

Clinical reactivity to beef in children allergic to cow's milk

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Background: Cow's milk is one of the most common food allergens in children. Limited information is available on the prevalence of reactivity to a related food source, beef. The purposes of this study were to examine the prevalence of symptomatic sensitivity to beef in a selected pediatric population and to determine the frequency of concomitant reactivity to cow's milk and beef.

Methods: Children referred for assessment of atopic dermatitis and possible food hypersensitivity were evaluated for symptomatic reactivity to beef by double-blind placebo-controlled food challenges (DBPCFCs) and subsequent open feedings of beef. Sodium dodecyl-sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), immunoblot, and immunodot blot analyses were performed with patients' sera on preparations of beef extracts subjected to different cooking conditions: raw (no heating), medium, and well-cooked.

Results: Eleven of 335 children referred for evaluation of atopic dermatitis and possible food hypersensitivity were found to have symptomatic sensitivity to beef; eight were also sensitive to milk, as demonstrated in previous DBPCFCs. Eight patients reacted to beef during DBPCFC, and three tolerated beef in a DBPCFC and well-cooked beef in an open challenge but reacted to ingestion of less well-cooked beef. SDS-PAGE of raw beef revealed at least 24 protein fractions. Several protein bands in raw beef appeared to denature with heating. Bovine serum albumin and bovine gamma globulin were heat-labile in the beef extract, but six protein fractions persisted even after heating the beef extract for 2 hours at 85° C. IgE from patients reacting to rare and well-cooked beef bound up to six of these heat-resistant fractions, but IgE from patients reacting only to rare beef failed to bind any of these fractions with one exception. In addition, patients reacting to rare and well-cooked beef had specific IgE to a 17.8 kd fraction, which was only weakly recognized by one patient reacting only to rare beef.

Conclusions: Specific IgE antibodies to heat-labile beef proteins might explain why some patients can tolerate well-cooked beef but not medium-rare and rare beef. Patients reacting only to rare beef may not need to maintain a complete beef elimination diet. (*J Allergy Clin Immunol* 1997;99:293-300.)

Key words: Beef allergy, milk allergy, heat-labile proteins, heat-stable proteins, immunoblot, BSA, BGG

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Abbreviations used

BGG:	Bovine gamma globulin
BSA:	Bovine serum albumin
DBPCFC:	Double-blind, placebo-controlled food challenge
P+:	Patients reacting to rare and well-cooked beef
P-:	Patients reacting only to rare beef
PBS:	Phosphate-buffered saline
SPT:	Skin prick test
SDS-PAGE:	Sodium dodecylsulfate–polyacrylamide gel electrophoresis

Little information has been published regarding allergic reactions to beef proteins. Clinically relevant sensitization appears to be rare even though beef is added to children's diets relatively early in life, and the average American ingests 65 pounds of beef per year (U.S. Bureau of the Census, 1991).¹ There appears to be no consensus on the allergenicity of beef. In one study by Ogle et al.,² beef was considered hypoallergenic, and children with allergic rhinitis and/or asthma were placed on an elimination diet, which included beef, to determine whether respiratory symptoms would improve. In contrast, beef was assumed to be a potent allergen and was consequently excluded from mothers' diets in a study by Chandra et al.,³ which evaluated the effect of maternal food allergen avoidance during pregnancy and breast feeding on the incidence of atopic eczema in infants.

Some adverse reactions after the ingestion of beef or pork were presumed to be caused by antibiotics, particularly penicillin and streptomycin.⁴ Tinkelman and Bock⁵ described a patient who experienced life-threatening reactions after meals containing beef but who failed to react during follow-up beef challenges and who had positive skin tests and PK test with streptomycin. At that time it was suspected that streptomycin was contained in the meat, and the authors concluded that the patient reacted to the drug. In contrast, Lindemayr et al.⁶ challenged volunteers allergic to penicillin with penicillin-contaminated meat and found that only two of nine patients reacted with pruritus or a sensation of tingling in the mouth. They concluded that it was unlikely that allergic reactions to penicillin were elicited by ingestion of penicillin-contaminated meat. The use of antibiotics in the meat industry has now been banned in the United States.

Interestingly, food allergy symptoms were partly mimicked by an outbreak of thyrotoxicosis after consumption

TABLE I. Patient characteristics

Patient No.	Age at diagnosis	History	Challenge dose (beef)*	SPT [mm] wheal/erythema	Clinical symptoms during challenge with beef	DBPCFC with milk
1 (P+)	14 yr	AD, asthma, rhinitis	30 gm	b: 8/16 m: 0	Ocular and nasal symptoms (rhinorrhea) pruritic erythematous rash, vomiting	—
2 (P+)	2.3 yr	AD, asthma, rhinitis	15 gm	b: 6/0 m: 13/0	Pruritic erythematous rash, urticaria (history of wheezing at home)	+
3 (P+)	20 mo	AD	50 gm	b: 6/6 m: 11/17	Urticaria	+
4 (P—)	16 mo	AD, asthma, rhinitis	10 gm	b: 8/20 m: 8/18	Oral pruritus, nasal pruritus, rhinorrhea	+
5 (P—)	5.5 yr	AD, asthma, rhinitis	60 gm	b: 8/224 m: 12/31	Erythematous rash, urticaria, dyspnea	+
6 (P—)	5.9 yr	AD, asthma	250 mg	b: 8/0 m: 11/0	Urticaria, oral pruritus, edema	—
10 (P+)	21 mo	AD	57 gm	b: 12/12 m: 0	Erythematous rash, pruritus, generalized urticaria, periorbital and perioral edema	—
11 (P+)	21 mo	AD	10 gm	b: 0 m: 10/14	Pruritic erythematous rash, vomiting, asthma (history $\times$'s 2)	+
12 (P+)	10 yr	AD, asthma, rhinitis	60 gm	b: 4/24 m: 7/36	Pruritic erythematous rash on face and neck, pruritic lips	+
13 (P+)	19 mo	AD, asthma, rhinitis	16 gm	b: 4/16 m: 6/23	Vomiting	—
14 (P+)	2.5 yr	AD, asthma, rhinitis	57 gm	b: 5/0 m: 10/0	Pruritic erythematous rash, pruritus	+

Negative control sera were obtained from patients 7, 8, and 9.

Sera of patients 1 to 9 were used in immunoblot and immunodot experiments. Each patient's number corresponds to the label number in Figs. 2, 4, and 5. AD, Atopic dermatitis; b, beef; m, milk; +, positive result; —, negative result.

*Challenge dose is documented in hydrated beef.

of beef from neck trimmings,⁷ but gullet trimming is now also prohibited by the U.S. Department of Agriculture.

Various reports in the literature have implicated beef ingestion as a cause of infantile colitis,⁸ chronic diarrhea,⁹ urticaria,¹⁰ and glomerulonephritis.¹¹ However, the diagnoses in these studies were based on elimination diets and not confirmed by double-blind, placebo-controlled food challenges (DBPCFCs). Our study was prompted by the identification of a child who did not react to beef in a DBPCFC or to well-cooked beef in an open challenge but who reacted to a subsequent ingestion of less well-cooked beef. The aims of the study were to determine the prevalence of reactivity to beef in a select population and to determine whether heating had any effect on the antigenicity/allergenicity of beef extracts and whether results from immunoblot and immunodot blot experiments could explain the differential reactivity of patients to raw and well-cooked beef.

METHODS

Subjects and procedures

All patients were admitted to a Clinical Research Center (RR00052) for in-hospital oral food challenges to confirm the existence of beef and/or milk allergy, as previously described.¹² The DBPCFC was performed with well-cooked beef (Heinz baby food, Pittsburgh, Pa.) and nonfat dry milk. All negative results of blinded challenges were confirmed by subsequent open challenges before discharge. Patients con-

sumed a standard portion of the test food under observation. The percentage of challenge substance ingested before the onset of symptoms in the DBPCFCs and open challenges was also noted.

Of 335 patients referred for evaluation, 11 were diagnosed as having beef allergy. All were highly atopic and had atopic dermatitis, eight had allergic rhinitis, and eight of 11 had asthma. Three nonatopic control subjects were also studied. Control subjects showed no evidence of symptomatic allergy to beef or cow's milk and had negative skin prick test (SPT) responses. Two of the three normal control subjects were previously shown to possess very small amounts of IgG-specific antibodies to milk by a sensitive enzyme-linked immunoassay. None of the subjects had a history of penicillin allergy.

The SPTs were performed with a commercially available raw beef extract (1:20 wt/vol; Greer Laboratories, Lenoir, N.C.), saline solution, and histamine control (1 mg/ml) by using a bifurcated needle (Allergy Laboratories of Ohio, Columbus, Ohio). A positive SPT response was defined as a wheal $\geq$ 3 mm larger than the negative control wheal. All tests were applied and measured by the same nurse. Age, history, results of SPTs, and patients' clinical symptoms after DBPCFCs are summarized in Table I.

Extract preparation

Raw ground beef was pulverized in a Waring blender, and 15 gm of meat was mixed with 150 ml of phosphate-buffered saline (PBS) (pH 7.4). The sample was placed on a rotating platform overnight in a cold room at 4° C and then centrifuged at 1100 g for 30 minutes, defatted by removing the fat from the liquid surface, and clarified at 17,000 g for 15 minutes. Supernatants

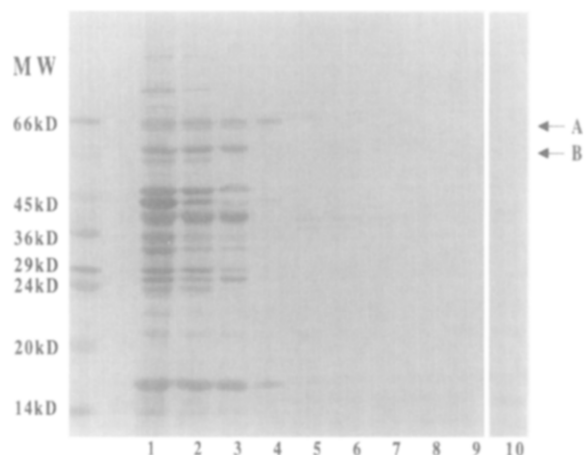


FIG. 1. Separation of raw ground beef extract protein fractions by SDS-PAGE and effect of heating at various temperatures for variable periods. Beef proteins were transferred onto nitrocellulose and subsequently stained with amido black (0.1%). Method 1 (time/temperature): Lane 1, 0 min/25° C; lane 2, 2 min/53° C; lane 3, 3 min/73° C; lane 4, 5 min/79.5° C; lane 5, 10 min/80° C; lane 6, 20 min/84.5° C; lane 7, 30 min/85° C; lane 8, 60 min/85° C; lane 9, 120 min/85° C; lane 10, same extract as in lane 9 but protein concentration was adjusted from 0.6 to 25 μ g, as in lane 1, to demonstrate the six heat-resistant fractions. In lane 1 equal volumes of heated extracts were loaded in each lane; therefore, protein content decreased from 25 μ g of protein in lane 1 to 0.6 μ g in lane 9, demonstrating loss of detectable water-soluble protein by heating. Arrow A, Heat-labile BSA; arrow B, heat-labile BGG. MW, Molecular weight.

were removed, and the protein content of the extracts was determined with the Coomassie blue–based protein determination kit (Bio-Rad Laboratories, Richmond, Calif). Milk proteins were extracted in a similar fashion.

Heating procedure

The extracts were heated in three different ways. The kinetics of protein denaturation were studied in method 1. Fifteen milliliters of the aqueous beef or milk extract was transferred to separate 50 ml tubes, which were placed in a boiling water bath and continuously stirred with a magnetic stir rod. During the first 5 minutes of this procedure, a sample of 500 μ l was removed every 30 seconds, and thereafter every minute. Temperatures were measured at each time point.

In method 2, 2 ml of beef (40 mg/ml in PBS), bovine serum albumin (BSA, 2 mg/ml in PBS; Sigma, St. Louis) or bovine gamma globulin (BGG, 2 mg/ml in PBS; Sigma) was heated at defined temperatures (60°, 65°, 70°, up to 95° C) for 15 minutes.

In method 3, ground beef was prepared in a frying pan for 2 different time and temperature intervals. Raw (0 minutes at room temperature), medium-rare (4 minutes at 45° C) and well-cooked (20 minutes up to 80° C) hamburger were extracted in the same way as the raw ground beef described above. The protein content was evaluated with a Coomassie blue–based protein determination kit (Bio-Rad).

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot

Physicochemical properties of the different beef extracts, milk, BSA, and BGG were evaluated by SDS-PAGE (13.5%) as

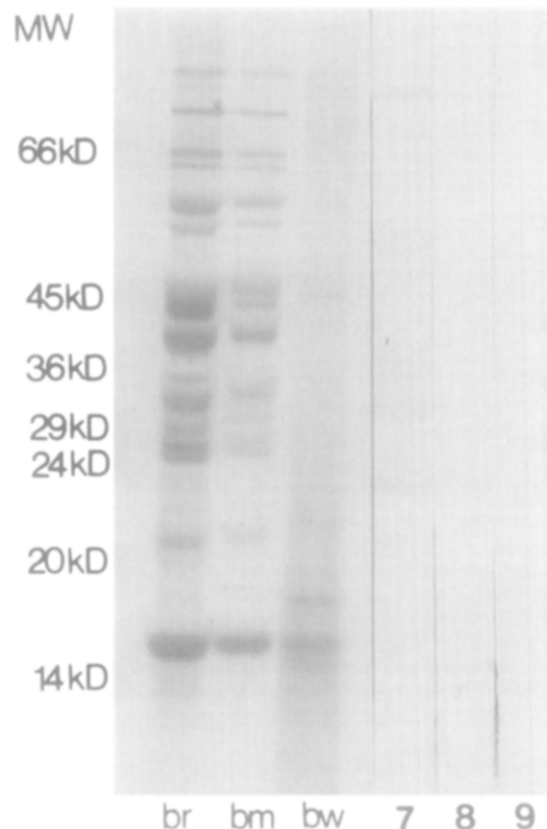


FIG. 2. Protein fractions of raw (*br*), medium-rare (*bm*), and well-cooked (*bw*) beef produced by SDS-PAGE. The protein concentration of different extracts was adjusted to 1 mg/ml, and 25 μ g of protein was loaded in each lane. Method 3: Beef protein fractions were transferred onto nitrocellulose overnight and stained with amido black (0.1%). Lanes 7, 8, and 9: Immunoblots with three different nonatopic control sera. Numbers correspond to patient numbers in Table I. MW, Molecular weight.

previously described.^{13, 14} Samples from the kinetics of heating study (Method 1) were diluted 1:10 in PBS and 1:2 in sample buffer, containing β -mercaptoethanol. Twenty-five microliters of each sample were loaded in separate gel lanes. Beef proteins and protein subunits were electrophoretically separated and transferred overnight onto nitrocellulose paper with 0.15 A in 50 mmol/L Tris-glycine buffer (pH 9.1) containing 20% methanol. The nitrocellulose was either stained with 0.1% amido black, to define specific protein bands and to ensure that proteins were transferred, or blocked with PBS–Tween-20 (0.05%) and porcine gelatin (0.5%) for immunologic studies.

For immunoblot studies, 25 μ g of each protein extract (method 3) was loaded in each gel lane, electrophoretically fractionated, and transferred onto nitrocellulose. As described previously, the blocked nitrocellulose blots were probed with a 1:20 dilution (in PBS–Tween and gelatin) of sera from patients allergic to beef (3 patients reacting to rare and well-cooked beef [P+] and 3 patients reacting only to rare beef [P–]) and control subjects to determine antigen-specific IgE binding to the raw and heat-treated beef protein bands. Bound human IgE was identified with affinity-purified, goat anti-human IgE antibody conjugated with alkaline phosphatase (Kirkegaard & Perry Laboratories).

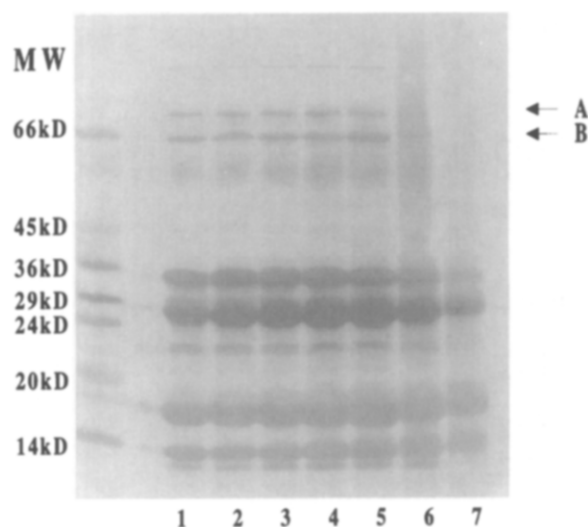


FIG. 3. Kinetics of thermal denaturation of milk proteins. Method 1 (time/temperature): *Lane 1*, 0 min/25° C; *lane 2*, 5 min/77.5° C; *lane 3*, 10 min/78.5° C; *lane 4*, 20 min/80° C; *lane 5*, 30 min/82° C; *lane 6*, 60 min/85° C; *lane 7*, 120 min/85° C. Twenty-five micrograms of protein was loaded in each lane. Arrow A, Heat-labile BSA; arrow B, heat-labile BGG. MW, Molecular weight.

Immunodot blot analyses

Briefly, 10 µg of each protein in 200 µl of PBS was applied directly to nitrocellulose paper with a 96-well minifold dot-blotter apparatus (Schleicher & Schuell, Inc., Keene, N.H.), as previously described in detail.¹⁴ The nitrocellulose was then stained with amido black, or later incubated with patients' and/or control subjects' sera (1:20).

Proteins investigated with this method were not reduced by β-mercaptoethanol in contrast to SDS-PAGE. A goat anti-human IgE antibody conjugated with alkaline phosphatase was used as the secondary indicator antibody in immunoblot and immunodot blot analyses.

IgE inhibition ELISA

Immunochemical analysis of the beef proteins was performed by an inhibition ELISA, as recently described.¹³ A raw beef protein extract was diluted in a sodium carbonate-bicarbonate buffer (pH 9.6), and 500 ng of protein was used per well to coat 96-well microtiter plates (Immunolon II plates; Dynatech Laboratories, Inc., Chantilly, Va.). Plates were incubated overnight at 4° C, washed with PBS-Tween, and blocked for 1 hour with 1% human serum albumin-PBS-Tween at room temperature.

The inhibition assay was performed by pipetting a fixed dilution (1:10) of sera from patients allergic to beef into a series of 1.5 ml microcentrifuge tubes containing dilutions of raw and cooked beef extract ranging from 500 µg to 2 µg/ml. The antigen-antibody mixture was then incubated at 37° C for 2 hours. One hundred microliters of the antigen (beef)-antibody (sera) solution was added to microtiter wells, previously coated with raw beef, and incubated for 2 hours at room temperature. Plates were washed and developed with a biotin-avidin system and read on a kinetic microplate reader (Molecular Devices Corp., Menlo Park, Calif.).

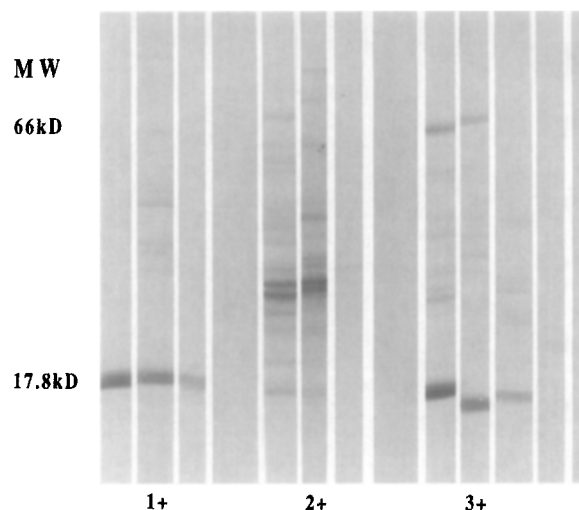


FIG. 4. Immunoblot analyses of sera from patients (P+) who reacted in DBPCFCs to well-cooked beef. For every patient: *lane 1*, serum IgE binding to rare beef; *lane 2*, serum IgE binding to medium-rare beef; *lane 3*, serum IgE binding to well-cooked beef. MW, Molecular weight.

RESULTS

SPTs and DBPCFCs

Eighty-nine of 335 patients (27%) evaluated for food hypersensitivity had a positive SPT response to beef. Sixty-six of the 89 patients underwent a DBPCFC with beef; the remaining 23 patients were ingesting beef with no apparent symptoms. Only eight of the 66 patients challenged with beef (12%) reacted during the DBPCFCs (P+) to the well-cooked beef; 58 of 66 tolerated the DBPCFC and well-cooked beef in an open challenge, but three of 58 reacted to subsequent ingestion of less well-cooked beef (P-).

Eight of the 11 children who reacted to beef (73%) were documented to be allergic to milk in previous DBPCFCs. All 11 children reacted with dermatologic symptoms, and none had life-threatening reactions to beef. In contrast, in a study group of 25 subjects with milk allergy verified by DBPCFC, only five (20%) had positive DBPCFC responses to beef, even though 21 (84%) had a positive SPT response to beef. Follow-up DBPCFCs documented that four patients lost their adverse reaction to beef within 2 years after the diagnosis; two of these four patients were not allergic to milk, and one patient lost clinical reactivity to both beef and milk. Only one patient showed persistent milk allergy once beef was tolerated. Challenge doses of beef that provoked allergic reactions, clinical symptoms elicited, and results of the SPTs are presented in Table I.

Characterization of BSA, BGG, beef, and milk by SDS-PAGE gels and effect of heat treatment on these proteins

The amount of water-extractable protein from the heat-treated beef extracts was significantly less than that

from the raw starting material: 40 mg/ml for raw beef (br), 30 mg/ml for medium-rare beef (bm) and 1 mg/ml for well-cooked beef protein (bw), respectively (Figs. 1 and 2).

After SDS-PAGE of raw beef and transfer to nitrocellulose, at least 24 protein fractions (Fig. 1, lane 1) were identified by amido black staining. The separated protein fractions ranged in molecular weight from greater than 66 kd to less than 14 kd. Six of 24 fractions persisted during heating to 85° C (Fig. 1, lanes 6 to 9), two strongly staining bands at 17.8 and 19 kd and four weakly staining bands at 14, 20, 45, and over 60 kd (Fig. 2). These bands were heat-stable for up to 2 hours (85° C) of heat treatment (Fig. 1). The decrease of detectable water-soluble protein (40 mg/ml to 1 mg/ml) by heating explains the loss of identifiable protein fractions on SDS-PAGE at later time points (Fig. 1, lanes 8 and 9). When method 1 was used, temperature increased slowly from room temperature to 85° C but did not reach 100° C. BSA and BGG were heat-labile in the beef extract (marked with arrows in Fig. 1, BSA: 66 kd, lane 5: 80° C/10 min, BGG: 60 kd, lane 4: 79.5° C/3 min) and in milk at 85° C (120 minutes) (Fig. 3, last lane). Likewise, the casein group, and especially kappa-casein, was partly heat-modified in milk with this degree of heat treatment (Fig. 3). Purified BSA (Sigma) in PBS (pH 7.2) was heat-resistant up to 95° C for 15 minutes, but purified BGG (Sigma) was heat-denatured within 15 minutes at 65° C (SDS-PAGE not shown).

Results from immunoblots

Water-soluble protein extracts from raw (br), medium-rare (bm), and well-cooked beef (bw) were adjusted to a concentration of 1 mg/ml and then resolved in a discontinuous SDS-PAGE system (Figs. 2, 4, and 5). Immunoblotting studies were performed with sera from 3P+ (nos. 1 to 3 in Table I), 3P- (nos. 4 to 6 in Table I), and three control subjects (nos. 7 to 9 in Table I). The nitrocellulose was cut into strips after transferring the proteins overnight in order to utilize small serum samples. Consequently, specific protein fractions in the three different extracts appear minimally shifted in Figs. 4 and 5. All patients allergic to beef possessed IgE antibodies specific to various beef protein fractions (Figs. 4 and 5). Up to six beef fractions, which persisted after heating, were bound by IgE from P+ (Fig. 4, Pt. 3+, third lane), but not P- (Fig. 5). A 17.8 kd fraction of raw, medium-rare and well-cooked beef appeared to be bound maximally by beef-specific IgE antibodies from P+ but only minimally by one P- (Fig. 5, Pt 4, third lane). Three of six patients (Pts #2 [P+], #3 [P+] and #4 [P-]) had IgE antibodies to the heat-labile BSA fraction (66 kd). One control serum (Fig. 2, lane 7) showed nonspecific binding to a band with high molecular weight (≥ 66 kd).

Results from immunodot blots

On immunodot blot analyses, nonspecific binding of the alkaline phosphatase-labeled goat anti-human IgE antibody to raw beef was seen (Fig. 6; control no. 10).

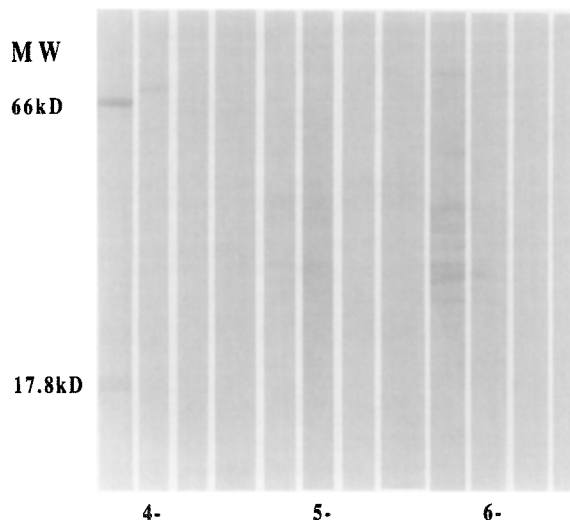


FIG. 5. Immunoblot analyses of sera from patients (3 P-) who reacted clinically only to rare and medium-rare beef but not to well-cooked beef. Clinical data of patients (nos. 1 to 9) are summarized in Table I. For every patient: *lane 1*, serum IgE binding to rare beef; *lane 2*, serum IgE binding to medium-rare beef; *lane 3*, serum IgE binding to well-cooked beef. MW, Molecular weight.

Patients allergic to milk (nos. 2 and 3) had significant IgE antibodies binding to all six extracts (raw and well-cooked beef, BSA, BGG, raw and heated milk). Patient 1 had significant binding only to BGG. The other patients and control subjects with negative results (nos. 7, 8, and 9) showed no significant binding to raw or well-cooked beef. One of the nonatopic control subjects showed binding to BGG.

Inhibition ELISAs

Inhibition ELISAs were performed with extracts from raw and well-cooked beef with patients' sera (P+ and P-). The concentration of raw beef protein required to achieve 50% inhibition was 8 μ g/ml in P+ (Fig. 7, A), whereas the curve with a well-cooked beef extract never reached 50% inhibition.

When serum from a patient who was clinically reactive to rare beef only was used, 50% inhibition of beef-specific IgE antibody required 2 μ g/ml well-cooked beef extract, whereas higher concentrations of raw beef were required to achieve similar inhibition. Similar results were seen with serum from a second patient in each group. Representative IgE-inhibition-ELISA curves from one P+ and one P- are shown in Fig. 7. It appears that patients reacting to well-cooked beef have higher levels of IgE antibodies to residual proteins of cooked beef compared with patients reacting only to rare beef.

DISCUSSION

A review of the literature suggests that beef allergy is rare. In a selected population, 11 of 335 patients (3.3%) evaluated were diagnosed as having clinical sensitivity to beef proteins. However, three of these reacted to rare

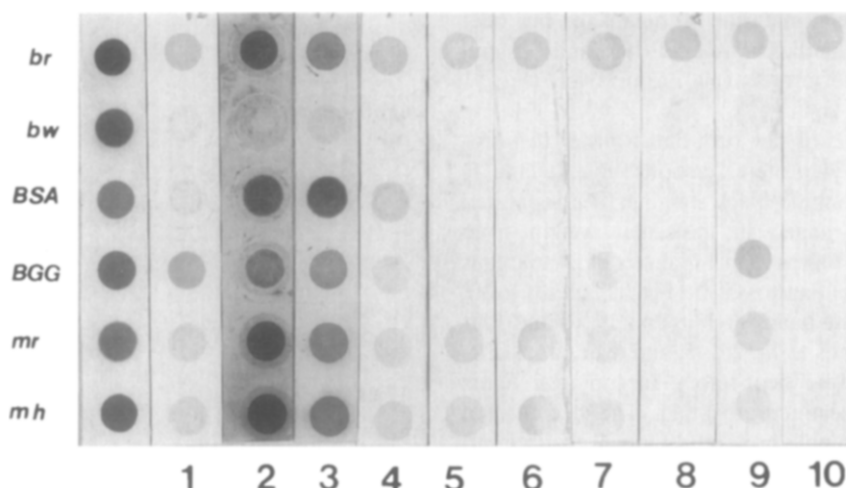


FIG. 6. Immunodot blot results. Numbers of lanes correspond to patient numbers in Table I. First lane shows amido black staining (0.1%) of protein loaded onto nitrocellulose. Lanes 7, 8, and 9: Incubation with nonatopic control sera; lane 10, incubation with the secondary antibody alone, which revealed some nonspecific binding to fractions of the raw beef (*br*) extract. *mr*, Raw milk; *mh*, heated milk.

beef only, having tolerated the DBPCFC and open feeding of well-cooked beef. A higher prevalence of beef allergy might be expected among children allergic to milk, because both foods contain BSA, BGG, and other proteins in significant quantities. Eight of the children allergic to beef reacted to milk in DBPCFCs. Bock et al.¹⁴ studied 460 subjects and did not report any positive reactions to beef in 1014 DBPCFCs, suggesting that beef is not a common allergen.

In agreement with previous published data, SPTs with a commercial beef extract are sensitive and have a good negative predictive value but have poor specificity and a poor positive predictive value. The loss of beef allergy was documented in four of 11 children within 2 to 3 years. However, because the number of cases of beef allergy is small, it is hard to determine whether the loss of clinical reactivity to beef and milk proteins correlates over time.

Concern has been raised that some proteins may change their characteristics with various processing treatments. To date, the number of false-negative DBPCFC results in which lyophilized food antigens were used in our studies is 0.9%. A study by Broadbent et al.¹⁴ demonstrated a 21% false-negative response rate with lyophilized fish used in DBPCFCs when compared with the subsequent open feeding, which indicated an unexpected lability of major fish allergens to lyophilization. This study, in which three patients were found who tolerated the blinded challenge but reacted to rare beef, also shows the importance of verifying negative DBPCFC results with a subsequent open challenge in which a standard portion of food is used. Furthermore it emphasizes the need to process the food in the same manner in which it is generally prepared for consumption by the patient in his or her diet.

The glycoprotein portion of a food is generally implicated as the allergenic component. These glycoproteins

are characteristically water-soluble, largely heat-resistant and acid-stable, and commonly in the molecular weight range of 10 to 60 kd. However, a report by Fisher¹⁶ supports the hypothesis that heat may influence the antigenicity/allergenicity of beef. He reported a patient who had anaphylactic symptoms after eating medium-rare beef but tolerated well-cooked beef. Scratch testing with raw beef produced a wheal-and-flare response, but well-cooked beef did not.

Thermal stability of proteins can be influenced by the pH,¹⁷ divalent cations,^{18,19} sugar content,¹⁹ and lipid concentration of the solution. Milk proteins are reported to vary in their heat stability in various milieus. BSA was documented as a more heat-labile protein in colostrum than in mature milk.^{20,21} With some proteins, such as β -lactoglobulin in cow's milk, some stages of heat modification are reversible.²² BSA and BGG are described to be the most heat-labile proteins in milk,²³⁻²⁶ and data from our study with beef would support this (Figs. 1, 4, and 7). Hanson and Johansson²³ demonstrated heat modification of BGG in 15 minutes at 75° C in mature milk and BSA at 80° C. In our study it was also necessary to heat the beef (pH 6) or milk extracts over 75° C to alter BSA and BGG (Figs. 1, 4, and 7). In contrast, purified BSA in PBS (pH 7.2) was resistant to heat (up to 95° C) for over 15 minutes. Although higher temperatures are necessary to denature protein in more acidic environments,²⁷ BSA was also more heat-labile in the beef extract (i.e., in a neutral pH). It is possible that the fresh beef extract used in our study contained residual enzymatic activity, which could have promoted BSA and BGG denaturation at lower temperatures, or that the lipid concentration remains high enough to destabilize the BSA protein in the beef extract, even after the defatting procedure.

Immunoblotting with sera from children allergic to milk has identified IgE antibodies to BGG²⁸ and cross-

reactivity between milk proteins from different species.^{29,30} Two of three patients who had IgE antibodies to BSA in the beef extract also had antibodies to BSA in milk and reacted clinically to milk. The number of patients allergic to milk who are clinically reactive to BSA and whether BSA has the same conformation and allergenicity in beef and milk remain unknown, but evaluation of our patient population suggests that isolated sensitization to BSA and BGG is the exception. Goldman et al.³¹ tried to study the frequency of reactions to isolated milk proteins. Only two of six patients with alleged BSA sensitivity could tolerate heat-processed milk. Nevertheless, it is difficult to draw any firm conclusions from these results, because it is unlikely that Goldman et al.³¹ were working with highly purified allergens.³² Ebbeling and Buckley³³ challenged five patients allergic to milk with a heat-treated whey protein-based formula. Four of five patients who were clinically sensitive to milk had immediate allergic reactions to the heated whey preparation. However, the authors did not indicate to what extent these patients were sensitized only to whey proteins or to what extent the preparation was free of casein proteins. In addition, whether the whey extract was heated for a sufficient time at an adequate temperature is not specified by the authors.

For our study, "well-cooked" beef consisted of thorough "browning" of the meat throughout its thickness, whereas in "rare" beef, the majority of a cut section appeared red centrally and pink peripherally with browning of the outer edges. "Less-well-cooked" beef consisted of pink meat centrally with no obvious "red" meat. The IgE-binding pattern correlated with the clinical symptoms of patients reacting to the rare and well-cooked beef (P+). However, one patient reacting only to less-well-cooked beef (P-, Fig. 5) showed weak serum IgE binding to the heat-resistant 17.8 kd band in the beef extract. Results of the immunodot blots and inhibition ELISAs failed to correlate with the challenge results. Previous studies with other foods have also shown that results of *in vitro* tests often do not correlate with results of DBPCFCs. A study by Bernhisel-Broadbent et al.¹³ showed that immunoblot analyses and inhibition ELISAs with sera from patients allergic to fish did not correlate with the patient's clinical reactivity. With the exception of a flounder extract, changes in allergenicity of the fish extracts as a result of lyophilization could not be demonstrated by SDS-PAGE, immunoblotting, or inhibition ELISA.³⁴ Consequently, the diagnosis of food allergy cannot be based on results of these *in vitro* assays.

Alternatively, concern has been raised that some proteins may become more allergenic with heating. One study by Bleumink and Young³⁵ suggested that heating might increase the allergenicity of β -lactoglobulin by incorporation of lactulose residues within the β -lactoglobulin molecule during pasteurization. With RAST inhibition, heat-stable shrimp allergens have been found to be more potent inhibitors than the raw extract.³⁶ However, IgE specificity to boiled shrimp may simply reflect the form

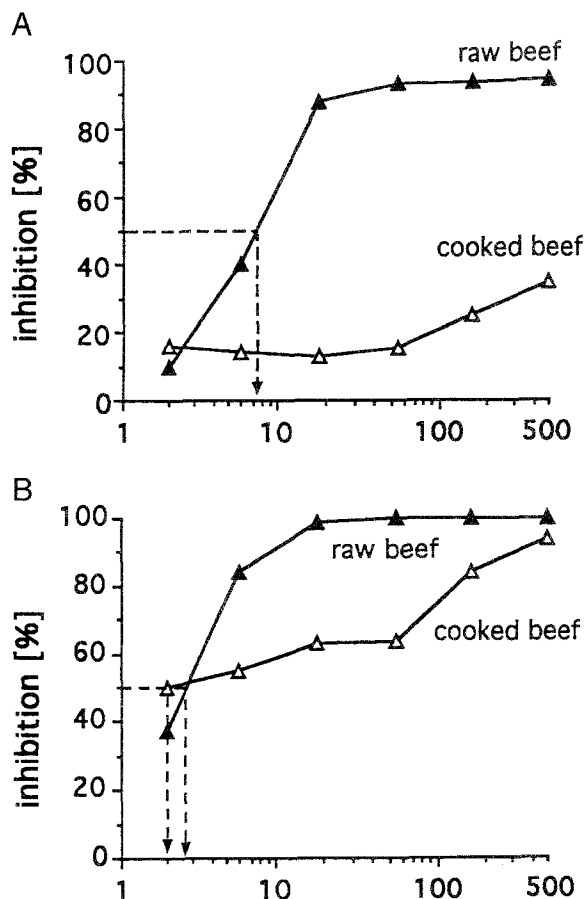


FIG. 7. A, Inhibition ELISA performed with serum from a P+. B, Inhibition ELISA performed with serum from a P-.

in which shrimp are consumed.³⁷ Similarly, IgE binding to certain soy proteins (20 kd band) also seemed to be enhanced by roasting the beans.³⁸ However, the presumption that "neo-antigens" are formed has never been confirmed to be of clinical significance.

In summary, our study emphasizes the need to verify a negative DBPCFC result with an open challenge. The food should be processed in the way it is normally consumed by the patient and should be provided in a standard portion. A marked loss in definable protein bands was noted in heated beef extracts. Patients clinically reactive only to rare beef had decreased IgE binding to beef fractions compared with patients reactive to well-cooked beef. We conclude that immunoblot analyses may help to distinguish which patients are likely to tolerate well-cooked beef, but the diagnosis cannot be based on the *in vitro* test results alone. Finally, patients reacting only to rare beef would not necessarily have to maintain a complete beef elimination diet.

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