

## ANTIBODIES AS PREDICTORS OF COMPLEX AUTOIMMUNE DISEASES AND CANCER

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The pathologic role of autoantibodies in many autoimmune diseases is widely accepted. An enzyme immunoassay was used for measurement of antibodies against disease-specific antigens and etiologic agents for cross-reactive antigens associated with them. This antibody assay was applied to a panel of antigens for the detection of different neuroautoimmune diseases that included multiple sclerosis, motor peripheral neuropathies, multifocal motor neuropathy, amyotrophic lateral sclerosis, pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection. We studied women with pregnancies complicated by neural tube defect, neuroborreliosis, autism and patients with possible somatic hypermutation. Antibodies were also measured against antigens and etiologic agents associated with primary biliary cirrhosis and chronic obstructive pulmonary disease. And, finally, antibodies were measured against several tumor antigens or peptides which are expressed in prostatic, breast and colon tissues. This panel of different autoantibodies was applied to 290 patients with neuroautoimmune disorders, cancer, and possible somatic hypermutation. The levels of these antibodies against different tissue-specific antigens and etiologic agents associated with them were significantly elevated in patients versus controls. We hope that this novel 96 antigen-specific ELISA will be used in additional studies that will prove its clinical efficacy, not only for the early diagnosis of many neuroautoimmune, liver and lung autoimmune disorders, but also for prognosis and the implementation of preventive steps for many complex diseases.

The understanding of autoimmune disease, including neuroautoimmune disorders, has expanded considerably in recent years. Autoimmune neurological disorders occur when immunological tolerance to antigens of Schwann cells, axons, motor neurons, receptors and synapses is lost. The resulting demyelinating diseases share pathological features characterized by destruction of myelin and other neural cell antigens accompanied by neural inflammation in the brain, spinal cord or optic nerve (1-2). It is commonly accepted that the early steps of neuroimmune disorders such as multiple sclerosis

(MS) are mediated by T cells, in particular the  $T_H17$  phenotype, followed by the production of antibodies against different neural antigens. (3). Disruption of the blood-brain barrier (BBB) is the key factor in lymphocyte transmigration and the entry of unwanted molecules across BBB endothelial cells (BBB-ECs) (4). Different environmental factors, such as xenobiotics, infections, dietary peptides, toll-like receptors, adhesion molecules, cytokines and antibodies also play a significant role in BBB dysfunction (5-8).  $T_H17$  transmigration across the BBB-ECs highly expresses granzyme-B, kills human

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neurons, and promotes central nervous system (CNS) inflammation through microglia CD4<sup>+</sup> lymphocyte recruitment (7). Granzyme-B, the killing of neurons, and possibly astrocytes and microglia, can induce the release of neural cell antigens such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), myelin associated glycoprotein (MAG), PLP, alpha-B-crystallin, tubulin, neurofilaments, glutamate receptors and other antigens. Immune response against these neural antigens and their cross-reactive epitopes can result in different neuroautoimmune disorders such as MS, peripheral neuropathy, Guillain-Barre syndrome (GBS), amyotrophic lateral sclerosis (ALS), Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal infections (PANDAS/OCD), and neural tube defect (NTD) (9-11).

NTD is an example of environmental factors inducing autoimmunity in women with complicated pregnancies. Exposure to fumonisins from contaminated corn and its consumption in a form of tortilla bread was found to be responsible for the occurrence of this disease (9). Antibodies against folate receptor have been reported in pregnant women who fell victim to this disorder (10).

Although Lyme disease is induced by infectious agents, if it is not detected at the earliest stage it can result in inflammation and autoimmunity, including Lyme arthritis and neuroborreliosis (11).

To date, science has failed to detect a single etiologic agent responsible for the spectrum of autistic disorders (12). Accordingly, there is no single medical or laboratory marker that could be used for the diagnosis or follow-up treatment of children with autism. Therefore, the protocol of testing for autism spectrum disorders is for the management and not diagnosis of autism (12).

Primary biliary cirrhosis (PBC) is another example of autoimmune disease induced by toxic chemicals. Recent evidence has suggested that environmental factors such as chemical xenobiotics 2-octynoic acid in cosmetics and food additives is responsible for modification of pyruvate dehydrogenase (PDH) and autoantibody production against PDH, octynoic acid and cytochrome P-450 (13).

Understanding the factors that lead to emphysema is useful for predicting individuals at risk. A very recent report indicated the involvement of cigarette

smoke in the induction of anti-elastin immunity in T-helper-1 response and the release of elastolytic matrix metalloproteinase (MMPs). This immune reaction may result in antibody production against chemicals bound to elastin and MMPs (14).

Cell-mediated immunity is a critical component of the immune response to a growing tumor. The immune response to tumors is complex, and in many cases is directed against tumor-specific antigens or tumor-specific transplantation antigens expressed by the tumor cells (15).

During the past ten years researchers have made the intriguing discovery that autoantibodies can appear in the blood of some cancer patients. These antibodies are produced due to overexpression of tumor antigens or peptides and mutated proteins (16-22). Immune response against tumors results in autoantibody production against mutated P53, Her-2/neu, ganglioside GD-3 and prostatic peptides (17-18, 20-21).

Indeed, autoantibody signatures against different peptides were suggested as biomarkers for early detection of prostate cancer (22). It was concluded that autoantibodies against peptides derived from prostate cancer tissue could be used as the basis for a screening test for prostate cancer (23).

While most of the antibodies presented in this study are detected in patients with different autoimmune diseases and some types of cancer, we should remember that there are patients who are universal reactors and produce pathogenic multi-reactive antibodies due to somatic hypermutation and defect in B-cell checkpoint (24). For this reason the test results of three patients with somatic hypermutation are presented.

## MATERIALS AND METHODS

The list of antigens, peptides and chemicals bound to human serum albumin (HSA) and their sources were described in the Materials and Methods section of the previous manuscript.

Human sera - Pooled normal human serum was purchased from Innovative Research, Southfield, MI. Disease state human sera from patients with MS, ALS, GBS, emphysema, autism, PANDAS/OCD, cancer, and multi-reactive antibodies were sent by different clinicians to our laboratory, and, after removal of their identification, were kept in the freezer at  $-70^{\circ}$  until used. Sera from

patients with known tick-borne diseases were purchased from the Centers for Disease Control, Atlanta, GA.

*Enzyme-linked immunosorbent assay (ELISA) procedure*

ELISA was used for measurement of antibodies in different sera against 96 antigens simultaneously, as described in the previous article. Briefly, after antigen coating, serum samples were added to duplicate wells and incubated 1h at room temperature. Plates were washed and secondary antibody was added to each well. The plates were then incubated for an additional hour, and after washing and addition of substrate, color development was measured at 405 nm. To detect non-specific binding, several plates containing all reagents except human serum were used for color development. Pooled normal human serum was examined on additional plates coated with the same 96 wells for the purpose of specificity of reaction and calculation of stimulation indices. For examination of assay reproducibility, each serum (patient and healthy control) was run on duplicate plates, each coated with 96 antigens. Index was calculated according to the following formula:

$$\text{Index} = \frac{\text{mean optical density of clinical specimen} - \text{optical density of blank}}{\text{mean optical density of normal human serum} - \text{optical density of blank}}$$

RESULTS

In this antibody array, 96 different antigens were used for measurement of disease-specific antibodies and etiologic agents or cross-reactive antigens associated with them. This autoimmune reaction may occur against any tissue antigen, including gastrointestinal, peripheral, or even CNS.

In MS, the autoimmune response is directed against different neural cell antigens and multiple microbial peptides (25-29). When blood samples of patients with MS were applied to 96 different antigens, in comparison to controls significant elevation was observed not only against specific antigens such as MBP, MOG, proteolipid protein (PLP), transaldolase and alpha-B-crystallin, but also against human herpesvirus 6 (HHV-6), *C. pneumoniae* heat shock protein 60 (HSP-60), acinetobacter, glutamate receptor, ion channels, milk butyrophilin, gluteomorphin and Hg-HSA (Table I, Fig. 1).

In patients with chronic motor peripheral neuropathies, antibodies were detected against MBP, MAG, ganglioside GM1, sulfatide and Hg-HSA

(Table II, Figure 2). Other antibodies detected in these patients were isocyanate-HSA, aflatoxin and fumonisin (29, 30). In comparison to patients with chronic motor peripheral neuropathies, GBS patients showed significant elevation in IgM antibodies against ganglioside GM1 and *Campylobacter jejuni* toxin (29, 30), with some cross-reactivity with parietal cell, tropomyosin, glutamate receptor and synapsin-I (Table III, Fig. 3).

ALS is a devastating motor neuron autoimmune disease resulting in paralysis and death within 3-5 years of diagnosis. Antibodies against extracellular autoantigen glutamate receptor-3 were reported in these patients (31-32). Results depicted in Table IV and Fig. 4 show that high levels of antibodies are detected against glutamate receptor-3, SOD1 peptide,

**Table I.** Detection of antibodies against different antigens in 3 representative patients with MS expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
MBP	3.6	1.9	4.8
MOG	1.2	4.3	3.7
PLP	1.4	4.5	3.1
Alpha-B-crystallin	4.9	1.3	0.9
Transaldolase	3.5	1.4	1.0
HHV-6	4.6	1.1	1.5
Acinetobacter	3.1	2.8	2.5
<i>C. pneumoniae</i> HSP-60	0.8	2.7	3.8
Cow's Milk Protein	4.8	1.6	0.9
Gluteomorphin	4.5	1.3	1.1
Glutamate Receptor	1.0	1.5	2.3
Ion Channel	0.9	2.4	0.8
Hg-HSA	3.7	1.5	1.0

**Table II.** Detection of antibodies against different antigens in 3 representative patients with peripheral neuropathy expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
MBP	1.8	0.9	4.3
MAG	2.7	3.4	2.9
Ganglioside	3.8	4.2	2.6
Sulfatide	2.5	1.9	3.4
Hg-HSA	0.8	3.6	1.2

**Table III.** Detection of antibodies against different antigens in 3 representative patients with Guillain-Barre syndrome expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
Ganglioside GM <sub>1</sub>	5.9	1.6	6.3
<i>Campylobacter jejuni</i> Toxin	4.5	0.9	5.5
Parietal Cell	4.3	1.0	1.3
Tropomyosin	3.7	1.2	4.1
Glutamate Receptor	1.5	0.9	3.9
Synapsin-I	2.8	1.5	1.4

BBB protein and ion channel. Furthermore, some elevation in antibodies was also observed against MBP, endothelial cell, MMPs and neurofilaments (Table IV).

PANDAS has been described in children with Obsessive-Compulsive Disorder, Anorexia Nervosa and/or tic disorders subsequent to streptococcal infections (33-34). Detection of antibodies against strep M proteins, strep enzymes, ganglioside, MBP, tubulin and B-cell antigens were confirmed in our laboratory, as is presented in Table V and Figure 5. In addition, antibodies were also detected against

**Table IV.** Detection of antibodies against different antigens in 3 representative patients with ALS expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
Glutamate Receptor	1.6	4.5	1.2
Ion Channel	3.3	1.4	1.0
SOD <sub>1</sub> Peptide	2.8	4.1	1.3
BBB Protein	3.5	0.9	4.4
MBP	1.2	2.0	3.5
Endothelial Cell	4.1	1.3	1.6
MMPs	3.9	0.9	1.1
Neurofilaments	1.6	1.3	3.9

arthritis-induced peptide, rheumatic fever peptide and ganglioside GD3.

In women with pregnancies complicated by NTD, antibodies against folate receptors and their causal relation has been reported (9). Exposure to mycotoxin fumonisins was suggested as an environmental factor responsible for NTD (10). Our results, summarized in Table VI, show that both folate receptor and mycotoxin antibodies were detected in the blood of women with pregnancies resulting in NTD. Antibodies against these additional antigens, neurofilaments, glutamate receptor, ion channel and alpha-B-crystallin, were also detected (Table VI).

In Lyme disease, if antibodies are not tested against all major antigens of *Borrelia*, false negative results will be obtained (35-36). Therefore, based on in vivo induced antigen technology (IVIAT), we developed the Multi-Peptide ELISA for increased sensitivity of Lyme disease diagnosis (11). In this study we selected peptides from different components of *Borrelia* during specific stages of its life cycle, including outer surface proteins, leukocyte function associated antigen, and immunodominant antigens.

**Table V.** Detection of antibodies against different antigens in 3 representative patients with OCD/PANDAS expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
Streptozyme	0.9	4.7	5.6
Strep M Protein	1.3	6.8	4.3
D8/17	1.7	5.4	4.5
MBP	4.9	1.7	1.2
Ganglioside GM <sub>1</sub>	1.1	3.6	4.8
Tubulin	0.7	4.0	2.5
Arthritis Peptide	1.7	2.4	3.1
Rheumatic Fever Peptide	1.2	2.8	3.5
Ganglioside GD <sub>3</sub>	1.6	2.1	1.8

The antibody signature of three patients with Lyme disease is shown in Table VII. In two out of three patients antibodies against Babesia, Ehrlichia and Bartonella were detected. In addition, in patients #1 and #3 with Lyme arthritis and neuroborreliosis elevation, RF, immune complex elevation, and high levels of antibodies against *Mycoplasma arthritidis*, MBP and BBB proteins were detected (Table VII).

Application of this ELISA to the sera of patients with autism resulted in the detection of antibodies against wheat, gliadin peptide, gluteomorphin, milk butyrophilin, dipeptidyl peptidase IV (DPP IV), trypsin, secretin, vasoactive intestinal peptide (VIP), serotonin receptor, motilin, neuropeptides Y, MBP, neurofilaments, synapsin, glutamate receptor, strep-M-protein, *Yersinia enterocolitica*, Rotavirus, HHV-6, tubulin, parietal cell and tropomyosin (Table VIII, Fig. 6). Simultaneous detection of antibodies against multi-antigens could be related to somatic

**Table VI.** Detection of antibodies against different antigens in 3 representative patients with neural tube defect expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
Folate Receptor	5.1	5.6	1.1
Aflatoxin	4.8	1.3	0.8
Fumonisin	1.5	1.0	4.2
Neurofilaments	3.1	1.3	4.9
Glutamate Receptor	1.5	3.8	1.0
Ion Channel	2.3	2.0	1.1
Alpha-B-crystallin	2.9	1.4	4.3

hypermutation in these patients (24). An example of three patients with possible somatic hypermutation with reactivity against 28 out of 90 antigens is shown in Table IX.

One of the best-documented relationships between the presence of autoantibodies and the subsequent development of autoimmune disease is that of mitochondrial PDH in PBC (13). Data presented in Table X and Figure 7 show that two patients with PBC produced antibodies against both PDH and PDH-octynoic acid, while the third patient was negative for both, but reacted with cytochrome P-450 peptide. Additionally, high total immune complexes and antibodies against aflatoxin, fumonisins and mitochondrial dehydrogenase were detected (Table X).

Chronic obstructive pulmonary disease and emphysema are common destructive inflammatory diseases associated with cigarette smoke that

**Table VII.** Detection of antibodies against different antigens in 3 representative patients with Lyme disease expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
Borrelia	5.8	4.7	3.4
OsPA + OsPC	3.7	1.0	4.1
OsPE	1.9	1.2	3.6
LFA	4.5	0.8	4.4
C2 + C6	5.4	3.5	3.9
Babesia	1.2	3.3	2.6
<i>Ehrlichia</i>	1.1	2.9	2.3
<i>Bartonella</i>	1.6	3.6	2.8
Rheumatoid Factor	2.7	0.8	4.7
Immune Complex	3.8	1.3	4.0
<i>Mycoplasma arthritidis</i>	4.6	1.0	3.5
MBP	3.9	0.9	1.8
BBB Protein	2.5	0.7	1.6

are leading causes of death worldwide (14). We measured antibodies against elastin and MMPs. Also, since occupational lung disease is prevalent in patients exposed to provocative agents such as toluene diisocyanate (TDI) (37), we included TDI-HSA in the list of tested antigens. We found that in patients with obstructive pulmonary disease, in addition to elastin, antibodies against MMPs and TDI-HSA were detected (Table XI, Fig. 8). Sera

**Table VIII.** Detection of antibodies against different antigens in 3 representative patients with autism expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
Wheat	5.9	1.6	1.4
Gliadin	5.5	1.2	1.8
Gluteomorphin	5.2	1.1	1.6
Cow's Milk Protein	2.7	1.0	5.6
DPP IV	4.6	2.6	1.1
Trypsin	1.2	3.7	0.8
Secretin	1.1	4.5	0.9
VIP	3.9	1.2	1.0
Serotonin Receptor	4.8	1.4	1.4
Motilin	4.3	0.9	1.5
Neuropeptide Y	4.1	0.8	1.6
Neurofilaments	1.2	0.7	1.2
Synapsin	2.8	0.8	4.8
MBP	3.6	1.0	0.9
Glutamate Receptor	1.2	1.7	4.0
Strep M Protein	1.1	5.5	1.2
<i>Yersinia enterocolitica</i>	3.3	1.4	1.0
Rotavirus	4.4	1.7	0.9
HHV-6	1.0	5.2	1.5
Tubulin	3.0	1.3	1.2
Parietal Cell	2.5	1.0	1.7
Tropomyosin	1.6	1.8	1.3

**Table IX.** Detection of antibodies against different antigens in 3 representative patients with possible somatic hypermutation expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
Wheat	3.8	2.1	4.7
Gliadin Peptide	3.7	3.2	4.1
Cow's Milk Protein	2.6	2.3	3.7
Casomorphin	6.6	8.5	5.9
MBP	2.8	2.3	3.5
PLP	3.0	3.0	4.6
MOG	2.1	2.3	4.2
Tubulin	3.2	4.4	5.8
MAG	3.1	3.1	3.9
Sulfatide	3.3	2.7	3.8
Alpha-B-crystallin	3.2	3.3	3.1
BBB Protein	3.3	3.9	4.4
Adrenal Gland	4.5	3.0	6.2
Myosin-Alpha	4.2	4.2	5.4
Immune Complex	2.0	5.2	4.0
Lupus Peptide	5.5	4.9	5.5
Borrelia	2.6	2.2	3.7
Parietal	3.3	3.6	2.9
DPP IV	4.7	2.2	5.2
Streptozyme	3.6	4.2	3.6
Strep M Protein	4.2	3.0	1.9
<i>Campylobacter jejuni</i>	3.0	1.7	1.3
<i>Mycoplasma</i>	5.2	3.0	4.8
Rheumatic Fever Peptide	5.6	4.3	5.3
Tropomyosin	3.6	4.0	2.6
Acinetobacter	4.5	2.9	5.7
C. pneumoniae	3.1	5.4	3.5
HSP-60	4.8	4.1	4.8

**Table X.** Detection of antibodies against different antigens in 3 representative patients with biliary cirrhosis expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
PDH	2.9	5.8	1.3
PDH-Octynoic Acid	3.7	4.1	1.8
Cytochrome P-450	0.8	1.7	3.6
Aflatoxin	0.6	1.4	4.1
Fumonisin	1.2	0.9	3.3
M. Dehydrogenase	1.7	0.7	3.5
Immune Complex	4.4	3.2	5.3

**Table XI.** Detection of antibodies against different antigens in 3 representative patients with lung dysfunction and emphysema expressed by index.

Antigen	Patient # 1	Patient # 2	Patient # 3
Elastin	5.2	4.3	1.2
MMPs	1.8	2.9	0.9
Isocyanate-HSA	4.6	0.7	3.9
Hg-HSA	3.4	1.5	1.1
Endothelial Cell	1.9	3.8	1.0
Motilin	1.0	3.5	1.3

from some of these individuals also reacted with Hg-HSA, endothelial cells and motilin.

The overexpression of tumor antigens or peptides has been demonstrated in a number of tumors, including breast, colorectal, prostate and others (16-22). The results summarized in Table XII show that detection of these antibodies against one or more tumor antigens is possible in colorectal, breast and prostatic hyperplasia. Also, elevations in total immune complexes were detected in patients

**Table XII.** Detection of antibodies against different tumor-associated antigens in 3 representative patients with colorectal breast cancer and prostatic hyperplasia expressed by index.

Antigen	Colorectal			Breast			Prostatic Hyperplasia		
	# 1	# 2	# 3	# 1	# 2	# 3	# 1	# 2	# 3
P-53	4.8	3.7	1.3	0.9	1.6	0.8	1.8	1.6	1.1
Her-2/neu	0.9	1.1	1.5	0.8	4.1	1.1	1.1	1.4	0.9
Ganglioside GD <sub>3</sub>	1.3	1.2	1.7	3.5	1.4	1.0	0.7	1.3	1.5
Prostatic Antigen	1.9	1.3	2.0	1.5	1.2	0.9	1.7	5.3	4.8
<i>Mycoplasma Arthritis</i>	1.0	0.9	1.3	0.8	0.7	1.5	1.8	3.4	2.6
Immune Complex	2.8	2.2	1.5	3.9	1.1	0.8	1.0	0.9	1.3
Tropomyosin	4.1	3.9	1.6	1.6	1.4	1.0	1.8	1.5	1.2

with colorectal and breast cancer, tropomyosin antibodies were detected in one out of 3 colorectal cancer patients, and antibodies against *Mycoplasma arthritis* were detected in two out of three patients with prostatic hyperplasia.

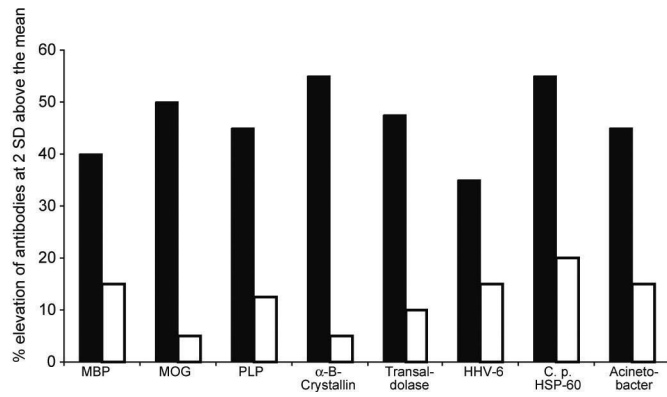
## DISCUSSION

In this article we attempt to provide a forum for future debate about the importance of predictive antibodies for the diagnosis and management of complex autoimmune diseases and cancer.

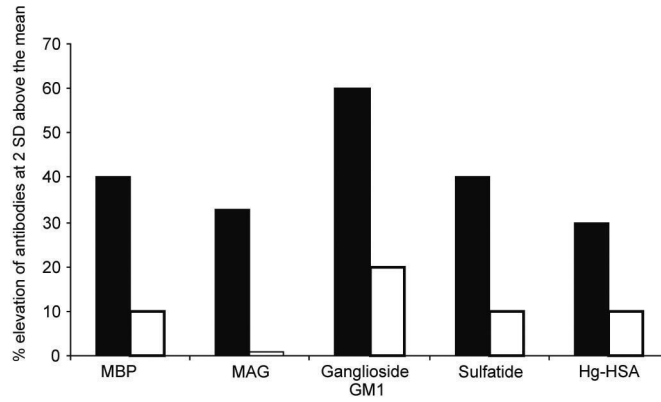
In MS, doctors are desperate to know which patient with early mild symptoms will progress to severe symptoms, so that they may take preventive measures. In addition to this early warning, some antibodies may help clinicians to measure the rate of progression of an already diagnosed autoimmune disease (38). Indeed, since 2003 many studies have used antibodies against two proteins that insulate the nerve, MBP and MOG, as demonstrations of autoantibodies with clinical association, specificity and sensitivity (25-29). However, a recent study showed no association between anti-myelin antibodies and the progression of MS (39). This lack of association between neural cell antibody and MS progression may stem from the analysis of antibodies in blood samples obtained between 46 to

59 days after the first clinical signs while 71% of the patients had undergone corticosteroid treatment (39). In our earlier study we found that the addition of alpha-B-crystallin antibody to MBP and MOG antibodies resulted in a sensitivity of 75% and a specificity of 70% (25). In the present study, the nature of the assay allowed us to detect antibodies against additional neuron-specific (PLP and TA) and non-specific antigens (HHV-6, *C. pneumoniae* HSP-60 and acinetobacter). These antibodies were found to be elevated in a significant percentage of MS patients (Fig. 1). Detection of antibodies against these and other antigens (Table I) should not be surprising, since these antigens were expressed in brain tissue and their administration to animal models resulted in an MS-like condition (28). Future studies may consider the inclusion of these autoantibodies in a panel with MBP and MOG for increased sensitivity of prediction.

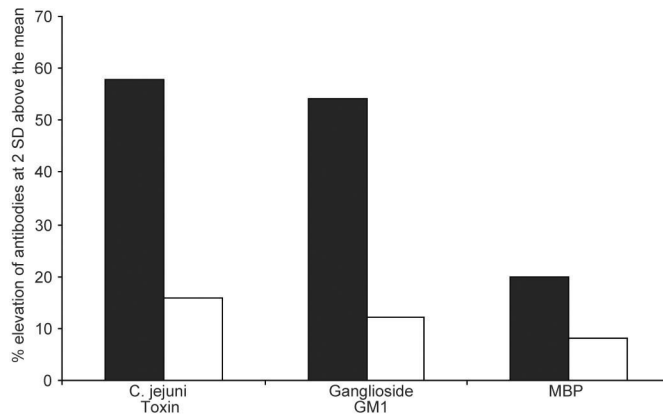
In patients with neuropathies, antibodies against MBP, MAG, ganglioside GM1, sulfatide and Hg-HSA were detected, which may differentiate this disorder from autoimmune CNS disorders (Table II, Fig. 2). Some of these antibodies, such as ganglioside GM1, are detected in patients with GBS (Table III, Fig. 3). Molecular mimicry between lipooligosaccharides in the *C. jejuni* cell wall and gangliosides in peripheral nerves plays a crucial role



**Fig. 1.** Elevation in IgG antibody in healthy controls  compared to afflicted patients  with multiple sclerosis;  $n = 40$  for each group,  $p < 0.001$ .

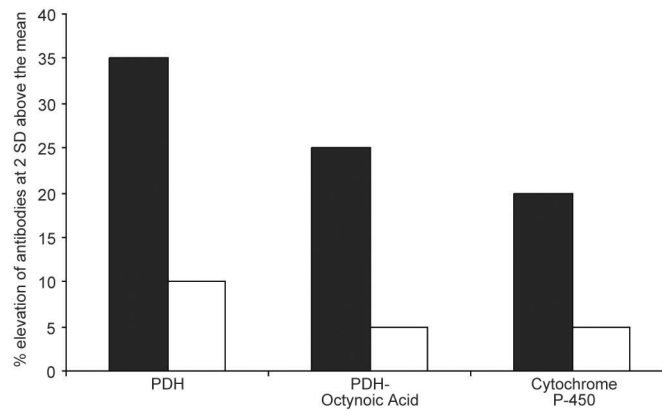


**Fig. 2.** Elevation in IgG antibody in healthy controls  compared to afflicted patients  with chronic motor peripheral neuropathy;  $n = 20$  for each group,  $p < 0.05$ .

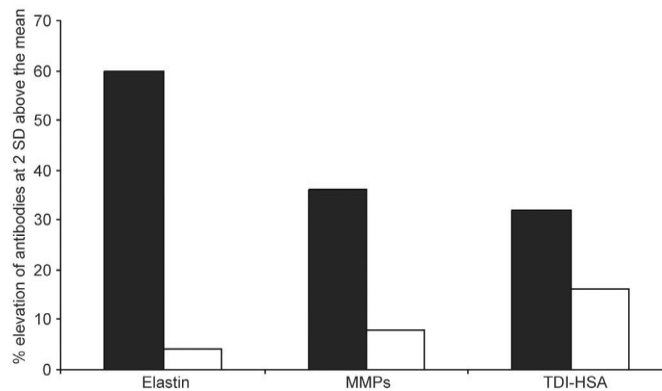


**Fig. 3.** Elevation in IgG antibody in healthy controls  compared to afflicted patients  with Guillain-Barre Syndrome;  $n = 25$  for each group,  $p < 0.001$ .





**Fig. 7.** Elevation in IgG antibody in healthy controls  compared to afflicted patients  with liver autoimmune disease;  $n = 20$  for each group,  $p < 0.005$ .



**Fig. 8.** Elevation in IgG antibody in healthy controls  compared to afflicted patients  with chronic pulmonary disease;  $n = 25$  for each group,  $p < 0.001$ .

in the pathogenesis of GBS (29, 30). Based on this mimicry, measurement of IgM antibody against *C. jejuni* toxin can differentiate between GBS versus chronic motor peripheral neuropathies (29). ALS is another motor neuron autoimmune disease in which antibodies are directed against glutamate receptors (31). Since recently misfolding of Cu/Zn-superoxide dismutase was described as a mechanism underlying motor neuron degeneration, we added SOD1 peptide to the tested antigens (32). Indeed, antibodies against GLU-R3, SOD1 peptide, ion channel and BBB protein were detected in a majority of 10 different tested ALS patients (Table IV, Fig. 4).

The unique clinical characteristics of the PANDAS subgroup are the presence of volumetric changes in the basal ganglia, increased titers of antibodies against the streptococcal M5, M12 and M19

proteins, and their cross-reactive epitopes on B cells (D8/17) and nerve cells, including the extracellular antigen lysoganglioside, and intracellular antigens such as tubulin, which have been described in a majority of these patients (33, 34). We would like to emphasize that this panel of antibodies is detected in a subgroup of patients with PANDAS who may benefit from antibiotic therapy, plasmapheresis or IVIG administration (Table V, Fig. 5).

Another example of environmental factors inducing autoimmunity is illustrated in women with pregnancies complicated by NTD. We detected antibodies against folate receptor, aflatoxin and fumonisin mycotoxins in the blood of such women (Table VI).

If not detected in time, Lyme disease becomes chronic and manifests itself in the forms of arthritis

and neuroborreliosis. The use of *in vivo* induced antigen technology in our earlier study (11), and in the present one, have further increased the sensitivity of Lyme disease diagnosis by about 25%. The varied pattern of antibody signatures in 3 patients with Lyme disease and its cross-infections is shown in Table VII.

At present, there is no single medical or laboratory marker that could be used for the diagnosis or follow-up treatment of children with autism. As has been observed earlier, then, the protocol of testing for autism spectrum disorders is for the management and not diagnosis of autism (12). The data presented in Table VIII and Fig. 6 show that in some patients with autism antibodies could be detected against up to 22 out of 96 tested antigens. Significant elevation of antibodies against 22 out of 96 tested antigens in patient #1 with autism, and many others whom we have observed, lead us to believe that somatic hypermutation and defect in B-cell checkpoint is a possibility in individuals producing multi-reactive antibodies (24). We selected sera from 3 patients who were known to our laboratory to be universal reactors. Application of their sera to the plates coated with 96 antigens resulted in antibody positivity against 28 out of 96 antigens (Table IX). We believe that in these individuals Ig hypermutation and/or class switch recombination through overexpression of activation-induced cytidine deaminase may be mechanisms enabling the development of pathogenic multi-reactive antibodies (24). Further research is needed to prove the involvement of AID in the development of these multi-reactive antibodies, as was proposed in experimental autoimmune encephalomyelitis (40).

Recent evidence has suggested that environmental factors, such as chemical xenobiotic 2-octynoic acid in cosmetics and food additives, are responsible for modification of PDH and autoantibody production (13). For this reason we included PDH, with and without octynoic acid, in the list of tested antigens. A majority of the patients' sera that reacted with PDH-octynoic acid also reacted with PDH, indicating that octynoic acid, as a cosmetic and food additive, is the major contributing factor for the induction of PBC (Table X, Fig. 7).

A very recent report indicated the involvement of anti-elastin immunity in T-helper-1 response and the

release of MMPs (14). Based on this mechanism of action, we measured antibodies against MMPs and elastin. A significant difference in the level of these antibodies was found between controls and patients in smoking-induced emphysema ( $p < 0.001$ ) (Table XI, Fig. 8). Since other provocative agents such as TDI can induce occupational lung disease, we found that a subgroup who were non-smokers produced antibodies against elastin MMPs as well as TDI, indicating TDI or other provocative chemicals (37) can induce autoimmunity against lung elastin and MMPs (Table XI, Fig. 8).

Reasoning that autoantibodies against peptides in cancer cases derived from cancer tissues, we included some of these tumor antigens in our ELISA assay and measured antibodies against mutated P53, Her-2/neu, ganglioside GD-3 and prostatic peptides. The clear potential of these antibodies in different cancers, particularly in prostatic hyperplasia, is shown in Table XII. More research could determine the predictive value of these antibodies in cancer diagnosis and treatment.

In conclusion, the data presented in this manuscript show that antibodies are detected against tissue-specific antigens as well as environmental factors associated with them. Many examples of such antibodies against cross-reactive or modified antigens are shown in Tables I-XII and Figs. 1-8.

Researchers and clinicians should then ask why the human body reacts to its own antigens, resulting in the production of potentially harmful autoantibodies. The cause may be environmental factors such as bacterial or viral infections, or haptenic toxic chemicals binding to human tissue, causing modification of self-antigens and the subsequent production of autoantibodies.

There would be many advantages in using a panel of different autoantibodies, some of which are related to the causative agents. In the first instance, the sensitivity of these assays would be increased. Additionally, in a modality that prescribes the removal of causative factors from the patient's environment, assays using these autoantibodies may allow the monitoring of the progression of the disease.

## REFERENCES

1. Steinman L. Multiple sclerosis: a coordinated

- immunological attack against myelin in the nervous system. *Cell* 1996; 85:299-302.
2. Raine CS, Cannella B, Hauser SL, Genain CP. Demyelination in primate autoimmune encephalomyelitis and acute multiple sclerosis lesions: a case for antigen-specific antibody mediation. *Ann Neurol* 1999; 46:144-60.
  3. Wilson, NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD, Basham B, Smith K, Chen T, Morel F, Lecron JC, Kastelein RA, Cua DJ, McClanahan TK, Bowman EP, de Waal Malefyt R. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* 2007; 8:950-7.
  4. Stone LA, Smith ME, Albert PS, Bash CN, Maloni H, Frank JA, McFarland HF. Blood-brain barrier disruption on contrast-enhanced MRI in patients with mild relapsing-remitting multiple sclerosis: relationship to course, gender, and age. *Neurol* 1995;45: 1122-6.
  5. Kim JV, Dustin ML. Innate response to focal necrotic injury outside the blood-brain barrier. *J Immunol* 2006; 177:5269-77.
  6. Phares TW, Fabis MJ, Brimer CM, Kean RB, Hooper DC. A peroxynitrite-dependent pathway is responsible for blood-brain barrier permeability changes during a central nervous system inflammatory response: TNF- $\alpha$  is neither necessary or sufficient. *J Immunol* 2007; 178:7334-7343.
  7. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A. Human T<sub>H</sub>17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nature Med* 2007; 13:1173-6.
  8. Ahmed S, Tsukahara S, Tin-Tin-Win-Shwe, Yamamoto S, Kunugita N, Arashidani K, Fujimaki H. Effects of low-level formaldehyde exposure on synaptic plasticity-related gene expression in hippocampus of immunized mice. *J Neuroimmunol* 2007; 186:101-11.
  9. Missmer SA, Suarez L, Felkner M, et al. Exposure to fumonisins and the occurrence of neural tube defects along the Texas-Mexico border. *Environ Health Perspect* 2006; 114:237-41
  10. Rothenberg SP, da Costa MP, Sequeira JM, et al. Autoantibodies against folate receptors in women with a pregnancy complicated by a neural-tube defect. *New Engl J Med* 2004; 350:134-42
  11. Vojdani A, Hebroni F, Raphael Y, et al. Novel diagnosis of Lyme disease: potential for CAM intervention. *eCAM* published online on 15 October 2007, doi:10.1093/ecam/nem138
  12. Vojdani A, Pangborn JB, Vojdani E, Cooper EL. Infections, toxic chemicals and dietary peptides binding to lymphocyte receptors and tissue enzymes are major instigators of autoimmunity in autism. *Int J Immunopathol Pharmacol* 2003; 16(3):189-99.
  13. Leung PSC, Park O, Tsuneyama K, et al. Induction of primary biliary cirrhosis in guinea pigs following chemical xenobiotic immunization. *J Immunol* 2007; 179:2651-7
  14. Lee S, Goswami S, Gurdo A, et al. Anti-elastin autoimmunity in tobacco smoking-induced emphysema. *Nature Med* 2007; 13:567-9
  15. Keogh E, Fikes J, Southwood S, Celis E, Chestnut R, Sette A. Identification of new epitopes from four different tumor-associated antigens: recognition of naturally processed epitopes correlates with HLA-A\*0201-binding affinity. *J Immunol* 2001; 167:787-96.
  16. Allen SD, Garrett JT, Rawale SV, et al. Peptide vaccines of the HER-2/neu dimerization loop are effective in inhibiting mammary tumor growth in vivo. *J Immunol* 2002; 179:472-82
  17. Kotlan B, Simsa P, Teillaud J-L, et al. Novel ganglioside antigen identified by B cells in human medullary breast carcinomas: the proof of principle concerning the tumor-infiltrating B lymphocytes. *J Immunol* 2005; 175:2278-85
  18. Penna G, Amuchastegui S, Cossetti C, et al. Spontaneous and prostatic steroid binding protein peptide-induced autoimmune prostatitis in the nonobese diabetic mouse. *J Immunol* 2007; 179: 1559-67
  19. Siegel S, Wagner A, Friedrichs B, et al. Identification of HLA-A\*0201-Presented T cell epitopes derived from the oncofetal antigen-immature laminin receptor protein in patients with hematological malignancies. *J Immunol* 2006; 176:6935-44
  20. Soussi T. P53 antibodies in the sera of patients with various types of cancer: a review. *Cancer Res* 2000; 60:1777-88

21. Nilsson BO, Carlsson L, Larsson A, Ronquist G. Autoantibodies to prostasomes as new markers for prostate cancer. *Ups J Med Sci* 2001; 106:43-9
22. Soares MM, Mehta V, Fin OJ. Three different vaccines based on the 140-amino acid MUC1 peptide with seven tandemly repeated tumor-specific epitopes elicit distinct immune effector mechanisms in wild-type versus MUC1-transgenic mice with different potential for tumor rejection. *J Immunol* 2001; 166:6555-63
23. Wang X, Yu J, Sreekumar A, Varambally S, Shen R, Giacherio D, Mehra R, Montie JE, Pienta KJ, Sanda MG, Kantoff PW, Rubin MA, Wei JT, Ghosh D, Chinnaiyan AM. Autoantibody signatures in prostate cancer. *N Engl J Med* 2005; 353:1224-35.
24. Hsu HC, Wu Y, Yang P, et al. Overexpression of activation-induced cytidine deaminase in B cells is associated with production of highly pathogenic autoantibodies. *J Immunol* 2007; 178:5357-65
25. Vojdani A, Vojdani E, Cooper EL. Antibodies to myelin basic protein, myelin oligodendrocytes peptides,  $\alpha$ - $\beta$ -crystallin, lymphocyte activation and cytokine production in patients with multiple sclerosis. *J Internal Med* 2003; 254:363-374.
26. Berger T, Rubner P, Schnautzer F, et al. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after first demyelinating event. *N Engl J Med* 2003; 349:139-45
27. Challoner PB, Smith KT, Parker JD, et al. Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. *Proc Natl Acad Sci USA* 1995; 92:7440-4
28. Tsunoda I, Libbey JE, Fujinami RS. Sequential polymicrobial infections lead to CNS inflammatory disease: possible involvement of bystander activation in heterologous immunity. *J Neuroimmunol* 2007; 188:22-33
29. Paterson G, Wilson G, Kennedy PGE, Willison HJ. Analysis of anti-GM1 ganglioside IgM antibodies cloned from motor neuropathy patients demonstrate diverse V region gene usage with extensive somatic mutation. *J Immunol* 1995; 155:3049-59
30. Peggy C, Godschalk R, Kujif ML, et al. Structural characterization of *Campylobacter jejuni* lipooligosaccharide outer cores associated with Guillain-Barre and Miller Fisher syndromes. *Infect Immun* 2007; 75:1245-54
31. Rogers SW, Twyman RE, Gahring LC. The role of autoimmunity to glutamate receptors in neurological disease. *Mol Med Today* 1996;2:76-81
32. Rakhit R, Robertson J, Velde CV, et al. An immunological epitope selective for pathological monomer – misfolded SOD1 in ALS. *Nature Med* 2007; 13:754-9
33. Mell LK, Davis RL, Owens D. Association between streptococcal infection and obsessive-compulsive disorder, Tourette's syndrome, and tic disorder. *Pediatr* 2005; 116:56-60
34. Kirvan CA, Cox CJ, Swedo SE, Cunningham MW. Tubulin is a neuronal target of autoantibodies in Sydenham's chorea. *J Immunol* 2007; 178:7412-21
35. Centers for Disease Control and Prevention. Recommendation for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR, Morb Mortal Wkly Rep* 1995; 44:590-591.
36. Engstrom SM, Shoop E, Johnson RC. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. *J Clin Microbiol* 1995; 33:419-27
37. Sequin P, Allard A, Cartier A, Malo JL. Prevalence of occupational asthma in spray painters exposed to several types of isocyanates. *J Occup Med* 1987; 29: 340
38. Notkins AL. New Predictor of diseases. *Sci Am* 2007; 296:72-79.
39. Kuhle J, Pohl C, Mehling M, et al. Lack of association between antimyelin antibodies and progression to multiple sclerosis. *N Engl J Med* 2007; 356:371-8.
40. Peterson LK, Tsunoda I, Masaki T, Fujinami RS. Polyreactive myelin oligodendrocyte glycoprotein antibodies: implications for systemic autoimmunity in progressive experimental autoimmune encephalomyelitis. *J Neuroimmunol* 2007; 183:69-80.