to the differences in the results obtained in this study and in Kozai's study. We used purified gliadins as an allergen, whereas Kozai used a gliadin rich fraction. The protocols of immunization also differed (e.g., doses of allergen, schedule of administration, and nature of the adjuvant used) as well as the origin of the mice and their husbandry conditions, including composition of the diet. Variations in those factors may affect the reproducibility of the animal responses and make a relevant and validated animal model of food allergy difficult to develop. The experiments presented in this study were performed at the same time; the three mouse strains were maintained in the same controlled environment and fed the same diet, and they were handled by the same experimenter so that the immune responses could be compared under the same conditions.

Although some authors successfully used the intradermal route (*19, 43*), most studies used oral or i.p. routes for mice sensitization (*44*). Gavage could be supposed to be a more appropriate route for assessing food allergy to wheat flour proteins, but the i.p. route proved to allow better discrimination between mice strain reactivity for a known allergen (*44, 45*). Moreover, i.p. or i.g. sensitization with peanut extract led to the production of IgE recognizing the same proteins (*18*), and i.p. sensitization with purified food allergen induced the production of IgE with epitopic specificity similar to those produced in allergic humans (*20*). Because wheat is part of the normal diet of mice, we minimized the induction of oral tolerance by feeding young weaned mice with a semisynthetic diet deprived of plant proteins. Different adjuvants can be used for i.p. immunization. Here, we used mineral oil-based aluminum hydroxide that preferentially stimulates Th2 cells, while complete Freund's adjuvant (CFA) preferentially stimulates a Th1-type response (*17, 46*). Incomplete Freund's adjuvant (IFA) has also been used previously (*17*). However, the

use of Freund's adjuvant can modify, e.g., partially denature, the structure of the allergen. This results in the production of IgE preferentially directed toward linear epitopes, whereas conformational epitopes are mainly recognized using alum (*17*). In this first study on a total gliadin extract, we chose to preserve the native conformation of the proteins by using alum. However, in further assessment of the allergenicity of purified gliadins, it may be worth adapting the adjuvant according to the type of epitopes recognized by allergic human IgE. Indeed, several linear epitopes of ω5-gliadin were identified in patients suffering from WDEIA, including a consensus QQX1PX2QQ motif, where X1 represents leucine, phenylalanine, serine, or isoleucine and X2 represents glutamine, glutamic acid, or glycine (*12, 33*). Conversely, linear epitopes were not evidenced in the case of children with AEDS in which wheat lipid transfer protein (LTP) is an important allergen.

As described by Battais et al., IgE specificity against individual isolated pure gliadins depends on the age of the patient (*7*). In children with WFA, the IgE response is mainly specific for α- and β-gliadin, then for γ-gliadin, and lastly for ω5-gliadin. Conversely, in allergic adults, IgE strongly binds ω5-gliadins. It is worth noting that mice sensitized with gliadin extract by the i.p. route demonstrated the same IgE specificity observed in allergic children. The intensity of the IgE responses against the different pure wheat gliadins correlates with their relative proportion in the gliadin extract, but all the gliadins are recognized by IgE (in both humans and mice), including ω5-gliadin despite its low concentration in the GE. This suggested the high allergenic potential of ω5-gliadins. Indeed, we observed high IgE levels in the allergic adult population, confirming the studies that identified ω5-gliadins as a major allergen in adults with WDEIA (*12, 33*).

In conclusion, this study outlines the multifactorial aspect of allergy and confirms the usefulness of animal models but also the need to interpret the results they generate with care. The genetic background of the mouse strains used is an important aspect, and the Balb/cJ mouse strain is an appropriate strain for studying the allergenicity of foods and of wheat flour gliadins in particular. Further studies are needed to adapt this model to reproduce the characteristics of the human allergic responses to the various gliadins and particularly to ω5-gliadin.

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