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Correlation of Tissue Antibodies and Food Immune Reactivity in Randomly Selected Patient Specimens

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Abstract

Objectives: Specific food antigens, such as gliadins and caseins, have been well documented as triggering immune reactivity to human tissues, due to cross-reactivity or molecular mimicry. Food antigens, such as agglutinins, are known to bind to human tissues, which can result in autoantibodies. This study aimed to examine the correlation between food protein antibodies and tissue antibodies.

Method: We selected 118 patients on whom food and tissue antibodies were simultaneously measured in our laboratory. Data was collected on patient IgG reactivity to wheat gliadins and glutenin; IgG+IgA reactivity to caseins, milk by tryophilin and other milk proteins; and IgG reactivity to wheat germ agglutinin (WGA) and IgG+IgA reactivity to other food lectins/agglutinins. We compared the tissue IgG+IgA positivity between patients positive for food antibodies versus patients negative for food antibodies.

Results: Of 45 patients negative for IgG against gluten proteins, 16 (35%) were reactive against one or more tissues, while of 45 positive for IgG against gluten proteins, 29 (64%) were reactive against tissues. Of 30 patients negative for dairy proteins antibodies, 9 (30%) were reactive against one or more tissues, while of 30 patients positive for dairy antibodies, 22 (73%) were reactive against tissues. Of 25 patients negative for IgG against WGA, 8 (22%) were reactive against one or more tissues, while of 25 patients positive for IgG against WGA, 19 (76%) were reactive against tissues.

Conclusion: Patients with antibody reactivity to specific food proteins showed higher co-occurrence of tissue autoantibodies than patients without food reactivity's. More studies are needed to assess the long-term role of diet on the onset and management of autoimmunity.

Keywords: Autoimmunity; Gluten; Dairy; Casein, Lectins; Agglutinins; Cross-reaction

Introduction

Autoimmune diseases are on the rise world-wide [1]. The devastating direct and indirect costs of autoimmune disease in the United States alone are conservatively estimated at over $100 billion [2]. In Europe, the Social Insurance Institution in Poland compiled statistics on the indirect costs, sick leave and disability payments, for only three autoimmune diseases, systemic lupus erythematosus, systemic sclerosis and sarcoidosis for 2012; for the 4800 patients included in the report, total indirect costs were as high as 7,260,595, 2,268,571 and 4,027,575 EUR, respectively [3]. The loss in quality of life cannot be quantified.

Prevention is key. Known environmental triggers of autoimmune reactivity include food proteins, for example, gluten instigates Celiac disease [4-7]; chemicals, for instance, bisphenol A plays a role in thyroid disorders [8-10]; and pathogens, such as, Porphyromonas gingivalis contributes to rheumatoid arthritis [11-13]. In this study we investigated the prevalence of specific food protein antibodies made simultaneously with human tissue antibodies. The food groups included here are gluten family protein antibodies and dairy family protein antibodies, both of which have been shown to cross-react with human tissues [14-17] and finally, food lectins and agglutinins, which are known to bind to human tissues [18,19].

The main mechanisms involved in food protein-induced autoimmunity are antibody cross-reactivity and covalent biding of food and human tissue proteins. Shared amino acid homology between gliadin and human tissues as well as dairy proteins and human tissues has been illustrated [20,21]. Due to this similarity, if antibodies are produced against gliadin, those gliadin antibodies could potentially mistake cerebellar tissue, or thyroid peroxidase for gliadin, and thus, pursue self-tissue as foreign material. Figure 1 illustrates

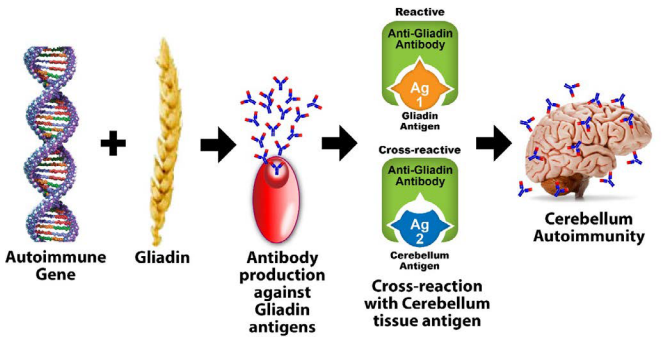

Figure 1: Mechanism of Cross-Reactivity Leading to Autoimmunity. The induction of environmentally-induced autoimmunity by cross-reactivity or molecular mimicry starts with a genetic susceptibility for joint disease. The person consumes wheat gluten. The person loses immune tolerance to gliadin and makes a myriad of antibodies to gluten family proteins. Some of those gliadin-specific antibodies mistake cerebellar tissue for gliadin and eventually neuoautoimmunity ensues.

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the mechanism of food contributing to autoimmunity *via* antibody cross-reactivity. In an earlier study, Vojdani and Tarash [15] found significant cross-reactivity between gliadin antibody and multiple human tissues including: asialoganglioside, cytochrome P450, glutamic acid decarboxylase 65, 21 hydroxylase, myelin basic protein, cerebellar, osteocyte, synapsin, myocardial peptide, ovary, thyroid peroxidase.

With this information, it is clear how potentially damaging gliadin can be to the human body. Dairy protein antibodies can also elicit autoimmune reactivity. Specifically, casein and milk butyrophilin have been shown to cross-react with glutamic acid decarboxylase-65 [22], cerebellar [14] and myelin [16,17].

The covalent binding of food lectins and agglutinins to human tissues is another mechanism of food contributing to autoimmune disorders (Figure 2). Plant lectins and agglutinins such as those found in wheat, lentils, beans, peanuts and soybeans, may covalently bind to a variety of human tissues (Table 1). The immune system may be alerted to the bound tissue, an alien antigen, tag it as non-self and generate antibodies against the new antigen. By attacking the new protein, the surrounding tissue can be destroyed, causing the presence of self-tissue proteins in circulation. In this scenario, auto-antibodies can be formed against the tissue, thus resulting in autoimmune disease.

Method

All testing was performed at Cyrex Laboratories, LLC, a clinical laboratory located in Phoenix, Arizona, USA. The testing method used was enzyme-linked immunosorbent assay (ELISA), as previously described by Vojdani [23,24], for the detection of IgG, IgA or IgG+IgA to the specific antigen. Specimens were assessed in side by side duplicate to ensure reproducibility. If correlation between the side by side wells was not achieved, the specimen was rerun. To be included in this study, a comprehensive predictive tissue antibody panel, Array 5 Multiple Autoimmune Reactivity Screen, had to be ordered simultaneously with at least one of three food immune reactivity panels: Array 3-Wheat/Gluten Proteome Reactivity and Autoimmune Screen, Array 4- Gluten-Associated Cross-Reactivity and Food Sensitivity, Array 10-Multiple Food Immune Reactivity Screen (Table 2 for antigen lists).

Results

A pool of 118 patient results was recorded. From this pool, equal numbers of positive and negative for each of three food proteins groups were compiled (45 gluten families, 30 dairy family, and 25 lectins/agglutinins). There is subject overlap, as some patient results fit in more

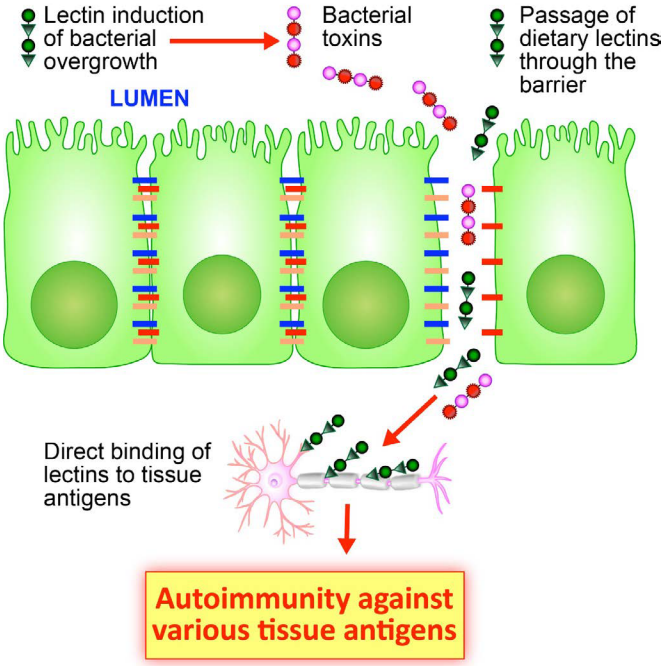


Figure 2: Mechanism of Lectin/Agglutinin Binding to Human tissue. Lectins/Agglutinins have an affinity for binding to multiple tissues in the human body. They can first induce gut dysbiosis, which results in gut inflammation. When the gut in inflamed, the intestinal barrier can breakdown allowing the passage of lectin/agglutinins and other antigens into circulation. Having infiltrated the body, lectins/agglutinins can attach to their target tissue, which can result in autoimmunity against that tissue.

Tissue	Wheat Germ Agglutinin	Soybean Agglutinin	Peanut Agglutinin	Lentil Lectin	Pea Lectin	Bean Agglutinins
Skin	*	*	*	*		*
Buccal mucosa	*	*	*	*		
Stomach	*					
Parietal cells		*	*			
Intestinal brush border	*	*				*
Colonic mucosa	*			*		
Connective tissue	*			*	*	*
Thyroid	*	*		*		*
Cartilage	*	*	*			
Liver	*	*	*			*
Pancreas	*					*
Kidney	*			*		*
Prostate	*		*	*		
Skeletal muscle	*	*	*		*	
Cardiac muscle	*	*				
Breast	*	*	*			
Pituitary			*			
Eye	*	*	*		*	*
Brain (myelin)	*			*		*

Table 1: Lectins/Agglutinins with affinity to specific tissues. Modified from Freed [18].

ARRAY 5 – MULTIPLE AUTOIMMUNE REACTIVITY SCREEN™ IgG+IgA Combined		
Parietal Cell+ATPase	Alpha-Myosin	Cytochrome P450
Intrinsic Factor	Phospholipid	Insulin+Islet Cell Antigen
ASCA+ANCA	Platelet Glycoprotein	Glutamic Acid Decarboxylase 65
Tropomyosin	Ovary/Testis	Myelin Basic Protein
Thryoglobulin	Fibulin	Asialoganglioside
Thyroid Peroxidase (TPO)	Collagen Complex	a+b Tubulin
21 Hydroxylase	Arthritic Peptide	Cerebellar
Myocardial Peptide	Osteocyte	Synapsin
ARRAY 3 – WHEAT/GLUTEN PROTEOME REACTIVITY & AUTOIMMUNITY™ IgG and IgA Separate		
Wheat	g-Gliadin-15-mer	Gliadin-Transglutaminase Complex
Wheat Germ Agglutinin	w-Gliadin-17-mer	Transglutaminase-2
Native+Deamidated a-Gliadin-33-mer	Glutenin-21-mer	Transglutaminase-3
a-Gliadin-17-mer	Gluteomorphin + Prodynorphin	Transglutaminase-6
ARRAY 4 – GLUTEN-ASSOCIATED CROSS-REACTIVE FOODS & FOOD SENSITIVITY™ IgG+IgA Combined		
Rye, Barley, Spelt, Polish Wheat	Yeast	Sesame
Instant Coffee	Oats	Amaranth
Cow's Milk	Millet	Quinoa
a+b Casein	Rice	Tapioca
Casomorphin	Corn	Teff
Milk Butyrophilin	Buckwheat	Potato
Whey Protein	Sorghum	Egg, Raw and Cooked
Milk Chocolate	Hemp	Soy
ARRAY 10 - MULTIPLE FOOD IMMUNE REACTIVITY SCREEN™ IgG+IgA Combined 180 food antigens tested in the following categories:		
Dairy and Eggs, Modified	Vegetables, Raw and Modified	Herbs, Raw
Grains, Raw and Modified	Fruit, Raw and Modified	Spices, Raw
Beans and Legumes, Modified	Fish and Seafood, Raw and Modified	Gums
Nuts and Seeds, Raw and Modified	Meat, Modified	Brewed Beverages and Additives

Table 2. Test Panels Ordered.

than one category. Although lectins/agglutinins have the capacity to bind to most of the tissues assessed on Array 5-Multiple Autoimmune Reactivity Screen (Table 2), we recorded only the reactivity to the tissues to which gliadin and/or dairy proteins antibodies cross-react. Thus, our tissue antibody list is narrowed to include: thyroid peroxidase (TPO), 21 hydroxylase (adrenal cortex), myocardial peptide, α-myosin, ovary/testis, fibulin, collagen complex, arthritic peptide, osteocyte, cytochrome P450 (hepatocyte), glutamic acid decarboxylase 65 (GAD65), myelin basic protein (MBP), asialoganglioside, α+β tubulin, cerebellar, synapsin. Patient results were recorded as being normal (N), which is considered immunologically within the reference range; equivocal (E), which falls between 1 and 2 standard deviations above the mean; or positive (P), which is out of range or greater than 2 standard deviations above the mean. In this study, E results were considered N and only P results were labeled as reactive.

Gluten family proteins

The Array 3-Wheat/Gluten Proteome Reactivity and Autoimmunity consists of multiple antigens (Table 2). For this study we recorded only the following: native+deamidated β-gliadin-33 mer, β-gliadin-17 mer, β-gliadin-15 mer, β-gliadin-17 mer, glutenin-21 mer. We selected 45 specimens resulting IgG negative for all gluten family proteins and 45 specimens IgG positive to at least one gluten family protein but IgA negative for all gluten family proteins. Our IgG positive group excluded IgA positive specimens so as to prevent using possible celiac patients in the group. The link between Celiac disease and additional extra-intestinal disorders has been well-established [25-27]. What hasn't been fully discussed is the link between non-celiac gluten sensitivity (NCGS) and autoimmunity.

The 45 IgG positive and 45 IgG negative for gluten family protein reactivity were compared to their corresponding tissue reactivity results.

In the negative group, 16 (35%) were positive for antibodies against one or more tissues. Although the tissue reactivity cannot be associated with IgG gluten reactivity, it is important to note that 7 out of these 16 patients were positive for dairy family proteins and 8 out of these 16 were positive for IgA gluten family proteins. Thus, all but one patient's tissue immune reactivity can be linked to either dairy reactivity or possible Celiac disease.

In the gluten family IgG positive group, 29 (64%) reacted to one or more tissues (Table 3). We found significantly more tissue immune reactivity in the IgG positive group compared to the IgG negative group (Figure 3).

Neurological tissues topped the list of autoimmune reactivity in patients IgG only positive for gluten family protein reactivity.

Dairy family proteins

Dairy family protein reactivity was assessed by looking at IgG+IgA measurements against dairy-specific antigens from Array 4-Gluten-Associated Cross-Reactive Foods (Table 2). The antigen results recorded included: cow's milk, α+βcasein, casomorphin, milk butyrophilin, whey protein, milk chocolate. We selected 30 specimens resulting IgG+IgA negative for all dairy family proteins and 30 specimens IgG+IgA positive to at least one dairy family protein.

The 30 IgG+IgA positive and 30 IgG+IgA negative for dairy

TPO	21 Hydro	HEART	Ova/Test	JOINT	BONE	Cyto P450	GAD65	NEURO
P	P	P	P	P	P	N	P	P
N	P	E	P	E	E	P	E	P
E	P	P	P	P	E	P	P	P
N	P	E	N	P	N	P	E	E
N	P	P	N	N	P	N	P	P
P	E	P	N	E	N	P	E	P
N	P	P	N	P	E	P	E	P
N	P	N	E	E	N	E	N	P
E	P	P	E	P	N	P	E	P
N	P	E	P	P	N	P	E	P
P	P	P	P	E	E	E	P	P
P	P	P	P	P	P	P	P	P
P	P	P	E	P	N	N	P	P
P	P	E	N	P	P	N	E	P
N	E	E	N	E	E	N	N	N
N	E	N	N	P	E	N	N	P
N	N	E	N	N	N	N	N	E
E	E	N	N	N	N	N	E	E
N	P	P	E	E	E	N	E	E
N	E	P	E	N	E	N	E	P
E	N	P	E	N	N	N	N	P
N	P	P	E	P	E	E	E	P
N	N	N	N	N	N	N	P	P
N	E	E	N	N	N	N	N	E
P	P	E	E	N	E	N	N	N
N	N	E	N	N	N	N	N	N
P	N	N	E	E	E	N	E	E
N	E	P	N	E	N	N	N	E
N	N	N	N	N	N	N	E	E
N	N	N	N	E	N	N	N	N
N	P	P	P	P	P	N	P	P
N	P	E	E	N	E	N	P	P
N	N	N	N	N	N	N	N	N
E	N	N	N	N	N	N	N	E
N	N	N	N	N	N	N	N	P
E	N	P	N	E	P	N	N	E
N	N	N	N	N	N	N	N	N
N	N	P	P	E	E	N	P	P
E	N	N	N	E	E	N	N	N
N	E	N	N	N	N	N	N	E
E	N	N	N	N	N	N	N	N
P	P	P	E	E	E	E	N	P
E	N	N	N	N	N	N	N	N
N	E	N	N	E	E	N	N	E
E	N	N	N	N	N	N	N	E

Table 3: Tissue Reactivity on 45 IgG Gluten Family Positive Patients. Gliadin antibodies can cross-react with multiple tissues in the body, which may result in autoantibody formation. Positive results are indicated as "P" or black, weak positive results are indicated as "E" or gray and Negative results are indicated as "N" or white.

family protein reactivity were compared to their corresponding tissue reactivity results.

In the negative group, 9 (30%) were positive for antibodies against one or more tissues. Although the tissue reactivity cannot be associated with IgG+IgA dairy protein reactivity, it is important to note that 7 out of these 9 patients were positive for gluten family antibodies. Thus, all but two patient's tissue immune reactivity can be linked to gluten reactivity.

In the dairy family IgG+IgA positive group, 30 (73%) reacted to one or more tissues (Table 4). We found predominantly more tissue immune reactivity in the IgG+IgA positive group compared to the IgG+IgA negative group (Figure 4).

Neurological tissues topped the list of autoimmune reactivity in patients IgG+IgA positive for dairy family protein reactivity.

Lectins/Agglutinins

Wheat germ agglutinin (WGA) IgG and IgA is assessed on Array 3-Wheat/Gluten Proteome Reactivity and Autoimmunity, while soybean agglutinin, peanut agglutinin, lentil lectin, pea lectin and bean agglutinin IgG+IgA are assessed on Array 10-Multiple Food Immune Reactivity Screen (Table 2). We selected 25 specimens resulting IgG negative for WGA and/or negative for IgG+IgA food lectin/agglutinins and 25 specimens IgG positive to WGA and IgG/IgA negative for all gluten family proteins and/or IgG+IgA positive for food lectins/

agglutinins. Our WGA positive group excluded gluten family positive specimens so as to prevent using possible CD or NCGS patients in the group.

The 25 positive and 25 negative for lectin/agglutinin protein reactivity were compared to their corresponding tissue reactivity results.

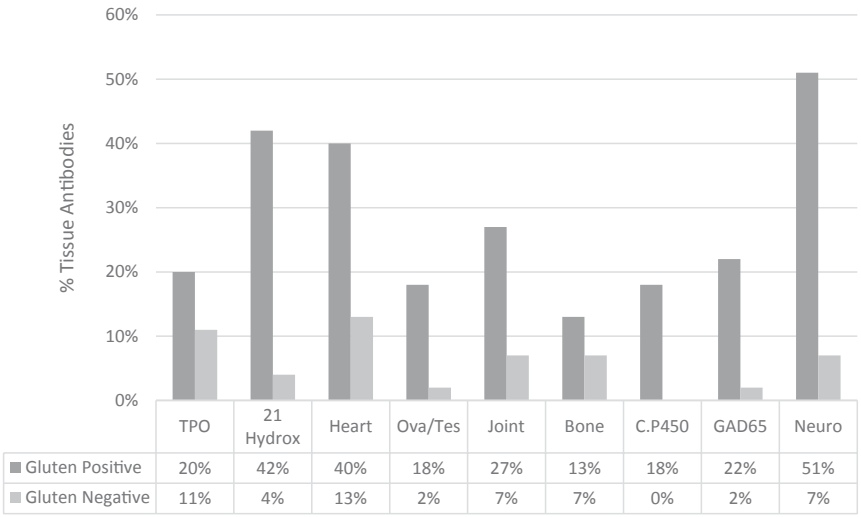


Figure 3: Comparison of Gluten Family Positive and Negative Tissue Reactivity. The shows a significant elevation of tissue antibodies in patients with gluten family protein IgG versus patients IgG negative for gluten family proteins. TPO=Thyroid Peroxidase, 21 Hydrox=21 Hydroxylase (adrenal cortex), heart=Myocardial Peptide and/or α-myosin, Ova/Tes=Ovary/Testis, Joint=Fibulin, Collagen and/or Arthritic Peptide, Bone=Osteocyte, C.P450=Cytochrome P450 (hepatocyte), GAD65=Glutamic Acid Decarboxylase-65, Neuro=Myelin Basic Protein, Asialoganglioside, α+β-Tubulin, Cerebellar and/or Synapsin.

TPO	21 Hydro	HEART	Ova/Test	JOINT	BONE	Cyto P450	GAD65	NEURO
N		E		E	E		E	
N		E	N		N		E	E
N			N	N		N		
	E		N	E	N		E	
N			N		E		E	
N		N	E	E	N	E	N	
				E	E	E		
N	N	N	N	N	N	N	N	
	N	N	N	N	E	N	N	N
			E			N		
	N	N	N		N	N	N	E
N			E			N	E	
N	E	E	E	N	N	N	E	
E		E	N		E	E		
E	E	N	N	N	N	N	E	
E	E	N	N	N		N	E	
N	E		E	N	E	N	E	
E	N		E	N	N	N	N	
N	N		N	N	N	N	N	E
N		E	E		E	E	E	
N	E	E	N	N	N	N	N	E
E	N	E	E	E	N	N	N	E
N	E		N	E	N	N	N	E
N			N				E	
N						N		
N		E	E	N	E	N	E	E
N	N	N	N	E	N	N	N	N
N	N	N	E	N	N	N	E	E
N	N	N	N	N	N	N	N	N
	N	N	N	N	N	N	N	N

Table 4: Tissue Reactivity on 30 IgG+IgA Dairy Family Positive Patients. Dairy protein antibodies can cross-react with multiple tissues in the body, which may result in autoantibody formation. Positive results are indicated as “P” or black, weak positive results are indicated as “E” or gray and Negative results are indicated as “N” or white.

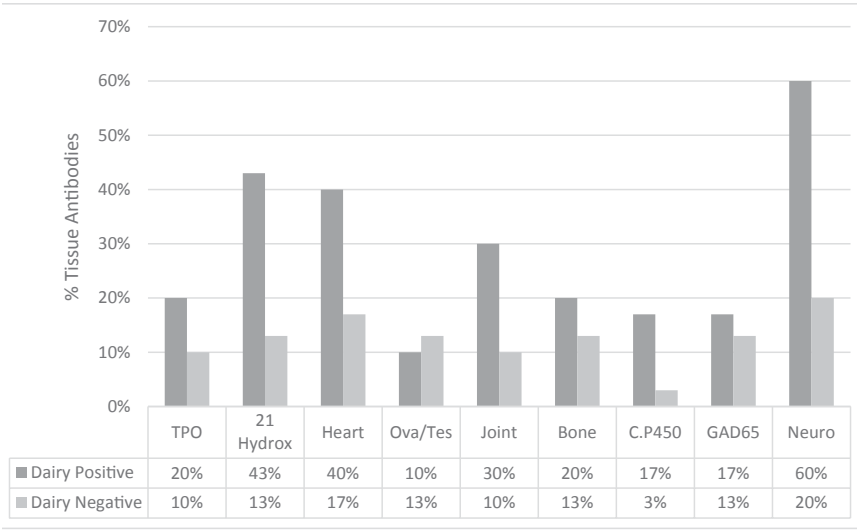


Figure 4: Comparison of Dairy Family Positive and Negative Tissue Reactivity. The shows a significant elevation of antibodies to most tissues in patients with Dairy family protein IgG+IgA versus patients IgG+IgA negative for Dairy family proteins. Ovary/Testis is the one tissue that resulted with 4 dairy negative patient reacting, and only 3 dairy positive patients reacting. In this instance, each of the 4 dairy negative patients was positive for gluten family proteins. TPO=Thyroid Peroxidase, 21 Hydrox=21 Hydroxylase (adrenal cortex), heart=Myocardial Peptide and/or α-myosin, Ova/Tes=Ovary/Testis, Joint=Fibulin, Collagen and/or Arthritic Peptide, Bone=Osteocyte, C.P450=Cytochrome P450 (hepatocyte), GAD65=Glutamic Acid Decarboxylase-65, Neuro=Myelin Basic Protein, Asialoganglioside, α+β-Tubulin, Cerebellar and/or Synapsin.

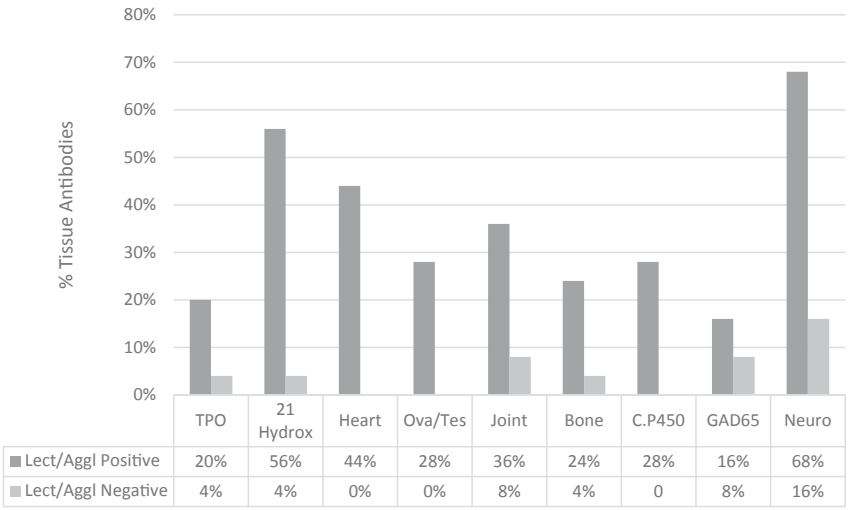
TPO	21 Hydro	HEART	Ova/Test	JOINT	BONE	Cyto P450	GAD65	NEURO
						N		
N		E		E	E		E	
N		E	N		N		E	E
	E		N	E	N		E	
N			N		E		E	
N		E			N		E	
				E	E	E		
N	N	N	N	N	N	N	N	
E	E	N	N	N	N	N	E	
N		E	E	E	E	N	E	E
N	E		E	N	E	N	E	
E	N		N	N	N	N	N	
		E	E	N	E	N	N	N
N						N		
E	N	N	N	E	E	N	N	N
N	N	N	N	N	N	N	N	N
E			E	E	E	E	N	
N	N	N	N	N	N	N	N	E
N	N	N	N	E	N	N	N	E
N	E	N	N		E	N	N	
E		E		E		N	E	
N			E			N	E	
N	N	E	N	N	N	N	N	E
N			N				E	

Table 5: Tissue Reactivity on 25 Lectin/Agglutinin Immune Reactivity Positive Patients. Lectin/Agglutinins are known to bind to multiple tissues in the body, which may result in autoantibody formation. Positive results are indicated as "P" or black, weak positive results are indicated as "E" or gray and Negative results are indicated as "N" or white.

In the negative group, 8 (22%) were positive for antibodies against one or more tissues. It is important to note that 3 out of these 8 patients were positive for dairy family protein/s. Thus, these tissue reactions may be linked to the dairy reactivity.

In the lectin/agglutinin positive group, 19 (76%) reacted to one or more tissues (Table 5). We found grave differences between the lectin/agglutinin positive groups compared to the lectin/agglutinin negative group (Figure 5).

Neurological tissues topped the list of autoimmune reactivity in patients positive for lectin/agglutinin family protein reactivity



	TPO	21 Hydrox	Heart	Ova/Tes	Joint	Bone	C.P450	GAD65	Neuro
Lect/Aggl Positive	20%	56%	44%	28%	36%	24%	28%	16%	68%
Lect/Aggl Negative	4%	4%	0%	0%	8%	4%	0	8%	16%

Figure 5: Comparison of Lectin/Agglutinin Family Positive and Negative Tissue Reactivity. The shows a significant elevation of antibodies to most tissues in patients with lectin/agglutinin family protein IgG or IgG+IgA versus patients IgG/IgA or IgG+IgA negative for lectin/agglutinin family proteins. TPO=Thyroid Peroxidase, 21 Hydrox=21 Hydroxylase (adrenal cortex), heart=Myocardial Peptide and/or α-myosin, Ova/Tes=Ovary/Testis, Joint=Fibulin, Collagen and/or Arthritic Peptide, Bone=Osteocyte, C.P450=Cytochrome P450 (hepatocyte), GAD65=Glutamic Acid Decarboxylase-65, Neuro=Myelin Basic Protein, Asialoganglioside, α+β-Tubulin, Cerebellar and/or Synapsin.

Discussion

The aim of this study was to show a correlation between food protein immune reactivity and autoantibody production. This information provides insight into the possibility that food may play a role in some environmentally-induced autoimmune disorder. It is interesting that the first, fully-defined autoimmune disorder involves food as its environmental trigger. The genes, the environmental trigger, the pathogenesis and the target tissue of Celiac disease (CD) have been elucidated. One would think this knowledge would ignite further studies with other foods and their potential link to a variety of autoimmune disorders. Unfortunately, judging by the lack of publication regarding the study of dietary proteins in the pathogenesis of autoimmune diseases is not a priority for researchers as a whole.

Based on the results of the gluten section of our study, gluten reactivity can be linked to extra-intestinal disorders beyond CD. Indeed, in our study, the most common tissue antibody found in subjects, with elevated IgG only to gliadin, was neurological. CD is known as the "gluten-associated disorder." To a much lesser degree, gluten family proteins from wheat and other grains have been shown to play a significant role in other disorders including neurological [22, 28-32], cardiovascular [33-35], thyroid [36-38] and joint disorders [39-41]. Many such studies show comorbidity between CD and other autoimmunities, however, a handful of studies are being done specifically on NCGS and/or gliadin IgG immune reactivity. While CD researcher's state there is no test for NCGS [42-44], others are boldly defining steps to differentiating CD from NCGS [45-48]. Indeed Vojdani and Tarash [15] show the affinity for gliadin antibodies to bind to human tissue beyond the gut, which can lead to autoimmunity. Interestingly, the gluten-free diet (GFD) is generally recommended for specific autoimmune disorders, as the GFD may reduce systemic inflammation, cut down on the frequency of flares, or arrest the autoantibody production against targeted tissues [49-52]. Unfortunately, much of the clinical world has not yet grasped the idea that gluten can trigger autoimmune disorders other than CD, which leads to misleading notions that only CD patients have to be strict with the gluten-free diet (GFD), while NCGS patients can "cheat" once-in-a-while. Statements such as, patients with "NCGS can be more liberal and titrate their exposure to gluten as needed to avoid symptoms," [53] can be harmful to the patient. CD is a gastrointestinal disorder in which patients make elevated IgA to gliadin and tissue transglutaminse-2. What if the NCGS patient makes significant levels of IgG against gliadin and cerebellar protein? Is protecting the brain really less important than protecting the gut?

Dairy products, especially cow's milk, are commonly one of the first non-human milk food products introduced to a newborn. Cow's milk allergy, an IgE-mediated immune response, can occur in infants and young children, but it usually resolves by adulthood [54,55]. Although rare in adults, cow's milk allergy is more severe [56] and females are more likely to develop cow's milk allergy in adulthood than males [57]. Lactose intolerance occurs in adulthood and is estimated to affect 75% of the population [58]. In spite of these statistics, for many families, dairy is still a significant part of the standard American diet (SAD). Our study on the more common delayed immune responses, IgG+IgA, illustrates a need for some individuals to abstain from consuming dairy products. To improve clinical conditions, the dairy-free diet has been recommended for patients with nephrotic syndrome [59], osteoarthritis [60], diabetes [61,62], and neurological disorders [63,64]. Indeed, dairy protein antibodies have been shown to cross-react with glutamic acid decarboxylase (GAD)-65 [22], GAD-67, and insulin receptor-α [65], an important serological marker for type 1 diabetes and latent autoimmune diabetes in adults (LADA) [66]. Cross-reactivity between casein and/or milk butyrophilin with neurological tissues cerebellar [14] and myelin [16,17] has been shown, which can link the consumption of these foods, to neuroautoimmunity, as seen in some patients.

An often forgotten detrimental food protein is the lectin/agglutinin, a natural binder. As described above, when a lectin/agglutinin binds to tissue, autoimmune reactivity against the tissue may occur. Of the food groups in this study, patient's positive for lectins/agglutinins reactivity had the most pronounced tissue antibody production. Lectins and agglutinins are present in a variety of plants, where they serve as defense mechanisms against other plants and fungi. Because of their ability to bind to virtually all cell types and cause damage to several organs [67],

lectins/agglutinins are sometimes categorized as food toxicants [68,69]. WGA IgG and IgA levels were significantly higher in patients with untreated Celiac disease compared to healthy controls [70]. Although Sollid and colleagues [69] found no cross-reactivity between WGA and gliadin proteins, others have shown that the combination of WGA and gliadin can increase intestinal permeability [71], which leads to an increase of translocating dietary proteins and other antigens into circulation, resulting in inflammation and autoimmunity.

This study also revealed co-occurrence of reactivity to different autoimmune-inducing food proteins. Vojdani et al. assessed wheat and milk antibodies in subjects to measure their prevalence and possible contribution to neuroimmune reactivities [22]. The two-way cluster analysis of the Pearson's correlation coefficients between the food proteins and the brain proteins showed significant clustering of wheat and dairy proteins compared to neurological tissue proteins. In a previous study, using both ELISA and dot blot, Vojdani and Tarash demonstrated the cross-reactivity between gliadin and casein [15], which may be a factor in the co-occurrence of gluten family and dairy family antibodies in some patients included in this study.

In our gluten positive group, 29 were simultaneously tested for gluten and dairy reactivity. From this subgroup, 14 tested positive for dairy family antibodies. Alternatively, in our dairy positive group, 25 were simultaneously tested for gluten and dairy reactivity. From this subgroup, 21 tested positive for gluten (13 IgG positive, 5 IgG and IgA positive, 3 IgA positive). Although studies on the cross-reactivity of lectin/agglutinin antibodies with dairy proteins have not been published, the co-occurrence of antibodies made against lectins/agglutinins and dairy family proteins in our study were minimal. In our lectin/agglutinin positive group, 15 subjects were simultaneously tested for lectins/agglutinins and dairy reactivity's. From this subgroup, 3 patients were positive for dairy reactivity. Our lectin/agglutinin positive group excluded all gluten family reactivities, thus, we do not have data on simultaneous antibody reactivity to lectins/agglutinins and gluten family proteins.

Tissue antibodies can show up in the bloodstream up to ten years before the clinical threshold of disease has been reached [24,72-74]. The detection of autoantibodies in this study indicates the patient is set up for autoimmune disease and the foods testing positive may have an impact on tissue antibody production. Dietary alternations may be necessary in order to prevent further tissue damage. There are three main ingredients for environmentally-induced autoimmunity, genetic susceptibility, dysfunctional body barrier and environmental trigger [75-78]. If the environmental trigger is removed, even with a broken body barrier and a genetic susceptibility, autoimmunity can be evaded. Alternatively, if the environmental trigger, in our case, food protein is still present, once the intestinal barrier has been breached, the offending food protein ignites the immune system and pathogenesis begins its slow progression toward tissue damage. Autoantibody biomarkers can be detected at this point. A patient is at greater risk for developing a disease, if certain markers are present and the more detectable markers, the higher the positive predictive value for disease [79,80]. Prevention of environmentally-induced autoimmunity is possible.

Conclusion

The aim of this study was to correlate food immune reactivity and autoantibody formation. We chose three defined food groups, gluten family, dairy family and lectins/agglutinins, which have been noted as playing a role in human disorders. If antibodies against both food and human tissue are found in a patient, one can surmise that the food might be fueling the tissue antibody formation. Autoimmune pathogenesis may have additional antigenic triggers, thus, the removal of the offending food from the diet, may improve the patient's condition, but not stop the autoimmune process.

The subjects included in this study were patients seeking professional health care. Thus, our data is not comparable to what one would find in a general population study. Patients with antibody reactivity to specific food proteins showed higher co-occurrence of tissue autoantibodies than patients without food reactivities. Our numbers are significant:

- 64% of IgG gluten family protein positive patients reacted to one or more human tissues tested
- 73% of IgG+IgA dairy family protein positive patients reacted to one or more human tissues tested
- 76% of IgG+IgA lectin/agglutinin protein positive patients reacted to one or more human tissues tested

In each category, the most common tissues reacted against were nervous system tissues.

By assessing food and human tissue antibodies simultaneously, insight into possible environmental triggers and subsequent tissue damage can be uncovered. This knowledge provides a therapeutic opportunity to arrest the disease process and prevent the onset of autoimmunity, or to improve the quality of life for patients already stricken with a disorder. More studies are needed to assess the long-term role of diet on the onset and management of autoimmunity.

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