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Reaction of Monoclonal and Polyclonal Antibodies Made against Infectious Agents with Various Food Antigens

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Abstract

Background: During the past 10 years in our clinical immunology laboratory, we have observed that some patients with high titers of antibodies against the Herpes family of viruses also exhibit elevation in antibodies against *Borrelia burgdorferi* antigens. Furthermore, when sera from patients with high IgG antibody levels against *B. burgdorferi* and Epstein-Barr virus were tested for food-specific antibodies, the degree of immune reactivity to food antigens was much higher in patients who were seropositive for *B. burgdorferi* and EBV antigens than in those who were seronegative. We purchased monoclonal and affinity-purified polyclonal antibodies against *B. burgdorferi*, Herpes family viruses and other infectious agents, and reacted them with 180 different food antigens so we could examine the degree of cross-reactivity between infectious agents and various food antigens.

Methods: Using ELISA methodology, we applied monoclonal and affinity-purified polyclonal antibodies against Epstein-Barr virus, cytomegalovirus, measles, rubella, herpes simplex type 1, *Varicella zoster*, *Chlamydia pneumoniae*, streptokinase, *Yersinia enterocolitica* and *Borrelia burgdorferi*, to 180 different food antigens in order to explain some baffling test results detected during lab testing.

Results: While some of these antibodies did not react to any of the tested food antigens, *B. burgdorferi* antibody reacted with 39 foods, EBV-VCA antibody with 20, EBNA-1 antibody with 10, EBV-EA antibody with 20, rotavirus antibody with 11, and measles antibody with 4 out of 180 food antigens. We demonstrated that these antibody-antigen reactions are specific, since only specific antigens and not non-specific antigens inhibited these immune reactivities.

Conclusions: Results indicate that the presence of some of the antibodies against infectious agents detected in human serum can result in false-positivity in serologic tests for food antigens used in the diagnosis of food adverse reactions. These results may explain antibody detection in the sera of many individuals against various food antigens that they have never actually eaten.

Keywords: Food IgG; Monoclonal antibody; Polyclonal antibody; Infectious agents; Cross-reactivity

Introduction

The false-positive rate of any laboratory test is the function of the specificity of the test and prevalence of the disease in the tested population [1]. In the performance of food antibody testing for the detection of food immune reactivities [2-4], the presence of antibodies that cross-react with other food antigens as well as with infectious agents can lead to false-positive results with deleterious consequences for the successful diagnosis of both food immune reaction and some infectious diseases. Although the immunological cross-reactivity between taxonomically related and unrelated foods has been described extensively and reviewed by Garcia and Lizaso in 2011 [5], very limited information is available about the role of infectious agents and how the presence of cross-reactive antibodies can result in false positivity in food antibody testing. This could be one major reason why there are often food extracts that test very high for antibody levels but do not actually provoke any clinical symptoms [2]. On the other hand, food-derived peptides have been shown to provoke antibody response in patients with Epstein-Barr virus (EBV) infection as well as in patients with autoimmune disorders [6]. For this reason, attempts have been made to characterize autoantigenic epitopes in different autoimmune diseases using random peptide phage libraries [7-9]. This approach, which identifies various cross-reactive epitopes, has been used to screen immunoglobulin fractions from patients with multiple sclerosis (MS) [10], type 1 diabetes [11], systemic lupus erythematosus (SLE) [12], and rheumatoid arthritis (RA) [13]. Interestingly, one of the peptides identified by affinity purification of an RA patient's serum showed homology with glycine-rich cell wall proteins (GRP) in cereals and with EBV nuclear antigen-1 (EBNA-1) [14,15]. Furthermore, this glycine-alanine repeated sequence (SGGGYGDGGAHGGGYGGGA) is shown to be homologous to cytokeratin, collagen, actin and human ribonucleoprotein [6,15]. Based on this mimicry, it was shown that EBNA-1 antibodies cross-react not only with French bean and related species but with different tissue antigens as well [6,14-17]. Serum IgG antibodies directed against the food-originated GRP are detected in different percentages of patients with several autoimmune disorders [6]. Researchers concluded that peptide originated from foods rich in glycine is able to elicit both a B- and T-cell immune response against EBNA-1 as well as a variety of tissues involved in various autoimmune diseases [6]. This B- and T-cell response to GRP peptide was also found in patients with food allergy [6]. Another example of antigenic mimicry between infectious agents, nutritional protein and human tissue antigen is the cross-reaction between rotavirus, bovine casein

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and retinal S-antigen, as shown by Wildner et al. [18]. In that study, while the injection of retinal S-antigen into animal models resulted in the development of uveitis in about 85% of the subjects, the injection of cross-reactive viral and food peptides resulted in severe uveitis in about 50% of the tested animals [18]. In further experiments, these authors showed that the sera of patients with iritis reacted with retinal S-antigen in 50% of the cases, followed by reaction with casein in 46%, and with rotavirus peptide in 38% of the cases [18]. Based on this and earlier studies, it was concluded that autoimmune responses to autoantigens can be induced by antigenic mimicry between the antigens of food and of infectious agents [18-23].

Borrelia burgdorferi, the spirochetal agent of Lyme disease, carries a peptide that cross-reacts with *E. coli*, *N. meningitides* and with *S. pyogenes* M5 protein [24,25]. *B. burgdorferi* is known to be involved in the induction of Lyme arthritis due to the cross-reactivity between human leukocyte antigen and cytokeratin 10 [26,27]. It has been postulated that infectious agents may trigger chronic inflammatory arthritis due to release of immunogenic material or by induction of anti-self reactivity from cross-reactive antibodies [25]. In addition, different studies have discussed the relevance of tick bites and tick-derived proteins to the production of antibodies against cross-reactive carbohydrate determinants (CCDs) such as mammalian oligosaccharide galactose-α-1,3-galactose (α-gal) in individuals with red meat allergy or in those who suffer from anaphylactic reactions to a chimeric monoclonal antibody containing α-gal epitope [28-31].

Yersinia enterocolitica and *Yersinia pseudotuberculosis* have been identified as the causative agents of reactive arthritis in humans. Evidence of serological cross-reactivity between Yersinia outer membrane protein and several proteins of *B. burgdorferi*, particularly proteins with the molecular size of 23, 33, 35, 37, 41, 44 and 45 kDa, has been demonstrated [32]. These two microorganisms could also be involved in oligoarthritis.

Interestingly, it has been shown that both Borrelia and Yersinia share amino acid sequence homology with various thyroid autoantigens; this means that the two microorganisms could be potential triggers for autoimmune thyroid disease through molecular mimicry [33].

Serological cross-reactivity between human tissue and various infectious agents such as rotavirus, *Yersinia enterocolitica*, *Borrelia burgdorferi*, *Candida albicans*, *Campylobacter jejuni*, and Epstein-Barr virus (EBV) has been established [15-20,23-35]; however, in relation to food protein cross-reactivity with different infectious agents, the existing knowledge as of this writing is limited only to the cross-reactivity of casein with rotavirus, milk with Chlamydia and Streptococcus, French beans with EBV [6,18,23], and tick-derived proteins with food-derived carbohydrate determinants [28,29]. For this reason, we studied possible serological cross-reactivity between 180 commonly used food items and, not just *B. burgdorferi* and EBV, but also rotavirus, *Y. enterocolitica*, measles, *Varicella zoster*, cytomegalovirus (CMV), and rubella. Understanding this cross-reactivity mechanism could allow the development of more accurate lab tests for Lyme disease, viral infections, and food immune reactions.

Experimental Section

Materials and methods

Antigens and antibodies: One hundred and eighty different food extracts or antigens, raw and/or cooked, (Table 1) were used in this study. Bean agglutinins, lectins, pea protein, wheat protein, rice protein, bromelain, porcine skin gelatin, transglutaminase, gums and food colorings were purchased from Sigma-Aldrich (St. Louis, MO). Cashew vicillin, latex hevein, parvalbumin, rice endochitinase, aquaporins and various oleosins were purchased from Bio-Synthesis (Lewisville, TX, USA). Other food items were purchased from Whole Foods.

The food proteins, glycoproteins, glycolipids and lipoproteins were extracted and purified according to the process described in previous studies [36] and used in our lab [37,38]. The protein concentration of each food was measured using a kit provided by Bio-Rad (Hercules, CA, USA).

Rotavirus antigen Strain SA-11 was obtained from MyBiosource.com. Epstein-Barr viral capsid antigen was obtained from Jena Bioscience (Germany), while *B. burgdorferi* lysate was obtained from Biodesign (Kennebunk, ME, USA).

Monoclonal anti-EBV VCA gp125 antibody clone L2, anti-EBV-EAD antibody clone R3, and anti-EBV-EBNA antibody clone E8.26 were purchased from EMD Millipore (Billerica, MA, USA). Monoclonal anti-HSV1+HSV2 capsid protein antibody clone 10B7 was purchased from Abcam (Cambridge, MA, USA). Affinity-purified goat anti-rotavirus was purchased from Genway Biotech Inc. (San Diego, CA, USA). Affinity-purified anti-CMV, measles, rubella, *Varicella zoster*, *Chlamydia pneumoniae*, human heat shock protein-60 (HSP-60) and streptokinase were prepared by Bio-Synthesis (Lewisville, TX, USA). Anti-*Yersinia enterocolitica* monoclonal antibody clone 8E9 was purchased from Mybiosource (San Diego, CA, USA). Monoclonal antibody against *B. burgdorferi* OspA, OspB, OspC, flab, VlsE, p18, p39, p66 and p93 were obtained from the CDC (Georgia, USA).

Reaction of antibodies with food antigens by ELISA: Each food antigen at a protein concentration of 1 mg/mL was dissolved in 0.1M of carbonate buffer (pH 9.6). After further dilution of 1:50, 100 µL of the extract or 2 µg of protein was added to duplicate wells of each microtiter plate and incubated overnight at 4°C. Plates were washed, and the unoccupied sites in the wells were saturated by adding 200 µl of 2% human serum albumin (HSA) and incubated overnight at 4°C. Plates were then washed again, and each monoclonal or affinity-purified polyclonal antibody was diluted in serum diluent buffer (0.1M PBS, pH 7.2, containing 2% HSA and 0.05% Tween 20) and added to the wells of the microtiter plates.

In addition, 100 µl of serum diluent alone was added to 4 different wells and used as controls. Plates were incubated for 2 hrs at room temperature (RT). This procedure was followed by washing, the addition of an optimal dilution of enzyme-labeled secondary antibody, and 1 hr incubation at RT. After another washing and the addition of the substrate the color development was measured by spectrophotometer at 405 nm.

Inhibition by specific and non-specific antigens: Twenty-seven mL of optimally diluted anti-EBV-VCA was divided among 3 different tubes (A, B, C) so that each tube contained 9 mL of antibody. One mL of serum diluent was added to Tube A; 5 mg of rabbit serum albumin (RSA) dissolved in 1 mL of diluent was added to Tube B; and 500 µg of each specific antigen dissolved in 1 mL of diluent was added to Tube C. The same procedure was done with optimally diluted anti-*B. burgdorferi* and anti-rotavirus antibody. All 9 tubes were incubated for 1 hr at 37°C, and then overnight at 4°C.

After centrifugation at 3000 g, the supernatant was removed and used for immune reactivity testing with different cross-reactive food antigens. For this, different strips of microtiter plate were coated with different food antigens that had shown high or moderate immune reactivity with these

Almond	Clam, cooked	Lima Bean, cooked	Rice, White+Brown, cooked
Almond, roasted	Clove	Macadamia Nut, raw+roasted	Rice Cake
Apple	Coffee Bean Protein, brewed	Mackerel, cooked	Rice Endochitinase
Apple Cider	Corn+Aquaporin, cooked	Mango	Rice Protein
Apricot	Corn Oleosin	Meat Glue	Rosemary
Artichoke, cooked	Corn, Popped	Mint	Safflower+Sunflower Oleosin
Asparagus	Crab+Lobster, cooked	Mushroom, raw+cooked	Salmon
Asparagus, cooked	Crab, Imitation, cooked	Mustard Seed	Salmon, cooked
Avocado	Cranberry	Nutmeg	Sardine+Anchovy, cooked
Banana	Cucumber, pickled	Okra, cooked	Scallops, cooked
Banana, cooked	Cumin	Olive, green+black, pickled	Sea Bass, cooked
Basil	Date	Onion+Scallion	Seaweed
Bean Agglutinins	Dill	Onion+Scallion, cooked	Sesame Albumin
Beef, cooked medium	Egg White, cooked	Orange	Sesame Oleosin
Beet, cooked	Egg Yolk, cooked	Orange Juice	Shrimp, cooked
Bell Pepper	Eggplant, cooked	Oregano	Shrimp Tropomyosin
Beta-Glucan	Fava Bean, cooked	Oyster, cooked	Soy Sauce, gluten-free
Black Bean, cooked	Fig	Papaya	Soybean Agglutinin
Blueberry	Flax Seed	Paprika	Soybean Oleosin
Brazil Nut, raw+roasted	Food Coloring	Parsley	Spinach+Aquaporin
Broccoli	Garbanzo Bean, cooked	Parvalbumin	Squid (Calamari), cooked
Broccoli, cooked	Garlic	Pea, cooked	Strawberry
Brussels Sprouts, cooked	Garlic, cooked	Pea Lectin	Sunflower Seeds, roasted
Cabbage, red/green	Gelatin	Pea Protein	Tea, Black, brewed
Cabbage, red/green, cooked	Ginger	Peach+Nectarine	Tea, Green, brewed
Canola Oleosin	Goat's Milk	Peanut, roasted	Thyme
Cantaloupe+Honeydew Melon	Grape, red+green	Peanut Agglutinin	Tilapia, cooked
Carrageenan	Grapefruit	Peanut Butter	Tofu
Carrot	Green Bean, cooked	Peanut Oleosin	Trout, cooked
Carrot, cooked	Gum Guar	Pear	Tomato+Aquaporin
Cashew	Gum, Locust Bean	Pecan, raw+roasted	Tomato Paste
Cashew, roasted	Gum, Mastic+Arabic	Pineapple	Tuna
Cashew Vicilin	Gum Tragacanth	Pineapple Bromelain	Tuna, cooked
Cauliflower, cooked	Gum, Xanthan	Pinto Bean, cooked	Turkey, cooked
Celery	Halibut, cooked	Pistachio, raw+roasted	Turmeric (Curcumin)
Cherry	Hazelnut, raw+roasted	Plum	Vanilla
Cheese	Honey, raw+processed	Pomegranate	Walnut
Chia Seed	Kidney Bean, cooked	Pork, cooked	Watermelon
Chicken, cooked	Kiwi	Potato, white, baked	Wheat
Chili Pepper	Lamb, cooked	Potato, white, fried	Whitefish, cooked
Chocolate, Dark,+Cocoa	Latex Hevein	Pumpkin+Squash, cooked	Wine, Red
Cilantro	Lemon+Lime	Pumpkin Seeds, roasted	Wine, White
Cinnamon	Lentil, cooked	Radish	Yam+Sweet Potato, cooked
Coconut, meat+water	Lentil Lectin	Red Snapper, cooked	Yogurt
Cod, cooked	Lettuce	Rice, Wild, cooked	Zucchini, cooked

Table 1: Food items used in this study.

antibodies. One hundred microliters of serum diluent only was added to each strip's A and B wells, which were then used as controls. One hundred microliters of unabsorbed antibody was added to wells C and D; 100 µl of antibody absorbed with RSA was added to wells E and F; and 100 µl of antibody absorbed with specific antigen (EBV-VCA, *B. burgdorferi*, rotavirus) was added to wells G and H. After 1 hr of incubation, the secondary antibody was added. Color development was measured at 405 nm. The percentage of inhibition was calculated by comparing the optical density of specific antibody reactivity to the specific antigen in the presence of RSA or microbial antigens.

Reactivity of sera with high titer of IgG antibody against EBV antigen, *B. burgdorferi* and rotavirus with various food antigens before and after absorption with specific antigens: Two different positive control sera with elevated IgG antibodies against *B. Burgdorferi* (MarDx Diagnostics), 2 different sera with elevated IgG antibodies against EBV antigen (Trinity Biotech), and 2 different sera with elevated IgG antibodies against rotavirus (MyBiosource) were each divided equally into 2 different test tubes for a total of 3 sets of 4 tubes each. The *B. burgdorferi* tubes were designated #s 1-4, the EBV tubes #s 5-8, and the rotavirus tubes #s 9-12. One hundred µg of RSA was added to tubes #1, 3, 5, 7, 9 and 11, 100 µg of *B. burgdorferi* antigen was added to tubes #2 and 4, 100 µg of EBV antigen was added to tubes #6 and 8, and 100 µg of rotavirus antigen was added and mixed to tubes #10 and 12. The tubes were mixed, kept 1 h at RT, and then overnight at 4°C. After incubation, the tubes were centrifuged at 3000 g; the supernatant was then removed and tested by ELISA for food IgG testing in triplicate, and the ODs were measured at 405 nm.

Results

To demonstrate possible cross-reactivity between infectious agents and food antigens, both monoclonal and affinity-purified antibodies were used. Each of these antibodies was applied to duplicate wells of ELISA plates coated with the antigens of specific infectious agents and of 180 different foods. While the application of specific antibodies against CMV and rubella to CMV- or rubella-coated wells resulted in a strong reaction or OD >3.0, the addition of these antibodies to wells coated with 180 different food antigens resulted in an OD of 0.1-0.3, which was equivalent to the ELISA background. Reaction of *Varicella zoster* antibody to these food antigens resulted in a very weak reaction with ginger (OD of 0.31) and ODs of <0.2 with the other 179 food antigens. The addition of anti-measles IgG antibody to measles and the same food antigens gave ODs of 3.3, 0.5, 0.5, 0.6, and 0.55 against measles antigen, seaweed, cashew, mustard seeds and shrimp respectively; no significant immune reaction (OD of 3SD above the mean of the ELISA background) was observed with the other 176 food antigens.

We examined the reactivity of monoclonal antibodies made against various antigenic components of *B. burgdorferi* to *B. burgdorferi* lysate-coated wells as well as to the 180 food antigens. The ELISA values of antibodies against *B. burgdorferi* and various food antigens are shown in Figures 1A and 1B. While the reaction of anti-*B. burgdorferi* with *B. burgdorferi* antigen reached an OD above 3.0, these antibodies also reacted strongly with 39 out of the 180 tested food antigens, including 28 plants and 11 different meats and seafood items. The highest reactivity (ODs>1.8) of anti-*B. burgdorferi* was observed with chicken, lamb, almond, mustard seeds, shrimp and parvalbumin. Reaction of this anti-*B. burgdorferi* with the other 141 food antigens resulted in the ELISA background color with a mean of 0.23 ± 0.21 (Figure 1B).

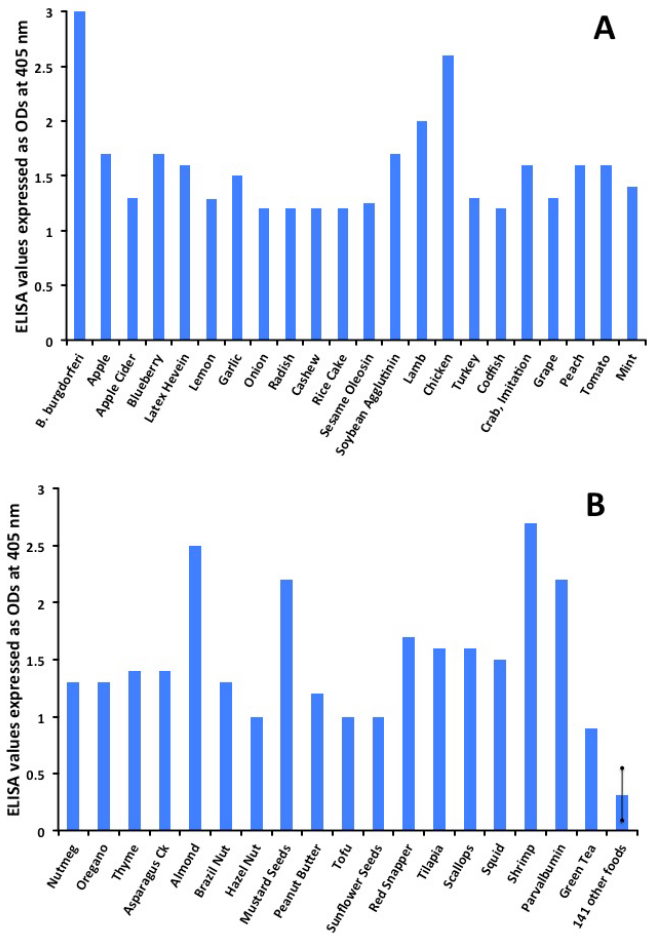

Figure 1: Reaction of antibodies against *B. burgdorferi* with 180 different food antigens. The reaction of anti-*B. burgdorferi* with *B. burgdorferi* lysate reached an OD above 3.0, but these antibodies also reacted strongly with 39 out of the 180 tested food antigens.

The major immunogenic antigens of EBV are viral capsid antigen (EBV-VCA), EB nuclear antigen (EBNA-1), and the early antigens (EBV-EA). Monoclonal antibodies generated against these viral antigens were tested for possible reactivity with various food antigens. Anti-EBV-VCA antibody reacted with 18 different plant antigens (fruits, vegetables, beans, nuts) and 2 seafood antigens (Figure 2). Anti-EBV-EA reacted with 15 different plant antigens and 5 seafood antigens (Figure 3). The anti-EBNA-1 antibody had the greatest reaction against egg white and moderate reaction against the antigens of 8 different plants and imitation crab (Figure 4).

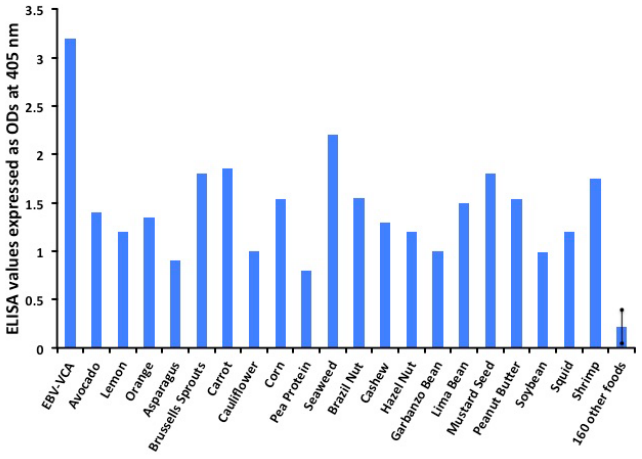

Figure 2: Reaction of antibodies against EBV-VCA with 180 different food antigens. Anti-EBV-VCA antibody reacted with 14 different plant antigens (fruits, vegetables, beans), 4 nut antigens and 2 seafood antigens.

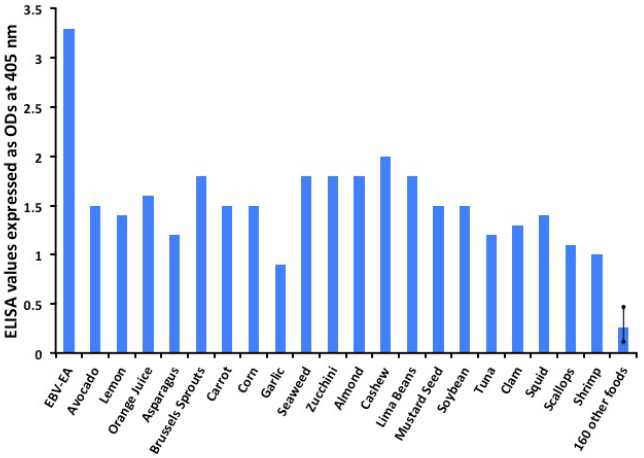

Figure 3: Reaction of antibodies against EBV, Early Antigen with 180 different food antigens. Anti-EBV-EA reacted with 13 different plant antigens, 2 nut antigens and 5 seafood antigens.

Rotavirus is the most common cause of severe gastroenteritis among children, and almost all children contract this infection. Affinity-purified anti-rotavirus antibody applied to rotavirus antigens and 180 different food extracts resulted in a very high OD of 3.45 with rotavirus antigen, and ODs ranging from 1.4 to 2.7 with 11 different food antigens, including 8 different plants, casein, tuna and shrimp (Figure 5).

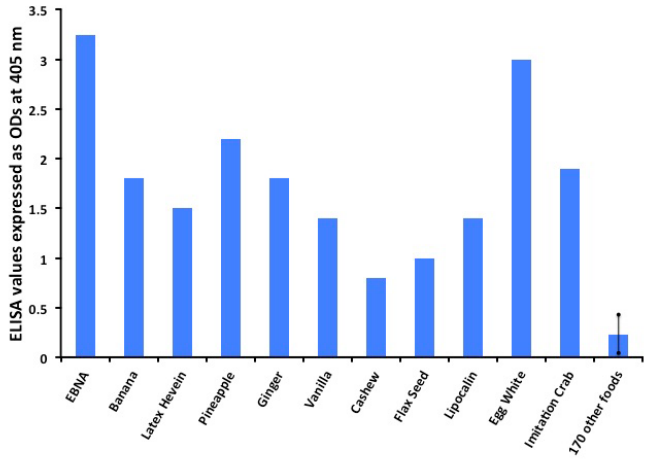

Figure 4: Reaction of antibodies against EBNA-1 with 180 different food antigens. The anti-EBNA-1 antibody had the greatest reaction against egg white and moderate reaction against the antigens of 8 different plants and imitation crab.

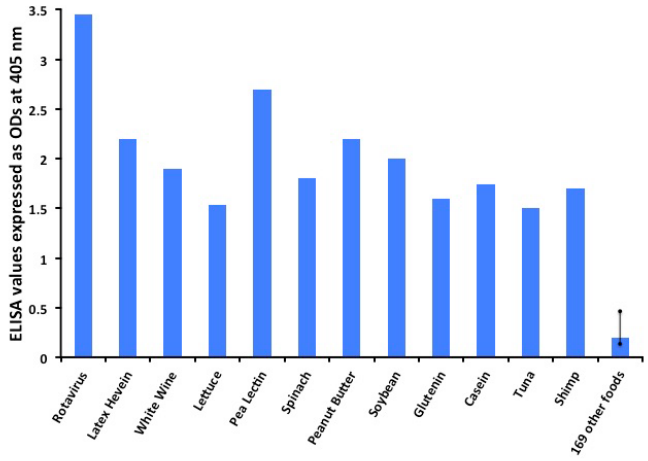

Figure 5: Reaction of antibodies against Rotavirus with 180 different food antigens. Anti-rotavirus antibody reacted with 11 different food antigens, including 8 different plants, casein, tuna and shrimp.

To demonstrate the specificity of these antibody-antigen reactions, the same monoclonal antibody made against *B. burgdorferi* was absorbed either with rabbit serum albumin (RSA) or *B. burgdorferi* antigens and was then reacted with the same food antigens. Results summarized in Table 2 showed that while absorption with RSA did not cause a significant change in the antibody levels, the addition of *B. burgdorferi* antigen to *B. burgdorferi*-positive serum resulted in 74% inhibition. The addition of various cross-reactive food antigens to the *B. burgdorferi*-positive serum resulted in an inhibition of antigen-antibody reaction of 35-63% (Table 2).

Absorption of EBV-VCA-positive serum with EBV-VCA antigen or with food antigens that have been shown to be reactive with this antibody is shown in Table 3. The addition of EBV-VCA in liquid phase resulted in 80% inhibition of EBV-VCA antibody binding to EBV-VCA antigen in solid phase. The addition of various immune-reactive food antigens resulted in 31-60% inhibition of this antigen-antibody reaction.

Similar results were obtained when anti-rotavirus antibody was absorbed with rotavirus antigen or with food antigens reacting to rotavirus antibody (Table 4).

To further demonstrate the specificity of these reactions, two different positive control human sera obtained from commercially available kits, each with high titers of IgG antibodies against *B. burgdorferi*, EBV-VCA and rotavirus and their immune reactivity against 180 foods, were tested before and after absorption with specific antigens as detailed in Materials and Methods. Results summarized in Table 5 show that after absorption with specific antigens the number of immune-reactive foods dropped 40% and 52% for *B. burgdorferi*, 28% and 32% for EBV-VCA, and 17% and 25% for rotavirus (Table 5).

Discussion

In the last ten years or so, in my own clinical immunology lab, I have performed hundreds of IgG and IgM antibody assays for the detection of Lyme disease and viral infection. And in the course of processing the continual parade of samples, antigens and antibodies, I made a personal observation of a curious correlation.

I noted that patients' samples with high titers of antibodies against the antigens of certain infectious diseases, specifically *Borrelia burgdorferi*, Epstein-Barr virus VCA, EBV-EA or EBNA consistently had significant reactions when tested against certain food antigens. These samples with high titers of antibodies against these infectious

	B. burgdorferi	**Apple**	**Blueberry**	**Latex Hevein**	**Lemon**	**Garlic**	**Almond**	**Cashew**	**Soybean Agglutinin**
Diluent	3.4	1.72	1.7	1.63	1.25	1.37	2.5	1.22	1.75
Diluent+1% RSA	3.5	1.8	1.5	1.7	1.2	1.4	2.4	1.1	1.6
Diluent + *B. burgdorferi* antigen	0.92	0.7	0.67	0.81	0.65	0.73	1.1	0.57	0.61
% Inhibition w/*B. burgdorferi* antigen	74	61	53	52	46	48	54	48	62
	Lamb	**Chicken**	**Turkey**	**Imitation Crab**	**Red Snapper**	**Tilapia**	**Shrimp**	**Scallops**	**Nutmeg**
Diluent	1.93	2.7	1.2	1.74	1.66	1.48	2.7	1.5	1.35
Diluent+1% RSA	1.81	2.2	1.3	1.5	1.5	1.4	2.2	1.31	1.2
Diluent+*B. burgdorferi* antigen	0.7	0.82	0.66	0.65	0.73	0.64	0.92	0.53	0.78
% Inhibition w/*B. burgdorferi* antigen	61	63	49	57	51	54	58	59	35

Table 2: Reaction of *B. burgdorferi* antibody with various food antigens before and after absorption with specific and non-specific antigens.

	EBV-VCA	Avocado	Lemon	Orange	Brussels Sprouts	Carrot	Corn	Seaweed	Brazil Nut
Diluent	3.3	1.4	1.2	1.35	1.8	1.86	1.54	2.2	1.55
Diluent+1% RSA	3.2	1.3	1.1	1.24	1.52	1.63	1.42	2.0	1.3
Diluent + EBV-VCA antigen	0.65	0.7	0.64	0.76	0.61	0.83	0.66	0.97	0.61
% Inhibition w/ EBV-VCA antigen	80	46	42	39	60	49	54	51	53
	Cashew	**Hazel Nut**	**Lima Bean**	**Mustard Seed**	**Peanut Butter**	**Soybean**	**Squid**	**Shrimp**	**Asparagus**
Diluent	1.3	1.2	1.5	1.8	1.54	0.99	1.2	1.75	0.9
Diluent+1% RSA	1.0	1.1	1.61	1.58	1.33	1.2	0.98	1.51	0.94
Diluent + EBV-VCA antigen	0.55	0.53	0.90	0.94	0.72	0.51	0.45	0.68	0.65
% Inhibition w/ EBV-VCA antigen	45	52	44	41	46	58	54	55	31

Table 3: Reaction of anti-EBV-VCA antibody with various food antigens before and after absorption with specific and non-specific antigens.

	Rotavirus	Latex Hevein	White Wine	Lettuce	Pea Lectin	Spinach
Diluent	3.4	2.2	1.9	1.53	2.7	1.8
Diluent+1% RSA	3.5	2.0	1.76	1.3	2.45	1.55
Diluent+Rotavirus antigen	0.86	0.85	0.73	0.61	0.98	0.69
% Inhibition w/Rotavirus antigen	75	47.5	59	53	60	45.5
	Peanut Butter	**Soybean**	**Glutenin**	**Casein**	**Tuna**	**Shrimp**
Diluent	2.2	2.0	1.6	1.8	1.5	1.7
Diluent+1% RSA	1.9	1.8	1.4	1.72	1.24	1.47
Diluent+Rotavirus antigen	0.82	1.0	0.59	0.81	0.61	0.75
% Inhibition w/Rotavirus antigen	57	45	58	51	53	49

Table 4: Reaction of anti-Rotavirus antibody with various food antigens before and after absorption with specific and non-specific antigen.

	***B. burgdorferi*-positive**		**Epstein-Barr Virus-positive**		**Rotavirus-positive**	
	Sample 1	**Sample 2**	**Sample 1**	**Sample 2**	**Sample 1**	**Sample 2**
Number of IgG immune-reactive foods before absorption with specific antigen	67/180	43/180	91/180	54/180	32/180	58/180
Number of IgG immune-reactive foods after absorption with specific antigen	32/180	26/180	66/180	37/180	24/180	48/180
Percentage of reduction	52%	40%	28%	32%	25%	17%

Table 5: 4 IgG sera reactive against *B. burgdorferi*, Epstein-Barr virus and Rotavirus and their reaction with various food antigens.

agents would react to 20-60% of tested food antigens. Conversely, samples with low levels of Borrelia and EBV antibodies reacted to only 5-20% of tested food antigens. What was the meaning of this correlation? With this in mind, I initiated this study in order to clarify the relevance of these antibodies to the food antibody testing that is currently performed in many laboratories for the detection of adverse reaction to foods.

The findings of this current study indicate that, indeed, some of the IgG immune reactivity test results reported by many labs for the detection of food immune reactions may be due to cross-reactivity with infectious agents, and, hence, could be false-positive. Since the introduction of food IgG testing in 1985, an increasing number of laboratories are marketing to healthcare practitioners and even directly to the consumers with unsubstantiated claims [38]. Because of my concern regarding this, I very recently published an article that discusses the history of food antibody testing, reviews the pertinent literature, tours the methodologies, analyzes and critiques them, and looks at why immunoglobulin G antibody testing for food may not be reproducible from one lab to another [38].

As discussed in the cited article [38], very often, unusual test results that are very difficult to comprehend are reported to the clinicians: for example, a patient reacting strongly to a food that he has never eaten, such as okra or seaweed; a patient who has been on a vegetarian diet for the past 12 years but who not only reacted strongly to 81 different plant-based food antigens, but also reacted to chicken, beef, shrimp, squid, tuna and several other fish; or an individual on a kosher diet who reacted to pork and various shellfish which were never part of his diet. Even if these particular assays had been performed by the laboratories accurately and reproducibly, it is precisely these kinds of baffling test results that have both patients and clinicians questioning the validity of antibody testing for foods. So far different authors have theorized that results such as these could possibly be due to the production of polyreactive antibodies that are presumably produced in response to gut microorganisms [39-42]. However, another possibility is unknown cross-reactivity. For example, the vegetarian patient that appears to be reacting to animal food proteins may actually be reacting to certain plant-based foods that the patient eats on a daily basis. These plant foods may be cross-reacting with chicken, beef or shellfish. This explanation is based on the cross-reactivity syndrome for IgE-mediated allergy to foods that are both related (e.g., eggs from different birds) and unrelated taxonomically (cross-reaction between milk and egg) [5]. While cross-reaction between taxonomically related or unrelated food antigens have been extensively studied and reviewed [5,43-46], by comparison the cross-reaction between infectious agents and food antigens has been the subject of only a few studies; for example, the cross-reaction between rotavirus and bovine casein [18], *Chlamydia pneumoniae* and Streptococcus Group A with milk [5], glycine-rich protein from foods provoking antibody response in patients with EBV

infection [6], or tick-derived proteins with red meat, red blood cells, and carbohydrate determinants in plant foodstuffs [29,30,47].

To demonstrate the contributions of infectious agents towards food immune reactivity, we applied a series of monoclonal and affinity-purified antibodies made against different infectious agents to 180 different food antigens in replicate studies by indirect quantitative ELISA. While the addition of specific antibodies to CMV, rubella, and VZV resulted in no or minimal reaction with food antigen-coated plates, the addition of anti-Borrelia antibody resulted in moderate to strong reactions with 39 out of the 180 tested food antigens. The degree of reactivity of these monoclonal antibodies to various food antigens was calculated using as 100% the OD obtained by binding anti-Borrelia antibody to Borrelia-coated plates, and removing the background OD from the resultant ODs. Based on this calculation, the binding percentage of anti-Borrelia antibody to green tea was 24%, to red snapper 52%, and to chicken 83% (Figure 1). While currently cross-reaction between *B. burgdorferi*, other infectious agents and human tissue has been demonstrated [25-27,32,33], we do not know about the nature of the cross-reaction between Borrelia and various food antigens, including red meat. However, by using Borrelia and food antigens in competitive ELISA, 74% inhibition was observed with the Borrelia antigens, and 35-63% inhibition was observed when food antigens were added. This is an indication of the specificity of this binding of Borrelia-specific antibody to various food antigens (Table 2). Sequence analysis of protein similarities between *B. burgdorferi* or other infectious agents and the various food antigens described here is recommended for future studies.

In relation to EBV, since it was demonstrated that the EBNA glycine-alanine repeat sequence cross-reacts with the protein sequences in some cereals [15-17], we reacted various EBV antibodies with 180 food antigens. Again, using the OD obtained by binding a particular EBV antibody to its specific EBV-coated plates as 100%, we found that EBNA-specific antibodies reacted with 10 different foods ranging from 20% for cashew to 92% for egg white (Figure 4), 20 different foods reacted with EBV-VCA antibody ranging from 20% for pea protein to 67% for seaweed (Figure 2), and 20 different foods reacted with EBV-EA antigen ranging from 23% for garlic to 58% for cashew (Figure 3). Thus, the chronic nature of EBV and prevalence of IgG antibodies in the blood of the general population may contribute to the elevation of antibodies against different food antigens, without an individual having adverse reactions to these food antigens.

Since EBV persists in blood lymphocytes [48], this repetitive stimulation may occur by repeated exposure to the cross-reactive food antigens shown in this study, or by repeated exposure to an enterovirus, such as rotavirus. Because rotavirus infections are common causes of gastroenteritis, and more than 90% of the general population have had rotavirus infection at least once in their lives, immune response to this virus might be elicited [49]. At the same time, oral tolerance to cross-reactive foods such as casein [18] and other food antigens shown in this study can be abrogated. The list of these food antigens that were shown to react with rotavirus antibody is summarized in Figure 5. Once more using the OD derived from the binding of rotavirus antibody to rotavirus-coated plates as 100%, the percentage of binding for rotavirus antibody was found to be 46% for shrimp, 47% for alpha-casein, the known cross-reactive antigen, and 77% for pea lectin. No immune reaction was observed with the other 169 foods.

With regards to other cross-reacting substances that may obfuscate accurate test results, we have already mentioned studies that have investigated the role of cross-reactive carbohydrate determinants (CCDs) in tick-borne disorders and infectious diseases [28-31]. Although antibodies against CCDs have been detected in allergic individuals [47,50], the following evidence indicates that the antibodies in our present study are not directed against carbohydrate structure or cross-reactive carbohydrate determinants [1]:

- Not all monoclonal and affinity-purified polyclonal antibodies reacted against the food items used. For example, CMV and rubella antibodies did not react to any of the food antigens.
- The antibodies that did react against food antigens each had different patterns of reactivity.
- Monoclonal antibodies produced against various epitopes of *B. burgdorferi* reacted both with foods that contained α-gal and foods that did not.
- The monoclonal antibodies used are not chimeric and do not contain the α-gal epitope which has been implicated in meat allergy.

Usually, IgG production against food antigens is associated either with a failure in oral tolerance mechanisms or molecular mimicry [51,52]. In this case, considering the patterns of association between the infectious agents and the foods detailed in the preceding sections, the detected IgG antibodies against 69 out of 180 tested food antigens (38%) could be due to molecular mimicry and the persistence of cross-reactive IgG antibody in the blood, and not due to a breakdown in the oral tolerance mechanism against these food antigens.

The role of homology of food proteins with infectious agents remains to be elucidated further in future studies. Combining our present findings with those of such future investigations will allow better utilization of current diagnostic tools and the development of more accurate lab tests, not only for food immune reactivity, but for tick-borne disorders and viral infections as well.

Conclusions

Conventional food antibody testing occasionally results in positive findings against foods that the tested individual has never actually eaten. This is not only mystifying but, if left unexplained, would throw doubt on the validity of food antibody testing itself. In this report, however, we determined that these results can be attributed to the cross-reactivity between some foods and various infectious agents that are present in the tested patient's blood, either through chronic infection or immunization. This would explain why some patients who test positive for multiple foods may remove those foods from their diet but fail to experience any improvement of their symptoms, and still test positive upon subsequent testing. This is because the actual culprit, the cross-reactive antibody to an infectious agent, is still within the body. Based on the results presented here, the IgG antibody test results for foods reported by many laboratories should be interpreted by the healthcare practitioner in the context of cross-reactivity with various infectious agents, including *Borrelia burgdorferi* and Epstein-Barr virus.

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Conflicts of Interest

Aristo Vojdani is a co-owner of Immunosciences Lab., Inc. (ISL). ISL offers tests for Lyme disease and viral infection, but does not offer any tests for food antibody assays.

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