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Development of Animal Models and Sandwich-ELISA Tests to Detect the Allergenicity and Antigenicity of Fining Agent Residues in Wines

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Food allergy can cause food-related anaphylaxis. Food allergen labeling is the principal means of protecting sensitized individuals. This motivated European Directive 2003/89 on the labeling of ingredients or additives that could trigger adverse reactions, which has been in effect since 2005. During this study, we developed animal models with allergy to ovalbumin, caseinate, and isinglass in order to be able to detect fining agent residues that could induce anaphylactic reactions in sensitized mice. The second aim of the study was to design sandwich ELISA tests specific to each fining agent in order to detect their residue antigenicity, both during wine processing and in commercially available bottled wines. Sensitized mice and sandwich ELISA methods were established to test a vast panel of wines. The results showed that although they were positive to our highly sensitive sandwich-ELISA tests, some commercially available wines are not allergenic in sensitized mice. Commercially available bottled wines made using standardized processes, fining, maturation, and filtration, do not therefore represent any risk of anaphylactic reactions in sensitized mice.

KEYWORDS: Wine; food allergy; allergen; ovalbumin; caseinate; fish; mice; antibodies; anaphylactic reaction

INTRODUCTION

Fining is one of many different processing techniques used to clarify and stabilize wine. It enables the elimination of tannins (polyphenols), which modify gustatory characteristics. Fining agents such as egg white, isinglass, and caseinate are traditionally used in wine making. Egg white or albumin is used to treat red wines containing high levels of tannins that render them too astringent. Caseinate is used for white and rosé wines or musts and ciders, and can also be of value in oxidized white wines. Isinglass is used to clarify white wines, giving them their characteristic luster. These components of eggs, milk, and fish can cause allergic reactions in sensitized humans (*1*−*4*). Egg white mainly contains ovalbumin, which is a major egg allergen, and another major allergen called ovomucoid (*5, 6*). Isinglass contains primary collagen and may be contaminated by allergens such as parvalbumin, which has been identified as an important muscle allergen in fish (*7*−*9*). Cow's milk contains allergens such as caseins, α-lactalbumin, and α-lactoglobulin, which appear to be the principal allergens responsible for cow's milk allergies (*10*).

According to the European Federation of Allergy and Airways Diseases Patient Associations, 4% of adults and 8% of children suffer from food allergy. In France, cow's milk, eggs and fish are the main allergens of animal origin (*11, 12*). Reactions caused by these allergens are more common in childhood, while reactions to plant products are more frequently encountered among adults (*12*). Cow's milk and egg allergies in childhood generally resolve after the age of four years, but when they persist in adulthood, they can be very severe (*13*).

Wines have an extremely complex composition, containing many hundreds of components that have an important role in determining their flavor and characteristics. An adverse food reaction may be immunologically (IgE) or nonimmunologically (non-IgE) mediated. The former is a food allergy; the latter constitutes food intolerance. Only small numbers of severe adverse reactions to wine have been reported in the literature. They are commonly attributed to biogenic amines (histamine) and added preservatives (salicylates, sulfites), although no systematic studies have been performed to distinguish reactions due to allergens from those due to pharmacological responses (*14, 15*). Few IgE-mediated reactions to grape proteins have been reported, and these were observed around the Mediterranean basin (*16*−*18*).

Small concentrations of fining agents are used in wine processing. It has clearly been shown that animal fining agents are not completely removed by settling and/or filtration (*19, 20*). Traces may thus be present in wines at levels sufficient to cause a variety of clinical symptoms in allergic consumers. That is why Directive 2003/89 on the labeling of ingredients that can cause adverse reactions was adopted by the European Parliament.

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There has not yet been any recorded case of an anaphylactic reaction due to the ingestion of wines that could contain fining agent protein residues. Indeed, Rolland et al. demonstrated that no anaphylaxis or symptoms of an adverse reaction to a double-blind, placebo-controlled challenge of fined Australian wines due to the consumption of wine made using food allergens (egg or fish) were present (*21*). However, the study cohort included 5 egg-allergic, 10 fish-allergic, and 1 milk-allergic patients, which was not representative of the Australian population. Moneret-Vautrin et al. suggested that the lowest observed adverse-effect level for allergens such as egg or milk is commonly in the range of 1−2 mg of natural foods, representing a few hundred micrograms of protein (*22*). For this reason, other analytical methods with threshold values capable of detecting allergenic protein residues in fined wines were subsequently investigated. In order to measure the concentrations of allergenic proteins, enzyme-linked immunosorbant assays (ELISA) were applied. Weber et al. developed an in-house competitive ELISA test and were able to quantify residual fining agent proteins and lysozyme in fined German wines (*23*). Their results showed no detectable amounts of soluble fining agent proteins in German wines, except for dried egg white and lysozyme in four simulated German commercially available wines (*23*). Further investigations in a broader range of commercially available wines are thus required. Rolland et al. had previously developed a specific and highly sensitive ELISA test to evaluate residual processing aids in 153 commercially available bottled Australian wines (*24*). They found no wine with detectable casein, and two wines with detectable whole egg rather than detectable egg white.

In order to assist industry in choosing tests with a threshold value that is low enough to detect fining agents in wines, we have developed sandwich ELISA tests specific to each fining agent in order to detect their residual antigenicity, both in wine processing after fining and filtration, and in commercially available bottled wines. We then used an animal model (i.e., mice sensitized to fining agents) to assess the residual allergenicity demonstrated by anaphylactic reactions after wine challenges.

MATERIALS AND METHODS

Animals. The animal model protocol complied with NIH guidelines. Six-week old female mice of the C3H/HeSn (H-2^k), CBA/j (H-2^k), DBA/2 (H-2^b), and SJL/J (H-2^s) strains and weighing 21 g were purchased from Harlan Nederland France Laboratory. Fourteen-week old male rabbits were also used during this study (Hsdlf New Zealand White strains weighing 3 kg, purchased from Harlan). The animals were fed ad libitum with standard laboratory food, which did not contain any fining agent (data not shown). They were maintained on a 12 h/12 h light/dark cycle.

Protein Fining Agents: Origin and Characterization. The fining agents were provided by the French Oenologists Union. Casein is the primary protein in milk. White potassium caseinate powder was obtained by coagulating skimmed cow's milk. This is used to clarify white wines at doses that generally range from 0.25 to 0.5 mg/mL; higher levels may sometimes be employed, up to a maximum of 1 mg/mL if the grapes have retained a degree of rot. The wine is drawn off 1−2 weeks after the fining agent has been incorporated.

Isinglass, unprocessed and raw, is obtained from fish gills and swimming bladders using a specific extraction procedure. It mainly contains collagen fibers. This fining agent is supplied in the form of white chips or flakes, from which a solution is prepared in water acidified with tartaric acid just before use. It is used to clarify white wines using a dose range from 0.01 to 0.025 mg/mL, and to clarify some red wines at a dose of 0.03 to 0.05 mg/mL. The wines can be filtered 8−14 days after the addition of the fining agent.

Egg albumin is obtained from hen's egg white, which contains about 12.5% protein, including 9 mg/mL lysozyme. In some cases, lysozyme may be removed from the fining agent. Hen's egg white is supplied fresh, as freeze-dried powder, as white to yellowish chips, or in frozen form. Fresh egg white is mixed and dissolved in water, taking care not to create foam. Powdered egg white can be dissolved in a potassium carbonate solution. The usual doses are 2 to 3 egg whites per hectoliter of wine. Standard industrial powdered fining agent doses range from 0.05 to 0.1 mg/mL for egg albumin. Powdered egg albumin from which lysozyme has been removed is applied at doses of 0.06 to 0.1 mg/mL. Frozen egg whites are used immediately after thawing at doses of between 0.75 and 2 μL/mL. The wine is drawn off two to three weeks after introducing the fining agent.

Sensitization to the Fining Agent. Mice ($n = 4$ per group) were sensitized by intraperitoneal injection (ip). They received two injections of 280 μL containing 10 μg enological fining agent (egg white, caseinate, and isinglass) in PBS with aluminum hydroxide $Al(OH)_3$ (alun) as the adjuvant, at 10-day intervals. The injections were followed by challenge tests on days 8 and 16. Blood samples were collected from each group of mice before and after immunization, by retro-orbital puncture on day −7 and days 14 after the second immunization. Individual sera were collected after centrifugation and stored at −20 °C.

Rabbits ($n = 6$) were immunized by subcutaneous injection. They received three injections of 1 mg of enological pure fining agent (albumin with lysozyme, caseinate, and flake isinglass), emulsified in 1 mL PBS with Freund's complete on day 0 and with incomplete adjuvant on day 21 and day 42. Blood samples (15 mL/rabbit) were collected from the marginal veins in each group of rabbits ($n = 2$ rabbits/fining agent) before and after immunization at day −7, day 15, day 35, and day 57. Individual sera were collected after centrifugation and stored at −20 °C.

Characterization of Wines. *Characteristics of Wines Fined in the Laboratory.* One red wine (Cabernet-franc variety) and two white wines (Chenin and Sauvignon varieties) were treated in the laboratory using enological fining agents. These wines were purchased by VINIFLHOR and had not been fined. The doses of fining agents corresponded to those normally used and were determined using the fining point test. The different finings were performed in white and red wines: three white wines, two of which were fined with flake isinglass (0.02 mg/mL) and prehydrolyzed isinglass (0.02 mg/mL) respectively, and another with caseinate (1 mg/mL); two red wines were fined with liquid egg albumin with lysozyme (0.1 mg/mL) and liquid egg albumin from which lysozyme had been removed (0.1 mg/mL).

Four types of suspensions were collected from each wine: (1) untreated wine, containing no specific fining agent; (2) mixture of wine and its lees, in which the fining agents remained. This mixture was stirred, and a sample was collected after the addition of the fining agent. (3) Decanted wine (removed from above the fining agents), which was separated from fining agents after decanting overnight and then drawing-off. (4) Fining agent lees extracted from drawn-off wine after treatment with the fining agents. All samples were freeze-dried and then used for challenge tests in the sensitized mice.

Characteristics of Wines Made under Controlled Conditions. We studied wines that were produced by winemakers under commercial conditions and intended for marketing. These fined wines were made using normal wine-making processes with fining agents provided by the French Oenologists Union. The wines came from different areas of France: Bordeaux, Champagne, and Val de Loire.

The Bordeaux AOC red wine was fined with egg albumin 0.03 mg/mL; six samples were collected during the process: control wine before fining, supernatant of wine 3 h after the fining operation, supernatant of wine 3 days after the fining operation, wine after moderate filtration 7 days after the fining operation, wine after a second filtration on the same day (day 7), and wine after a third and final filtration the next day (day 8).

The Val de Loire white wine was fined with flake isinglass at a concentration of 0.025 mg/mL; 4 samples were collected during the process: control wine before fining, fined wine from the top of the tank, fined wine from the bottom of the tank, containing lees, and wine filtered 3 weeks after the fining operation.

The Champagne (Chardonnay white wines) was fined with caseinate: (1) 2001 Vintage (before the "prise de mousse") fined with 0.25 mg/mL caseinate and the addition 24 h later of 0.20 mg/mL bentonite. Filtration was performed 5 weeks after the fining operation. (2) 2002 Vintage (before the "prise de mousse") fined with 0.30 mg/mL caseinate and the addition 24 h later of 0.20 mg/mL bentonite. Filtration was performed 4 weeks after the fining operation. (3) 2003 Vintage (after the "prise de mousse") obtained from a must fined with a bentonite−caseinate complex at 0.6 mg/mL. Filtration was performed 4 weeks after the fining operation. One sample was collected from each vintage.

Characteristics of Commercially Available Wines and Ciders. The final step was to blindly analyze 400 wines and 38 ciders available to French consumers. This study was performed to provide a representation of the presence or absence of fining agents in French wines and ciders.

These wines and ciders came from three different sources: 38 ciders produced in 2005 by the only two French companies that use caseinate for fining; 98 wines were provided by winemakers and wine traders, and the fining agents employed were known; 265 wines were purchased in supermarkets and wine shops, and the presence and nature of the fining agents used during the production process were unknown; 37 organic wines purchased from organic retail outlets, and the presence and nature of the fining agents used during the production process was unknown.

All wines came from different French regions and comprised 54% red wines, 15% rosé wines, and 32% white wines.

Measurement of Immunoglobulins Specific to Fining Agents. In mice, the level of response to specific immunoglobulins (IgE, IgG, IgG1, and IgG2a) was determined by ELISA. Microtiter plates (Nunc-maxisorp, France) were coated with 50 μL of the fining agent (10 μg/mL in PBS pH 7.4). After one night at 4 °C, the plates were washed and blocked with 50 μL of 3% BSA PBS for 1 h at 37 °C. Fifty microliters of serum samples (Diluted in 1% BSA PBS) was added to the plates and incubated for 2 h at 37 °C. The plates were then washed and incubated for 1 h at 37 °C with 50 μL of 2 μg/mL biotinylated polyclonal specific antibody for mouse IgG (1:5000 Sigma B9904, France) or biotinylated monoclonal specific antibodies for mouse IgE, IgG1, and IgG2a (Pharmingen, France; 02232D:1/1000, 02122D:1/1000, and 02012D:1/1000, respectively). Fifty microliters of streptavidin-peroxydase (1:5000 dilution, Sigma E-2886, France) was then added to the plates and incubated for 30 min at 37 °C. After the addition of 50 μL of H_2O_2 (30%, 0.25 μL/mL, Sigma, France) associated with OPD (0.5 mg/mL, Sigma, France) in a sodium citrate 0.05 M, pH 5.1 buffer used as the substrate, the reaction was stopped by the addition of H_2SO_4 (1 M). Between each incubation, the plates were washed with PBS containing 0.05% Tween 20. All experiments were made in duplicate. Absorbance was measured at 490 nm using a microplate reader (Bio-Tek Instruments).

Mouse Challenges and the Assessment of Anaphylactic Symptoms. The level of allergy in the mice was evaluated by challenge tests with pure fining agents. In this case, anaphylactic symptoms in sensitized mice were assessed 15 min after an initial ip challenge with 1 mg of fining agent or after a gastric gavage (gg) challenge with 10 mg of fining agent. The allergenicity of fined wines was evaluated after an ip challenge with 1 mg of freeze-dried wine. Symptoms were quantified using a previously reported scoring system (*25*) as follows: 0, no symptoms; 1, scratching and rubbing around the nose and head; 2, puffiness around the eyes; pillar erect, reduced activity and/or decreased activity with increased respiratory rate; 3, wheezing, labored respiration, and cyanosis around the mouth and the tail; 4, no activity after prodding or tremor and convulsion; 5, death.

Characterization of Fining Agents. Electrophoresis on polyacrylamide gel in the presence of SDS was used to analyze the protein composition of each fining agent (albumin with lysozyme, caseinate, and isinglass flakes).

Purification of Antibodies with Affinity Chromatography. *Immunosorbent Preparation.* Fifty eight milligrams of each fining agent (albumin with lyzosyme, caseinate, and isinglass flakes) were solubilized in 0.1 M sodium bicarbonate buffer at pH 8.3. The volume of the buffer was 10.5 mL for albumin and caseinate, and 17 mL for isinglass. The solutions were agitated for 30 min and heated at 37 °C for 1 h. They were then centrifuged for 10 min at 280 g, and the nonsoluble fraction was withdrawn.

Gel and Column Preparation. Five grams of activated CH-Sepharose 4B was washed on a filter with 1 L of 1 mM HCl. The activated gel was placed in a bicarbonate buffer and shared between the three fining agent solutions. These solutions were then agitated for 2 h. The excess of nonfixed fining agents on the gels was washed with a volume of buffer bicarbonate corresponding to 5 times the gel volume. After five minutes, the supernatant of each mixture was eliminated and replaced with a 1 M ethanolamine solution at pH 8.0, and the test tubes were then agitated for 2 h. Finally, each suspension was transferred to a small chromatography column (10 mm diameter). The gel was washed successively with 0.1 M Tris-HCl buffer at pH 8 and 0.1 M acetate buffer at pH 4.0. The quantity of buffer used at each passage corresponded to 5 times the volume of the gel, i.e., 40 mL. The columns were stored at 4 °C.

Before use, the columns were washed with several buffers of different pH, in the following order: 0.1 M PBS buffer at pH 7.2 containing 0.5 M NaCl; 0.1 M acetate buffer at pH 4.5 containing 0.5 M NaCl; 0.1 M glycine-HCl buffer at pH 2.8 containing 0.5 M NaCl; 0.1 M glycine-HCl buffer at pH 2.2 containing 0.5 M NaCl; and 0.1 M PBS buffer at pH 7.2 containing 0.5 M NaCl.

Antibody Purification. The purification of anti-isinglass, anticaseinate, and antialbumin antibodies present in the serum of rabbits was performed in our laboratory as follows: 3 to 6 mL of serum from rabbits previously immunized with the different fining agents was placed in each column. The nonabsorbed fraction was recovered, and the column was washed successively with 15 mL of 0.1 M PBS buffer at pH 7.2 containing 0.5 M NaCl; 0.1 M acetate buffer at pH 4.5 containing 0.5 M NaCl; 0.1 M glycine-HCl buffer at pH 2.8 containing 0.5 M NaCl; 0.1 M glycine-HCl buffer at pH 2.2 containing 0.5 M NaCl; and 0.1 M PBS buffer at pH 7.2 containing 0.5 M NaCl. The eluates of glycine-HCL at pH 2.8 containing 0.5 M NaCl and glycine-HCl at pH 2.2 containing 0.5 M NaCl were recovered in 1 mL fractions, and around 100 to 150 μL of 1 M Tris buffer at pH 8 was added to the fractions at pH 2.8 and 2.2 to neutralize and preserve the structure of the immunoglobulins. These eluates were mixed and stored at −20 °C.

Detection of Fining Agents by Sandwich ELISA. Fining agents were solubilized according the manufacturer's recommendations and diluted with PBS at pH 7.4. Twenty milliliters of each wine was lyophilized and then dissolved with 2 mL of PBS 7.4 containing 0.05% Tween 20.

Aliquots of affinity-purified rabbit antibodies were biotinylated using act-biotin dissolved in *N*-dimethylformamide. The ELISA-sandwich (ELISA-sw) assay was tested for each fining agent. Microplates (Nunc-Maxisorp, France) were coated overnight at 4 °C with 50 μL of antifining agent purified rabbit antibodies (pooled fractions of glycine-HCL at pH 2.8 and 2.2) and diluted in PBS at pH 7.4. After blocking with 3% BSA PBS for 1 h at 37 °C and washing, 50 μL of fining agents and/or lyophilized wine samples were added to the plates and incubated for 2 h at 37 °C. The plates were then washed and incubated for 1 h at 37 °C with 50 μL of biotin-conjugated-antifining agent antibodies diluted in PBS at pH 7.4 with 1% BSA. After washing, extravidine peroxidase (Sigma) (diluted in PBS at pH 7.4, 1% BSA, 1:5000 dilution) was added for a 30-min incubation at 37 °C. Fifty microliters of H_2O_2 (30%, 0.25 μL/mL, Sigma, France) associated with OPD (0.5 mg/mL, Sigma, France) in a 0.05 M sodium citrate buffer at pH 5.1 was used as the substrate, and the reaction was stopped by the addition of H_2SO_4 (1 M). Between each incubation, the plates were washed with PBS containing 0.05% Tween 20. All experiments were made in duplicate. The plates were read at 490 nm using a microplate detector (Bio-Tek Instruments). A sample was considered positive when absorbance was higher than the mean of the absorbance of wine produced under controlled conditions (wines before fining or unfined white, rosé, or red wines) plus 1.96 standard deviations ($p < 0.05$). Standard curves were developed with the fining agents. The limits of detection with the fining agents were 4 ng/mL for caseinate, 1 ng/mL for egg white, and 150 ng/mL for isinglass.

Statistical analysis. The statistical significance of data was determined using the *t* test, a value of $p < 0.05$ being considered as significant.

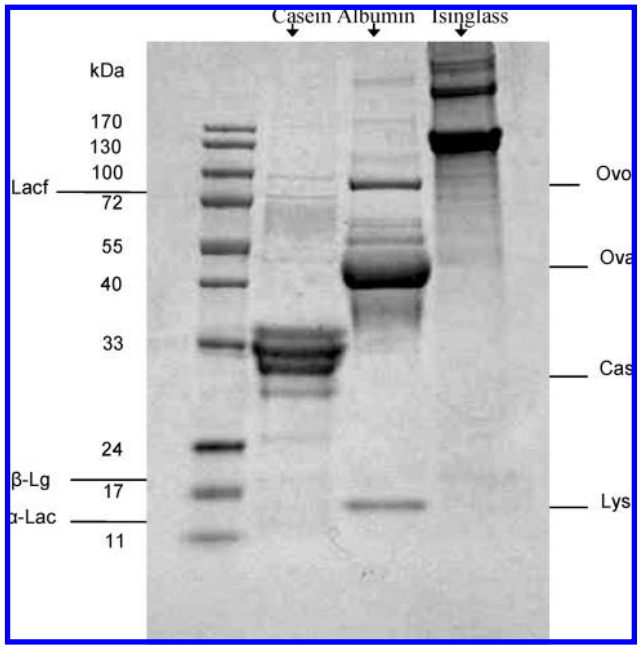

Figure 1. SDS−PAGE staining with Coomassie Brillant blue of the fining agents caseinate, albumin with lysozyme, and flake isinglass. On the left: the marker of molecular mass. Lact, lactoferrine; β-Lg, β-lactoglobulin; α-Lac, α-Lactalbumin; Ovot, ovotransferrin; Ova, ovalbumin; Lys, lysozyme; Cas, caseinate.

RESULTS

Characterization of Fining Agents. After characterization of the fining agents by unidimensional electrophoretic gel SDS−PAGE, we noted several bands stained with Coomassie blue. For caseinate, we detected two light bands between 72 and 100 kDa, four bands observed around 33 kDa, a light band around 24 kDa, two light bands above 17 kDa, and the last below 11 kDa. For albumin, we observed one band between 40 and 55 kDa, another between 11 and 17 kDa, and two others between 72 and 100 kDa, with two light bands above 120 kDa. For isinglass, around six bands could be distinguished with high molecular masses above 130 kDa (**Figure 1**).

Lack of Overall Toxicity of the Wines. Before developing mouse models for allergy to the fining agents, the potential toxicity of the wines was verified. Four strains of in-bred mice with different genetic inheritance were thus selected: C3H (H-2^k), CBA (H-2^k), SJL (H-2^s), and DBA/2 (H-2^d). All mice received an intraperitoneal injection of lyophilizate (1 mg) from wine produced under controlled conditions with or without fining agents. No detectable clinical symptoms were observed in any of the strains after the injection of these lyophilized wines. These results indicated that this mouse model could therefore be used to study the allergenicity of enological fining agents.

Development of Mouse Models of Allergy to Fining Agents. We determined the allergic status of the four mouse strains regarding egg albumin, isinglass and caseinate. Specific antibodies (IgG, IgG1, IgG2a and IgE) were analyzed in each strain in the different groups, before and after sensitization. Evaluation of the biological signs due to fining agents showed that mice of all four strains presented specific IgG, IgG1, and IgG2a responses to the enological fining agents. However, the specific IgE response was variable (**Table 1**), different as a function of mouse strain and antigen. Specific IgG levels were higher than those of specific IgE. The specific IgE titer was higher in mice sensitized with albumin (with or without lysozyme) than in those sensitized with flake isinglass. No measurable specific IgE responses were observed in mice immunized with prehydrolyzed isinglass and caseinate (**Table 1**). Only DBA/2 mice sensitized with caseinate in the presence of aluminum hydroxide produced significantly increased levels of both anticaseinate-specific IgG1 and IgE, two weeks after the initial caseinate sensitization (**Table 1**).

The clinical signs appearing after challenge tests with fining agents were studied in sensitized mice (**Table 2**). After the first challenge on day 8, C3H mice presented clinical signs with albumin containing no lysozyme, flake isinglass, and prehydrolyzed isinglass (stages 3, 3, and 3, respectively), and CBA mice reacted with albumin without lysozyme and flake isinglass (stages 3 and 2, respectively), but SJL mice did not exhibit any detectable clinical signs with the different fining agents. After the second challenge on day 16, C3H, SJL, and CBA mice presented with anaphylactic reactions due to the challenge with albumin (with or without lysozyme) and isinglass (flakes and prehydrolyzed). We also noted mortality rates of 100% among C3H mice sensitized with albumin without lysozyme, 66.6% among C3H mice sensitized to albumin with lysozyme, and 33.3% among SJL mice sensitized to albumin without lysozyme. However, no deaths were reported in CBA mice. As for caseinate, no anaphylactic reactions were observed following fining agent challenges in C3H, CBA, and SJL mice. Anaphylactic reactions were observed in DBA/2 mice after a challenge with caseinate on day 16, with a mortality rate of 25% (**Table 2**).

In conclusion, we thus established a mouse model that is allergic to fining agents. On the one hand, C3H, CBA, and SJL mice were allergic to albumin and isinglass with elevated serum levels of specific IgG and IgE, and severe anaphylactic reactions after challenge tests with albumin and flake isinglass so that for this study, we chose the C3H strain with the most severe anaphylactic reactions. On the other hand, only DBA/2 mice were allergic to caseinate, with elevated caseinate-specific IgG and IgE levels and severe anaphylactic reactions after challenge tests with caseinate.

Characterization of Wines Prepared in the Laboratory. *Clinical Signs after Challenge in Sensitized Mice.* This experiment was designed to investigate whether different samples of lyophilized wine prepared in the laboratory could induce anaphylactic reactions in mice sensitized to fining agents. The wines were lyophilized in order to eliminate alcohol and concentrate all of the antigens. Mice sensitized with fining agents were placed in four groups ($n = 5$ per group) and challenged on day 21. In the first group, the challenge was performed with the control, nonfined, lyophilized wine. In the second group, the challenge was performed with a lyophilized mixture of fined wine and its lees. In the third group, the challenge was performed with lyophilized lees of the fining agents, while lyophilized wine that had been decanted and filtered was used in the final group (**Table 3**). No anaphylactic reactions were observed following a challenge with the control wines. CH3 mice sensitized with albumin (with or without lysozyme) and challenged with fined wines mixed with their lees exhibited anaphylactic reactions (stages 2 and 1, respectively). CH3 mice sensitized with albumin (with or without lysozyme) and challenged with fining agent lees (stages 3 and 2, respectively) and prehydrolyzed isinglass (stage 3) exhibited severe anaphylactic reactions, while no clinical signs were noted in C3H mice sensitized with flake isinglass or DBA/2 mice sensitized with caseinate. Anaphylactic reactions are prevented in all mice challenged with decanted and filtered wines. Sensitization to

Table 1. In Vitro Reactivity to Fining Agents (Albumin with Lysozyme, Albumin without Lysozyme, Prehydrolyzed Isinglass, Flake Isinglass, and Caseinate) of Intra-Peritoneal Fining Agent-Sensitized Mice (C3H, CBA SJL, and DBA/2; $n = 3$)[a]

fining agents	antibodies	strains	day −7	day 14	fining agents	antibodies	strains	day −7	day 14
albumin with lysozyme		C3H	0.07 ± 0.003	0.39 ± 0.21		IgG2a	CBA	0.1 ± 0.01	0.24 ± 0.13
	IgE	CBA	0.06 ± 0.001	0.46 ± 0.14			SJL	0.1 ± 0.02	0.4 ± 0.2
		SJL	0.06 ± 0.002	0.18 ± 0.01					
							C3H	0.16 ± 0.05	2.32 ± 0.5
		C3H	0.08 ± 0.009	2.26 ± 0.05		IgGt	CBA	0.17 ± 0.03	1.79 ± 0.8
	IgG1	CBA	0.08 ± 0.02	2.13 ± 0.07			SJL	0.2 ± 0.07	2.26 ± 0.41
		SJL	0.15 ± 0.08	2.14 ± 0.05	flake isinglass				
							C3H	0.08 ± 0.01	0.15 ± 0.01
		C3H	0.1 ± 0.01	2.34 ± 0.13		IgE	CBA	0.07 ± 0.001	0.37 ± 0.2
	IgG2a	CBA	0.07 ± 0.01	2.24 ± 0.05			SJL	0.06 ± 0.003	0.11 ± 0.008
		SJL	0.09 ± 0.008	2.27 ± 0.13					
							C3H	0.08 ± 0.01	2.42 ± 0.36
		C3H	0.12 ± 0.01	2.04 ± 0.05		IgG1	CBA	0.11 ± 0.04	2.55 ± 0.04
	IgGt	CBA	0.11 ± 0.02	2.07 ± 0.02			SJL	0.08 ± 0.15	2.32 ± 0.03
		SJL	0.25 ± 0.05	1.97 ± 0.07					
							C3H	0.14 ± 0.06	1.28 ± 1.2
		C3H	0.07 ± 0.003	0.61 ± 0.02		IgG2a	CBA	0.11 ± 0.03	1.54 ± 1.2
albumin without lysozyme	IgE	CBA	0.06 ± 0.001	0.82 ± 0.18			SJL	0.08 ± 0.01	2.51 ± 0.1
		SJL	0.06 ± 0.004	0.48 ± 0.04					
							C3H	0.14 ± 0.06	2.45 ± 0.1
		C3H	0.06 ± 0.002	2.34 ± 0.12		IgGt	CBA	0.13 ± 0.07	2.29 ± 0.12
	IgG1	CBA	0.07 ± 0.02	2.31 ± 0.01			SJL	0.08 ± 0.1	2.39 ± 0.1
		SJL	0.07 ± 0.004	2.33 ± 0.1					
					caseinate		C3H	0.08 ± 0.007	0.09 ± 0.01
		C3H	0.08 ± 0.003	2.47 ± 0.1		IgE	CBA	0.08 ± 0.009	0.08 ± 0.01
	IgG2a	CBA	0.06 ± 0.002	2.44 ± 0.06			SJL	0.12 ± 0.02	0.08 ± 0.003
		SJL	0.06 ± 0.002	2.36 ± 0.14			DBA/2	0.07 ± 0.009	0.11 ± 0.004
		C3H	0.09 ± 0.01	2.46 ± 0.11			C3H	0.07 ± 0.003	1.55 ± 1.12
	IgGt	CBA	0.07 ± 0.004	2.36 ± 0.01		IgG1	CBA	0.06 ± 0.003	0.55 ± 0.21
		SJL	0.1 ± 0.01	2.35 ± 0.13			SJL	0.07 ± 0.007	2.2 ± 0.19
							DBA/2	0.06 ± 0.006	2.4 ± 0.087
prehydrolyzed isinglass		C3H	0.08 ± 0.008	0.14 ± 0.003					
	IgE	CBA	0.07 ± 0.002	0.11 ± 0.01			C3H	0.09 ± 0.01	0.5 ± 0.2
		SJL	0.07 ± 0.001	0.09 ± 0.008		IgG2a	CBA	0.09 ± 0.008	0.23 ± 0.04
							SJL	0.08 ± 0.006	2.13 ± 0.5
		C3H	0.1 ± 0.006	1.82 ± 0.1			DBA/2	0.11 ± 0.04	0.10 ± 0.012
	IgG1	CBA	0.1 ± 0.01	0.71 ± 0.32					
		SJL	0.18 ± 0.03	0.2 ± 1.1			C3H	0.09 ± 0.001	2.03 ± 1.07
						IgGt	CBA	0.1 ± 0.02	1.22 ± 0.4
		C3H	0.16 ± 0.05	1.78 ± 1.3			SJL	0.07 ± 0.002	2.33 ± 0.2
							DBA/2	0.12 ± 0.03	2.50 ± 0.05

[a] Serum specific IgE (serum dilution: 1/10), IgG1, IgGt (1/100), and IgG2a (1/25) measured on days −7 and 14: mean and standard deviation of ELISA absorbance.

Table 2. Evaluation of Clinical Stages after Challenge Tests by Intra-Peritoneal Injection with Different Fining Agents (1 mg/mouse) on Day 8 and Day 16

antigens	mouse strains	number	systemic anaphylactic reactions score on day 8	% deaths	systemic anaphylactic reactions score on day16	% deaths
albumin without lysozyme (AwL)	C3H	3	**3**	0	**4−5**	100%
	CBA	3	**3**	0	**4**	0
	SJL	3	0	0	**4−5**	**33.3%**
albumin with lysozyme (AL)	C3H	3	0	0	4−5	**66.6%**
	CBA	3	0	0	**3**	0
	SJL	3	0	0	**3**	0
flake isinglass (FI)	C3H	3	**3**	0	**3**	0
	CBA	3	**2**	0	**3**	0
	SJL	3	0	0	**3**	0
prehydrolyzed isinglass (HI)	C3H	3	**3**	0	**3**	0
	CBA	3	0	0	**3**	0
	SJL	3	0	0	**3**	0
caseinate (C)	C3H	3	0	0	0	0
	CBA	3	0	0	0	0
	SJL	3	0	0	0	0
	DBA/2	4	0	0	**4−5**	**25%**

food antigens generally occurs by ingestion. This led us to challenge sensitized mice with fining agents by the oral route. C3H mice sensitized with albumin (with or without lysozyme) were challenged by intragastric gavage with 10 mg of fining agents and showed evident anaphylactic symptoms. However, C3H mice sensitized with isinglass (flakes or prehydrolyzed) and the DBA/2 mice sensitized with caseinate exhibited very mild clinical signs.

Table 3. Evaluation of Clinical Stages after Challenge Tests by Intra-Peritoneal Injection with Different Samples of Wines and after a Challenge with the Intragastric Gavage of Different Fining Agents (AL, Albumin with Lysozyme; AwL, Albumin without Lysozyme; Fl, Flake Isinglass; Hl, Prehydrolyzed Isinglass; C, Caseinate) on Day 35 (C) in Mouse Strains Sensitized to These Fining Agents

		C3H sensitized by different wine types				DBA/2 sensitized by
		AL ($n = 5$)	AwL ($n = 5$)	Fl ($n = 5$)	Hl ($n = 5$)	C ($n = 5$)
		Cabernet Franc	Cabernet Franc	Chenin	Sauvignon	Chenin
B: hypersensitivity responses after the second challenge with wine samples (i.p 1 mg/mouse; day 21)	nonfined wine	0	0	0	0	0
	fined wine mixed with its lees	**2**	1	0	0	0
	fining agent lees	**3**	**2**	0	**3**	0
	decanted and filtered wine	0	0	0	0	0
C: hypersensitivity responses after the third challenge with pure fining agents (10 mg/ig/mouse/ day 35)		**3**	**3**	**1**	**1**	**1**

Table 4. Sandwich-ELISA Test Results Compared with the Clinical Responses of Sensitized Mice after an ip Challenge with Wines Fined in the Laboratory (AL, Albumin with Lysozyme; AwL, Albumin without Lysozyme; Fl, Flake Isinglass; Hl, Prehydrolyzed Isinglass; C, Caseinate)

mouse strains	wines prepared in the laboratory	fining agents used during preparation	samples	ELISA-sw tests	clinical responses on day 21
C3H	Cabernet Franc red wine	AL	nonfined wine	−	**0**
			fined wine	+	**2**
			wine dregs	+	**3**
			filtered wine	+	**0**
	Cabernet Franc red wine	AwL	nonfined wine	−	**0**
			fined wine	+	**1**
			wine dregs	+	**2**
			filtered wine	+	**0**
	Sauvignon white wine	Hl	nonfined wine	−	**0**
			fined wine	+	**0**
			wine dregs	+	**3**
			filtered wine	+	**0**
	Chenin white wine	Fl	nonfined wine	−	**0**
			fined wine	+	**0**
			wine dregs	+	**0**
			filtered wine	+	**0**
DBA/2	Chenin White wine	C	nonfined wine	−	**0**
			fined wine	+	**0**
			wine dregs	+	**0**
			filtered wine	+	**0**

Sandwich ELISA Tests. The results of the sandwich-ELISA tests used to detect fining agents in different samples of lyophilized wines prepared in the laboratory were positive with fined wine, wine dregs, and decanted wine. These results differed from those observed during clinical tests on mice on day 21 (**Table 4**).

Characterization of Commercially Available Wines. *Clinical Signs after Challenge in Sensitized Mice.* Our mouse models thus enabled us to test the allergenicity of wines from different cellars and observe the effects of maturation and aging on their allergenicity. Two groups of C3H mice sensitized with albumin with lysozyme ($n = 3$) and flake isinglass ($n = 3$), and two groups $n = 4$/group) of DBA/2 mice sensitized with caseinate-bentonite and caseinate (**Table 5**) were subjected to an ip challenge with lyophilized wines at different stages of fining. In C3H mice, no anaphylactic reactions were observed after a challenge with the control wine. They exhibited anaphylactic reactions that were stronger after a challenge with the supernatant 3 h and 3 days after fining, than after a challenge with wine collected after the first filtration. No clinical signs were observed after the second and third filtration. As for C3H mice sensitized with flake isinglass, no anaphylactic reactions were seen after a challenge with the control wine before fining. After fining, we noted more severe anaphylactic reactions with wine sampled from the bottom of the tank than with wine taken from the top of the tank. No clinical signs were observed after the filtration of fined wine. No anaphylactic reactions were observed after a challenge with caseinate in DBA/2 mice.

Sandwich ELISA Tests. Overall, the results of sandwich-ELISA tests with albumin plus lysozyme used to detect fining agents in different samples of lyophilized wines from different cellars were parallel to those observed in sensitized mice. Indeed, sandwich-ELISA tests and challenge tests were positive in the supernatant 3 h and 3 days after fining, and in wine collected after the first filtration (**Table 5**). The results of sandwich-ELISA tests with wines fined with flake isinglass were negative when the clinical tests were positive.

Characterization of Commercially Available Wines. *Sandwich ELISA Tests and Clinical Signs.* A panel of around 400 wines and 38 ciders were lyophilized and analyzed using the sandwich-ELISA method in order to confirm or invalidate the presence or absence of fining agents. We noted that 44 bottles

Table 5. Sandwich-ELISA Test Results Compared with the Clinical Responses of Sensitized Mice after an Intra-Peritoneal Challenge with Commercially Produced Wines Fined with Albumin with Lysozyme (AL), Flake Isinglass (FI), and caseinate (C) (Nd, Not Identified)

strains	fining agents	wine samples	Sw-ELISA tests	challenge tests (ip/1 mg/mouse on day 15)
C3H ($n = 3$)	albumin with lysozyme (AL)	wine before fining	−	**0**
		supernatant 3 h after fining	+	**2**
		supernatant 3 days after fining	+	**2**
		wine after the first filtration 7 days after fining	+	**1**
		wine after the second filtration 8 days after fining	−	**0**
		wine after the third filtration 9 days after fining	−	**0**
($n = 3$)	flake isinglass (FI)	wine before fining	−	**0**
		wine sampled from the bottom of the tank	−	**2**
		wine sampled from the top of the tank	−	**1**
		filtered fined wine	−	**0**
DBA/2($n = 4$)	bentonite-caseinate	2001 vintage, 5 weeks after fining	**Ni**	**0**
($n = 4$)	caseinate (C)	2002 vintage, 4 weeks after fining	**Ni**	**0**
		2003 vintage, filtered wine from fined must	**Ni**	**0**

Table 6. Wines Positive According to Sandwich-ELISA Tests As a Function of Different Thresholds: $0.01 < p < 0.05$; $0.0049 < p < 0.0001$; and $p < 0.00099$

		0.01 − 0.05	0.0049 − 0.0001	<0.00099	total
organic wines	albumin	0	0	2	2
	caseinate	1	0	2	3
	isinglass	2	1	0	3
nonorganic wines with known fining agents	albumin	1	0	0	1
	caseinate	0	0	0	0
	isinglass	2	0	0	2
nonorganic wines with unknown fining agents	albumin	5	3	1	9
	caseinate	3	6	1	10
	isinglass	2	7	5	14
total		**16**	**17**	**11**	**44/400**

Table 7. Percentage of Wines Positive According to Sandwich-ELISA Tests As a Function of Source

	Sw-ELISA test with albumin	Sw-ELISA test with caseinate	Sw-ELISA test with isinglass	Positive sw-ELISA tests
organic wines (37)	5%	8%	8%	21%
wines with known fining history (98)	1%	0%	2%	3%
wines with unknown fining history (265)	3%	4%	5%	12%

of commercially available wines were positive at the threshold value of 0.05%, with values in 16 of them between 0.01% and 0.05% (**Table 6**). As a function of source, we observed that organic wines had the highest percentage of positive sandwich-ELISA tests. Among these organic wines, 5% of bottles were positive to albumin, 8% to caseinate, and 8% to isinglass. Of the wines with a known fining history, 1% of bottles were positive to hen's egg albumin, 2% to isinglass, and none positive to caseinate. Of the wines with an unknown fining history, 3.4% were positive to hen's egg albumin, 5.3% to isinglass, and 3.7% positive to caseinate (**Table 7**). All wines with positive sandwich-ELISA test results were injected into mice sensitized to the corresponding fining agent. No anaphylactic reactions were noted. All sandwich-ELISA tests on ciders produced negative results.

DISCUSSION

Few allergic reactions to wines and grapes have been reported in the literature (*16*−*18*). Alcoholic beverages are capable of triggering a broad variety of allergy-like responses. Wines are extremely complex in their composition, consisting of hundreds of components in addition to ethanol, which alone is widely known to be linked to the triggering of adverse responses. Hypersensitivities to wine appear to be due to pharmacological intolerances toward specific components in this beverage (*26*). For example, sulfite additives (*15*) and biogenic amines (*27*) have been implicated in hypersensitivity to wine. Indeed, sulfite additives are used to ensure the preservation of wine. They have clearly been shown to play a role in the asthmatic responses to wine displayed by certain individuals (*15*). However, in many people who present enhanced hypersensitivity to the sulfites in wine, reactivity to these additives has not been demonstrated when they are subjected to controlled challenges (*15*). Biogenic amines have also been suggested as possible triggers for a variety of adverse responses to wine, with histamine and tyramine being implicated most frequently in red wine (*28, 29*). Histamine is a potent mediator of the allergic response, and its ingestion has been shown to induce a broad range of allergic reactions in susceptible individuals (*30*). Other sensitivities have been described, such as those to ethanol and spirits (*31*−*33*). The presence of ovalbumin in wines has also been demonstrated (*23, 24*), but that of isinglass and caseinate has never been reported in the literature.

The aim of our study was thus to document whether allergenic residues originating from the products used during the fining

process were still present and active in an animal model of sensitized mice.

We first of all verified the absence of toxicity from the wines before fining. Indeed, toxic substances and some pseudoallergen components might have been the principal causal factors for the deleterious effects of the wines, possibly confusing the allergic reactions induced by the fining products. To invalidate these hypotheses, all strains of mice received the lyophilized control wine via the ip route, and no clinical signs were noted in any of the animals. We could thus conclude from the toxicity tests that nonfined wines are apparently safe for the mice. Furthermore, as well as a lack of any detectable deleterious effects in mice prior to sensitization, fining agents did not cause any allergic responses after the ip challenge. This allowed us to use our models and protocols to study the antigenic and allergenic properties of pure fining agents and different samples of fined wine.

Four strains of mice (C3H, CBA, SJL, and DBA/2) were used. C3H (H-2^k), CBA (H-2^k), and SJL (H-2^s) mice sensitized with albumin, caseinate, and isinglass developed fining agent-specific IgG1, IgG2a, and IgGt antibodies (**Table 1**), thus demonstrating the effectiveness of the sensitization protocol. IgE specific responses differed as a function of the mouse strain and antigen. The levels of specific IgE were lower than those of specific IgG.

Concerning albumin, the three strains developed IgE specific to albumin with and without lysozyme. SJL mice were distinctive from the others because of the absence of albumin-with-lysozyme-specific IgE (**Table 1**). Our findings on the production of albumin-specific IgE were consistent with the results obtained by Dearman after albumin sensitization in BALB/c (H-2^b) mice (*34, 35*). Furthermore, we noted that antibody responses (IgGt and IgE-specific) in our model were stronger in the absence of lysozyme, although this substance is one of the major allergens present in hen's eggs. In addition, anaphylactic reactions were more severe when testing albumin without lysozyme than albumin with lysozyme.

The C3H, CBA, and SJL mice sensitized by flake isinglass failed to produce isinglass-specific IgE but developed anaphylactic reactions. Our findings were compatible with those published by Untersmayr (*36*), during whose study BALB/c mice were sensitized intraperitoneally with caviar proteins and produced high levels of specific IgG1 and IgG2a antibodies. Although these mice did not produce any specific IgE antibodies against caviar proteins, they exhibited specific type I skin reactivity to caviar extracts. Indeed, previous studies, have shown that IgG1 induces anaphylaxis in some strains of mice even though in those models serum antigen-specific IgE was not present (*37*−*39*).

We selected C3H mice to study albumin and isinglass allergenicity. The clinical signs were more marked in C3H mice than in CBA mice, even though isinglass-specific IgE titers were higher in CBA mice than in C3H mice. The allergic response was thus strain-dependent.

As for caseinate, only DBA/2 mice induced caseinate-specific IgE. Our results confirmed those published by Ito et al., who indeed suggested that the oral administration of adjuvant-free casein increased the levels of serum IgE anticasein antibodies (*40*). They also examined antigen-specific IgE production in other mouse strains such as BALB/c and B10A, which exhibited no IgE response to casein, and a casein-specific IgE response was only observed in DBA/2 mice.

In conclusion, the IgE specific response showed that C3H and DBA/2 mice were sensitized to fining agents, and the effectiveness of this sensitization was demonstrated by clinical signs. Furthermore, the reproducibility of these results was verified and confirmed during a series of experiments.

As a second step, we studied the antigenicity (sw-ELISA tests) and allergenicity (clinical signs) of fined wine samples from different sources. Positive reactions (sw-ELISA tests) revealed the presence of antigens (fining agents) in all samples of these wines fined under laboratory conditions. In parallel, the systemic anaphylactic reactions obtained using dreg samples of these wines were positive and severe with albumin (with or without lysozyme) and prehydrolyzed isinglass (stages 3, 2, and 3, respectively). These reactions were due to the presence of allergens, thus demonstrating the value of decantation (**Table 3**). With respect to flake isinglass and caseinate, the absence of reactions with dregs did not indicate that they were absent. We hypothesize that a smaller quantity of fining agents was used and/or the antigens were hidden by the formation of complexes with tannins, thus preventing anaphylactic reactions. We conclude that the challenge tests were more relevant to the sw-ELISA results because the mouse model allowed the expression of the allergenicity of fining agent residues rather than their antigenicity.

Furthermore, clinical signs were prevented when the wines were filtered, thus demonstrating the effectiveness of filtration in eliminating allergenic residues, although antigenic residues were still detectable (see positive results to sw-ELISA tests).

Because wine is consumed per os, we analyzed the clinical signs in the animal models challenged on day 35 by gastric gavage with each pure fining agent at a rate of 10 mg per mouse. We observed that albumin (with and without lysozyme) was more allergenic than isinglass and caseinate because it provoked more severe clinical signs (stage 3). However, these anaphylactic reactions were less marked than those observed after an ip challenge (stage 4), even though the dose administered was 10 times higher (10 mg gg and 1 mg ip). These results could be explained by the resistance and stability of certain proteins to digestion but which nonetheless remain potentially allergenic. Indeed, stability during digestion is a significant and valid parameter that distinguishes allergenic from nonallergenic foods (*41*). Food allergens are known to be resistant to food processing and gastrointestinal digestion (*42*). During the present study, the pepsin/protein ratio in albumin could be markedly modified, a deterioration of albumin during digestion and the formation of proteolytic fragments that would remain allergenic. However, not all food allergens are more resistant to digestion than nonallergenic proteins. Indeed, Fu et al. did not find any clear relationship between digestibility (measured in vitro) and protein allergenicity (*43, 44*).

When studying isinglass, we observed mild clinical signs (stage 1) after an oral challenge (10 mg) in C3H mice sensitized to flake and prehydrolyzed isinglass; although isinglass mainly contains collagen, we suggest that some traces of allergen may remain. The parvalbumin (Gad C 1: 11 kD, a major codfish allergen) has always been described as a typically digestion-resistant food antigen (*45, 46*). Nevertheless, Untersmayr et al. reported that parvalbumin and caviar proteins (30, 84, 100, and 118 kD) fed by oral route did not generate specific IgE and that they demonstrated negative skin tests and negative oral food challenges with caviar because they are immediately degraded under stimulated gastric conditions and lose their allergenic potential (*47*). For this reason, digestion could explain the reduced allergenic potential of isinglass. The same applies for caseinate (clinical demonstration of stage 1 reactions), which is known to be digestible at gastric pH (*48*).

Thus, our models could also be used to determine fining agent residues in commercially produced wines that have undergone fining, filtration, and maturation. In C3H mice sensitized with albumin and lysozyme, we noted no clinical sign when they were challenged with the control wine, although antigens (positive sw-ELISA tests) and allergens (stage 2 anaphylactic reactions three days after fining) were observed. After the first filtration, the antigens were still present, but allergen levels had declined (stage 1 clinical signs). As from the second filtration, these symptoms disappeared completely, and the sw-ELISA tests were negative. We thus conclude that in the case of albumin, the second filtration was important to ensure the removal of antigenic and allergenic residues. In C3H mice immunized with flake isinglass, the reactions observed after a challenge with wine sampled from the bottom of the tank were more severe (stage 2) than those noted after a challenge with wine from the top of the tank (stage 1), although no antigenic residue could be detected (**Table 5**). Filtered and fined wine did not induce any anaphylactic reactions, suggesting that the allergenic residues had been eliminated. We noted that wines fined with caseinate did not induce any clinical signs in DBA/2 mice sensitized with caseinate. The effectiveness of decantation followed by filtration was thus demonstrated. The results obtained with commercially produced wines were similar to those observed using wines prepared in the laboratory, although filtration in the laboratory was probably not as effective as that applied in wineries.

Four hundred wines and 38 ciders available to French consumers were analyzed using the sandwich ELISA method, and the tests were positive in 11% of these commercially available wines, thus demonstrating the presence of antigens. The positive wines were given to sensitized mice in order to verify their allergenicity. No anaphylactic reactions were recorded in any of the animals. Thus in mice, the antigens highlighted by sw-ELISA were not allergenic. Organic wines ($n = 37$) produced the highest percentage of positive sw-ELISA tests (**Table 6**). Some organic winemakers choose not to filter their wines after fining, which could explain the high level of detection of fining agents. It is worth noting that the percentages indicated in this article are not representative and only serve as an indication. None of the ciders were positive to caseinate during sw-ELISA tests.

We thus demonstrated that different wines (organic or with a known or unknown fining history) with positive sw-ELISA test results did not produce any anaphylactic reactions after challenges in mice sensitized to the corresponding fining agents. We showed that antigenic residues could persist in commercially available wines (positive sw ELISA tests) but that allergenic residues disappeared completely (no anaphylactic reactions).

This haze of uncertainty is due to the presence of macromolecular particles, including proteins, polysaccharide colloids, microorganisms, and polyphenol compounds. Our data suggest that settling, decantation, maturation, and filtration may play a considerable role in eliminating allergenic residues and preventing a risk of the development of allergy after wine consumption. Indeed, fining agents are used at very low concentrations (from 0.01 to 1 mg/mL), and when eliminated by decantation and/or filtering, their residues are limited to traces in the wine. These traces can then be almost entirely eliminated by means of successive filtrations.

As a general rule, wine undergoes one bulk filtration to remove the largest particles and then a further filtration prior to bottling in order to achieve perfect bulk filtration.

Our animal model could form part of the controls on the effectiveness of decantation and/or filtration methods and be of assistance in the definition of a protocol to ensure the elimination of fining agent residues. Our findings show that filtration can eliminate particles that might be allergenic in consumers. Indeed, none of the sensitized mice responded to the challenge with any of the commercially available wines, even though the sw-ELISA tests were positive. These results thus show that although some commercially available wines contain antigenic residues, they are not allergenic to mice. To confirm the validity of the results obtained using our mouse model, a DBPCFC study is planned in men and women sensitized to egg, milk, and fish.

In conclusion, the results obtained using our animal model showed that wines made according to a standardized process (fining, maturation, and filtration) are not allergenic and do not represent any risk of anaphylactic reactions in mice sensitized to ovalbumin, caseinate, and isinglass.

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