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Expert Opinion

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Antibodies as predictors of autoimmune diseases and cancer

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Background: Autoantibodies targeted against a variety of self-antigens are detected in autoimmune diseases and cancer. Emerging evidence has suggested the involvement of environmental factors such as infections and xenobiotics, and some dietary proteins and their antibodies in the pathogenesis of many autoimmune diseases. These antibodies appear in the blood years before presentation of symptoms in various disorders. Therefore, these antibodies may be used as biomarkers for early detection of various diseases. **Objective:** To provide an overview of antibody arrays that are measured against different human tissue antigens, crossreactive epitopes of infectious agents, dietary proteins, and haptenic chemicals in autoimmune diseases and cancer. **Method:** Microarray analysis of antigen–antibody reaction. **Conclusion:** The application of these antibody arrays to human autoimmune disease is expanding and is allowing for the identification of patterns or antibody signatures, thus establishing the premises for increased sensitivity and specificity of prediction, as well as positive predictive values. The presence of these antibodies would not necessarily mean that a patient would definitely become sick but may give a percentage of risk for different conditions that may develop over future months or years. Using this high-throughput microarray method, it is possible to screen rapidly for dozens of autoantibodies at low cost. This is an important factor in the implementation of autoantibody testing as a routine part of medical examinations.

Keywords: autoantibodies, autoimmune diseases, ELISA, environmental factors, predictive antibodies

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1. Introduction

Antibodies are molecules produced by plasma cells and B cells against bacteria, viruses, parasites, antigens, dietary proteins and peptides, and even haptenic chemicals such as medications [1-6]. In response to bacterial, viral and parasitic infection, the immune system jumps into action, deploying cells as well as antibodies in order to recognize and destroy the invaders. However, owing to molecular mimicry or antigenic similarity between these infectious agents and human tissue structure, in a genetically susceptible individual, components of the body's immune system target one or more types of the person's own tissue, which may result in autoimmunity [7-9]. In relation to dietary proteins and peptides, the mucosal immune system regulates responses to these substances in order to avoid harmful reactions to common mucosal antigens. This homeostasis between the host and antigenic stimulus is maintained by the mucosal immune system's induction of immunologic ignorance or oral tolerance against dietary proteins and commensal bacteria [10]. In the absence of oral tolerance, specific antibody-dependent protection is induced by secretory IgA and IgM, the predominant isotypes in human external secretions, including saliva. This breach of the intestinal barrier by dietary proteins through loss of tolerance can lead not only to antibody production in blood, but

56 also, owing to molecular mimicry, might lead to an immune
 response to different target organs and the induction of
 autoimmune diseases [11].

60 Being composed of small molecules, some medications
 and xenobiotics do not initiate an immune response
 unless they manage to bind to human tissue proteins and
 form hapten-coupled proteins. Diclofenac binding to tissue
 enzymes [12], formaldehyde, isocyanate and trimellitic
 65 anhydride binding to hemoglobin and human serum
 albumin [3,4], heavy metals binding to nucleoproteins [13,14],
 and chemicals generated from tobacco smoke binding to
 human elastin [6] are a few examples of many such immune
 reactions to drugs and xenobiotics.

70 Autoimmune reaction or autoimmune disease may be
 induced by drugs and chemical xenobiotics, which have the
 potential to form complexes with or otherwise alter self-
 proteins such that they become immunogenic. The loss of
 tolerance would involve the spreading of immune responses
 from the modified proteins to the unmodified native protein.
 75 The chronic presence of the self-protein would then
 serve to perpetuate the immune response initiated by the
 xenobiotically modified self-protein and lead to
 autoimmunity [2-5,8,9,12-15]. Detection of antibodies against
 these haptenic chemicals and modified tissue proteins
 80 requires special skills as well as highly pure antigens. That
 is partially why these tests are not performed at present
 in major clinical laboratories that perform routine testing.

85 In 'New predictors of disease', the work that
 inspired this manuscript, Notkins wrote of using molecules
 called predictive autoantibodies that appear in the blood
 years before symptoms of various disorders are actually
 presented [1]. As was stated by Notkins, 'This task is
 90 challenging because researchers will have to follow large
 populations for years to prove that particular autoantibodies
 can signal future disease'. That is, many thousands of healthy
 people must be recruited to give blood samples and then
 tracked carefully for 10 years or more to see if they
 become sick. Aside from posing logistical difficulties, these
 prospective studies can cost tens of millions of dollars.

95 An alternative to conducting prospective studies from
 scratch might be to tap into existing health databases and
 carry out retrospective studies. For example, blood samples
 and medical information have already been collected over
 many years from members of the US military and from
 100 subjects in the Women's Health Initiative, a vast continuing
 study of more than 100,000 women. Experts in autoimmunity
 could team up with investigators in these and other projects,
 identify individuals who have been diagnosed with an auto-
 immune disease and then examine their stored blood for the
 105 presence of predictive autoantibodies [1,16-18].

110 It is understood that a laboratory can introduce a
 new method as specified in Section 493.1202(a) or (b), or
 Section 493.1203(a) of the US Federal Clinical Laboratory
 Improvement Amendments of 1988 (9CLIA-88) regulations
 at 42CFR493.1213. Before reporting patient test results,

the laboratory must verify or establish performance 111
 specifications, including: accuracy; precision; analytical
 sensitivity and specificity if applicable; the reportable range
 of patient test results; the reference range(s) (normal values);
 115 and any other applicable performance characteristic. Under
 this condition, FDA approval is not required if the laboratory
 does not manufacture diagnostic kits. In this article, the
 terminology of predictive antibodies will be used, for some
 of which clinical sensitivity, specificity and predictive values
 have already been established, and for others only analytical 120
 sensitivity and analytical specificity are being defined.
 Hopefully, information summarized in this article will initiate
 more research and this antibody screening will become part
 of the standard medical examination in the near future.

2. Present diagnostic tools in autoimmune diseases based on tissue-specific antibodies and etiologic factors associated with them

125 Laboratory research is the main driving force behind
 producing medically relevant scientific observation and its
 successful translation into therapeutic modalities for complex
 autoimmune diseases [19-21]. The execution of such a noble
 idea, however, is being forestalled to some extent owing to
 the role played by regulatory agencies and an underlying 135
 division between clinicians and basic researchers. Mechanistic
 overinterpretation of studies based on patients' clinical results
 on one hand and an excessive faith in the validity of animal
 disease models on the other have helped to widen this gap
 between the two camps. 140

As both approaches have their fundamental limitations,
 there is no doubt that both animal and disease models have
 provided the rationale and platform for the development
 of laboratory assays that are in place for the diagnosis of
 autoimmune diseases today. 145

In this article, an attempt is made to provide a forum for
 future debate about the importance of predictive antibodies
 for the diagnosis and management of complex diseases, for
 some, with or without available clinical sensitivity, specificity
 and predictive values. 150

2.1 Gastrointestinal autoimmunity

155 In gastrointestinal autoimmunity such as pernicious anemia,
 celiac disease and inflammatory bowel disease (IBD),
 immune reaction against common mucosal tissue antigens,
 dietary proteins or peptides and commensal bacteria has
 been reported. This immune reaction against different
 gastrointestinal (GI) tissue antigens is due either to the binding
 of haptenic chemicals (e.g., medication) [12] or infectious
 agents' antigens to GI tissue antigens, or when the balance 160
 between tolerogenic and inflammatory responses is tipped [22].
 A breakdown in immune tolerance and the induction of
 Th1/Th2 imbalance could be because of change in production
 of TGF-beta and IL-17, which contributes to inflammation
 and autoimmunity. 165

166 Association between parietal cell and intrinsic factor
antibody has been reported previously in patients with
pernicious anemia (PA), which is an organ-specific auto-
immune disease. These autoantibodies are detected in
170 ~ 90% of patients with PA, but are also detected in ~ 13%
of their non-anemic first-degree relatives. It should be noted
that these data relate to the identification, not prediction, of
disease [23,24]. Based on our experience, patients with PA,
when tested against tropomyosin and many other non-
175 related antigens and peptides, not only showed significant
elevation against parietal cells plus intrinsic factor antibody,
but also reacted against Tropomyosin. Antibodies against
tropomyosin have been reported in patients with ulcerative
colitis and colorectal cancer [25,26]. Dipeptidyl peptidase IV
180 antibody was reported in patients with autoimmune diseases
as well as in children with autistic spectrum [9]. Celiac
disease is another autoimmune disease associated with tissue
antibodies against endomysial and reticulin, and antibodies
against gliadin [27,28]. Both IgG and IgA antibodies against
185 these antigens have been detected in patients with classical
celiac disease, with sensitivity, specificity and predictive
values of 70 – 100% [29-31]. These autoantibodies are detected
in the blood up to 7 years before presentation of symptoms,
suggesting that high-risk individuals may be able to prevent
190 celiac disease by eliminating gluten from their diet. However,
limitation of testing to gliadin and transglutaminase
completely ignores the consequences of gluten sensitivity
beyond the gut. Since 1966, in patients with both celiac
disease and gluten intolerance, antibodies against tissue
195 antigens from thyroid, joints, bone, heart, pancreas, brain
and even synapses have been reported [32-40].

In a very elegant study [41] an attempt was made to
demonstrate the involvement of infectious agents and innate
immunity in the pathogenesis of celiac disease. This study
200 involved a random peptide library with pooled sera of
patients affected by active disease after a prescreening with
the sera of the same patients on a gluten-free diet.
The study team identified a peptide recognized by serum
immunoglobulins of patients with active disease but not
205 by those of patients on a gluten-free diet. This peptide
shares homology with the rotavirus major neutralizing
protein VP-7 and with the self-antigens tissue transglutami-
nase, heat-shock protein 60 (HSP-60), desmoglein 1 and
Toll-like receptor 4. VP-7 is an outer capsid protein of
210 rotavirus that is known to induce polyclonal B-cell
activation [42]. It was shown that antibodies against the
peptide affinity-purified from the sera of patients with
active disease recognize the viral product and self-antigens
in ELISA and western blot. These antibodies were able to
215 induce increased epithelial cell permeability. Finally, the
purified antibodies induced monocyte activation upon
binding Toll-like receptor 4 and induced pro-inflammatory
cytokine production *in vitro* [41].

From these reports, we learn that testing for celiac disease
220 and gluten intolerance not only should include antibodies

against gliadin and transglutaminase, which limits their
221 findings only to the gut, but also should emphasize the
importance of extraintestinal consequences. In IBD, although
antibodies against *Saccharomyces cerevisiae* (ASCA) and
225 against neutropin cytoplasmic antigen (ANCA) have been in
studies for differentiation between Crohn's and ulcerative
colitis, no attempt has been made to demonstrate the
predictive value of these antibodies [43]. Furthermore, it is
well documented that different medications, bacterial
endotoxins and exotoxins play a significant role in the
230 development of Crohn's and ulcerative colitis [11-13,44-46].

2.2 Thyroiditis, lupus, arthritis and osteoarthritis

Thyroid autoantibodies thyroglobulin (TG) and thyroid
235 peroxidase (TPO) can also reflect disease activity and
progression and are valuable in disease prediction and the
classification of Hashimoto and Graves disease [47,48]. While
clinicians rely on the antibody level and elevation in
thyroid-stimulating hormone (TSH), little attention
has been given to the other enzymes, binding proteins
240 and receptors involved in thyroiditis [49-52]. This includes
TSH receptor (TSH-R), thyroxin-binding globulin,
thyroxin-binding prealbumin and thyroxine deiodinase.
Some patients may have negative or low levels of antibody
against TG or TPO but have a significant elevation
245 antibody against TSH-R, thyroid-binding globulin or
thyroid-binding prealbumin. This inclusion of tissue and
receptor antibodies in patients negative or positive for either
TG or TPO antibodies may increase the sensitivity of
thyroid autoimmunity detection. 250

Systemic lupus erythematosus (SLE) and rheumatoid
arthritis (RA) are autoimmune diseases affecting different
tissues and organs accompanied by the production of anti-
bodies against modified nucleic acids, nucleoproteins and
other self-proteins, which are typically present many years
255 before a diagnosis of SLE [53]. SLE is an autoimmune
disease affecting different organs accompanied by the
production of antibodies against ssDNA and extractable
nuclear antigens, including Sm, RNP, Ro, La and
phospholipids [54]. Furthermore, the appearance of auto-
260 antibodies in patients with SLE tends to follow a predictable
course. SLE autoantibodies were detected as much as
9.4 years earlier. For anti-Ro antibodies, the mean interval
between the earliest detection of autoantibodies and
diagnosis of lupus was 3.68 years; for ANA, antiphospho-
265 lipid and anti-La, 3.4 years; and antissDNA antibodies were
first detected a mean of 2.2 years before diagnosis [53].
Recently, highly specific peptides such as SmD3 [55] and
laminin, enzymes such as poly (ADP-ribose) polymerase,
metal binding proteins, and antibodies against them have
270 been reported in animal models [56,57].

Rheumatoid arthritis is another complex autoimmune
disease. Its characteristic feature is a chronic destructive
inflammation that is primarily localized in the synovial
lining of the joints. In patients with RA, inflammation in
275

276 different organs, including skin, lungs, heart and peripheral
 280 nerves, has been detected [58]. Serum antibodies specific for
 modified self-proteins are a hallmark for many complex
 autoimmune disorders, including RA. These disease-specific
 285 antibodies predominantly react with modified self-proteins
 such as IgG, citrullinated protein and collagen [59,60], and
 several mimic peptides [61-63]. Antibodies against aggregated
 IgG, RA-induced peptide, citrullinated peptide, collagen
 type 2 peptide, *Mycobacterium avium*, *Mycoplasma arthritidis*,
Chlamydia pneumoniae and HSP-60 are detected frequently
 in patients with RA [61,62].

Autoimmunity to chondrocyte-producing proteins such as
 fibulin-4 has been reported in patients with osteoarthritis [63-65].
 In reactive arthritis, Yersinia heat-shock protein has been
 290 identified as the target of HLA-B27-restricted CTL
 response [62]. That is why in addition to rheumatoid factor
 (RF), antibodies against fibulin and Yersinia HSP-60 are
 measured and detected in a significant percentage of patients
 with osteoarthritis.

295 In this regard, studies support the inclusion of newly
 reported antibodies against Smd3, laminin, poly (ADP-
 ribose) polymerase for lupus [53-55], and RA-induced peptide
 for arthritis [58,59], and chondrocyte-producing protein such
 as fibulin-4 for patients with osteoarthritis [64,65]. Factors
 300 responsible for the direct induction of systemic autoimmune
 disease and antibodies against them may be considered in
 future studies of predictive antibodies. For example, despite
 the fact that it is very well accepted that injection of HgCl₂
 can induce systemic autoimmunity including scleroderma in
 305 animal models [66-68], no attempt has been made to measure
 antibodies against Hg-HSA or Hg-binding nucleoproteins
 such as fibrillarin and chromatin in human lupus [9,14].
 Similarly, in the case of infections with *Mycoplasma*,
Chlamydia and *Mycobacteria*, their heat-shock proteins or mimic
 310 peptides are considered to be the most likely triggering
 factors for RA [61,62]. Inclusion of these protein-modifying
 factors and mimic peptides in the existing antigen-specific
 autoantibody test panels is likely to contribute to the early
 detection and prevention of systemic autoimmunities.

315 **2.3 Adrenalitis, type 1 diabetes and cardiac
 autoimmunity**

Adrenalitis is another organ-specific autoimmune disease
 that can lead to adrenal gland failure or Addison's disease.
 320 Adrenalitis-associated antibodies were measured against adrenal
 gland antigens, 17-hydroxylase, 21-hydroxylase, cytochrome
 P450 enzyme and glomerular basement membrane protein [69,70].
 Detection of these antibodies has been associated with high
 progression to clinical Addison's disease [69].

325 In type I diabetes, the immune system attacks the beta
 cells in the pancreas and manufactures antibodies against
 multiple beta cell antigens [71-74]. These antibodies precede
 the occurrence of clinically manifested hyperglycemia.
 Autoantibodies specific for insulin, glutamic acid decarboxylase
 330 (GAD), islet cell antigen-2 (IA2) and islet-specific

glucose-6-phosphate catalytic-subunit-related protein (IGRP) 331
 have been detected in humans [71-74]. These antigens are
 typically associated with pancreatic B cells but the presence
 of non-B-cell antigens such as glial fibrillary acidic protein
 (GFAP), Cocksackievirus-B and milk proteins has also been
 335 shown. The evolution of the autoimmune response to these
 antigens is sequential; consequently, when antibodies are tested
 against this panel of antigens, depending on the stage of the
 disease, some may be positive for antibodies against one or
 more antigens, whereas others may produce antibodies
 340 against two or three of the other antigens. Antibodies against
 insulin, GAD and IA2 are detected in the blood of patients
 5 – 10 years before the onset of the disease [72,74]. In the
 sera of patients with type 1 diabetes, in addition to insulin,
 GAD and IA2 antibodies, antibodies against Cocksackievirus-B
 345 and cow's milk were detected in our laboratory. This confirms
 earlier studies in which it was reported that consumption of
 milk and infection with Cocksackievirus could contribute to
 type 1 diabetes in genetically susceptible individuals [75,76].
 350 Detection of these antibodies against milk in some patients
 with type 1 diabetes may justify implementation of a milk-
 free diet, because high consumption of cow's milk during
 childhood can be diabetogenic in siblings of children with
 type 1 diabetes [76].

In cardiac autoimmunity, antibodies are produced against 355
 heart myosin, vascular endothelial cells, platelet glycoprotein,
 β₂-glycoprotein, phosphorylcholine, HSP-60, or against
 modified low-density lipoprotein (o-LDL) [77-84]. However,
 antibodies against some or all of these antigens are detected
 in a significant percentage of healthy controls [78-80]. 360

**3. Biomarkers for neuroautoimmune
 disorders**

Response to injury is often accompanied by protecting 365
 mechanisms that antagonize the damaging events or
 mediate repair.

Multiple sclerosis (MS) is an autoimmune neuro-
 degenerative disease leading to destruction of the myelin
 sheath that ultimately affects the ability of nerves to 370
 conduct electrical impulses. The development of effective
 therapeutics has been complicated by a poor understanding
 of the etiology of MS. Despite strong evidence for the
 contribution of T-cell responses to manifestations of auto-
 immunity in the central nervous system (CNS) of patients 375
 with MS, investigators have been encouraged by recent
 findings to search for B-cell-mediated biomarkers that
 contribute to the MS pathogenesis [85-92].

There is ample data showing that autoantibodies against
 myelin protein components being detected in the blood 380
 characterize a significant portion of MS cases. Also, high-
 resolution microscopic analysis detected myelin-specific
 autoantibodies in the regions of demyelination plaques in
 human MS and an MS-like disease in marmosets, suggesting
 their direct involvement in myelin destruction [85-87]. 385

386 In MS, doctors are desperate to know which patients with
 early mild symptoms will go on to suffer from severe
 symptoms so that they may be able to take preventive
 measures. In addition to this early warning, some antibodies
 390 may help clinicians to measure the rate of progression of an
 already diagnosed autoimmune disease to a severe one [1].
 Indeed, since 2003 many studies have used antibodies
 against two proteins that insulate the nerve, MBP and
 MOG, as demonstrations of autoantibodies with clinical
 395 association, specificity and sensitivity [85-92]. On the other
 hand, a recent study did not show any association between
 antimyelin antibodies and the progression of MS [93]. This
 lack of association between neural cell antibody and the
 progression of MS may stem from the analysis of antibodies
 400 in blood samples obtained between 46 and 59 days after the
 first clinical signs while 71% of the patients had undergone
 corticosteroid treatment. In an earlier study it was found
 that the addition of alpha-B-crystallin antibody to MBP and
 MOG antibody measurements resulted in a sensitivity of
 405 75% and a specificity of 70% [85]. More neuron-specific
 (such as proteolipid protein, transaldolase) and nonspecific
 antigens (human herpes type-6, *C. pneumoniae* HSP-60 and
 acinetobacter) were studied and found to be elevated in a
 significant percentage of MS patients. The detection of
 410 antibodies against these and other antigens was expected,
 because these antigens were expressed in brain tissue and
 their administration to animal models resulted in an
 MS-like condition [94-96].

In patients with neuropathies, antibodies against MBP,
 415 MAG, ganglioside GM₁ and sulfatide have been reported [97-99].
 Detection of these antibodies may differentiate this disorder
 from autoimmune CNS disorders. Some of these antibodies,
 such as ganglioside GM₁, are also detected in patients with
 Guillain-Barre syndrome (GBS) [97-99]. Molecular mimicry
 420 between lipooligosaccharides (LOS) in the *Campylobacter jejuni*
 cell wall and gangliosides in peripheral nerves plays a crucial
 role in the pathogenesis of GBS [97-101]. Based on this
 mimicry, measurement of IgM antibody against *C. jejuni*
 toxin can differentiate between GBS versus chronic motor
 425 peripheral neuropathies [102].

Amyotrophic lateral sclerosis (ALS) is another motor
 neuron autoimmune disease in which antibodies are directed
 against glutamate receptors [103-105]. Very recently, misfolding
 of Cu/Zn-superoxide dismutase was described as a mechanism
 430 underlying motor neuron degeneration [106].

The unique clinical characteristics of the pediatric auto-
 immune neuropsychiatric disorder associated with the group
 A streptococcal infection (PANDAS) subgroup are the presence
 of volumetric changes in the basal ganglia, increased titers of
 435 antibodies against the Streptococcal M proteins (ST.M), and
 their crossreactive epitopes on B cells (D8/17) and nerve
 cells. This includes the extracellular antigen lysoganglioside,
 and intracellular antigens such as tubulin, which have been
 described in a subgroup of patients with PANDAS [107-110].
 440 The detection of antibodies against a panel of antigens in a

subgroup of patients with PANDAS may justify antibiotic 441
 therapy, plasmapheresis or intravenous immunoglobulin
 administration. Furthermore, unpublished data from many
 patients in our lab showed that in a different subgroup of
 patients with PANDAS/OCD, the detected levels of anti- 445
 bodies against streptococcal or its crossreactive antigens were
 significantly lower than in the healthy control subjects. This
 indicates that streptococcal and other antigens are not
 associated with a subgroup of patients with OCD/PANDAS.
 Therefore, further studies are needed to map out more 450
 definitively the cause and effect relationship in this
 PANDAS/OCD subgroup. The lack of antibody detection
 from tests directed against streptococcal antigens and their
 crossreactive epitopes in human tissue in a subgroup of patients
 with PANDAS/OCD may warrant the design of different 455
 treatment modalities from the ones discussed above.

An example of environmental factors inducing neuroauto-
 immunity is illustrated in women with pregnancies complicated
 by a neural tube defect. Exposure to fumonisins from
 contaminated corn and its consumption in a form of tortilla 460
 bread was found to be associated with the occurrence of
 neural tube defect [111]. To investigate this causal relation,
 measurements of antibodies against folate receptor, aflatoxin
 and fumonisin mycotoxins in the blood of women with
 complicated pregnancies may be considered. Only the 465
 simultaneous detection of antibodies against folate
 receptor and mycotoxins may further clarify their roles
 in complicated pregnancies.

4. Search for antibodies as peripheral disease 470 markers in cancer

Cancer sera contain antibodies that react with a unique
 group of autologous cellular antigens called tumor-associated
 antigens (TAAs). During the past 10 years researchers have 475
 made the intriguing discovery that autoantibodies can appear
 in the blood of some cancer patients. These antibodies are
 produced as a result of overexpression of tumor antigens or
 peptides and mutated proteins [112-120]. Immune response
 against tumors results in autoantibody production. Reasoning 480
 that autoantibodies against peptides derived from cancer tissues,
 antibodies were measured 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, has been discussed [114-122]. 485

The tumor suppressor p53 is a phosphoprotein barely
 detectable in the nucleus of normal cells [123,124]. Mutations
 of the p53 tumor-suppressor gene are some of the most
 common genetic variations in human cancer with a prevalence
 that varies from 35 to 60% of different cancers [123,124]. This 490
 alteration in antigenic expression can result in cellular
 accumulation of p53 and the production of p53 antibody in
 serum [116]. In a review article based on more than 130 papers
 published in the field of cancer detection, it was demonstrated
 that p53 antibodies are found predominantly in human cancer 495

496 patients with a specificity of 96% [116]. Such antibodies were
predominantly associated with p53 gene missense mutations
and p53 accumulation in the tumor, but the sensitivity of
500 such detection was only 30%. It has been demonstrated that
this immune response is due to a self-immunization process
linked to the strong immunogenicity of the p53 protein.
The clinical value of these antibodies remains subject to
debate, but consistent results have been observed in breast,
colon, oral, uterine, ovarian and gastric cancers, in which
505 they have been associated with high-grade tumors and poor
survival [124-127]. The finding of p53-Abs in the sera of
individuals who are at high risk of cancer, such as exposed
workers or heavy smokers, indicates that they have promising
potential in the early detection of cancer [123-127].

510 HER-2/neu is a transmembrane glycoprotein with tyrosine
kinase activity, the overexpression of which contributes to
uncontrolled growth signal transduction and, therefore,
cellular transformation [128]. It has been shown that
overexpression of HER-2/neu protein on tumor cells
515 results in specific antibody immunity in patients with breast
cancer [129]. The presence of antibodies to HER-2/neu
correlated with the presence of breast cancer. HER-2/neu
antibodies at titers of $\geq 1:100$ were detected in 12 of 107
(11%) breast cancer patients versus none of the 200 (0%)
520 normal controls ($p < 0.01$). The presence of antibodies to
HER-2/neu also correlated to overexpression of HER-2/neu
protein in the patient's primary tumor. Nine of 44 (20%)
patients with HER-2/neu-positive tumors had HER-2/
neu-specific antibodies, whereas 3 of 63 (5%) patients
525 with HER-2/neu-negative tumors had antibodies. It was
concluded that antibodies in breast cancer patients and the
correlation with HER-2/neu-positive cancer imply that
immunity to HER-2/neu develops as a result of exposure
of patients to HER-2/neu protein expressed by their
530 own cancer [129].

Tumor gangliosides are membrane glycosphingolipids
that shed into the tumor cell microenvironment, resulting
in antitumor immune response [113]. Antibodies against
ganglioside GD3 have been reported in patients with breast
535 carcinomas [130], in human gliomas [131] and in patients
with differentiated thyroid cancer [132].

The past decade has seen a resurgence of interest and
exciting new research on chronic prostatitis and related
syndromes [121], in particular, the detection of autoanti-
bodies in prostate cancer [133]. In one study a decision tree
540 was constructed for classifying prostate cancer using seven
TAAs. These antibody measurements resulted in 79%
sensitivity and 86% specificity [133]. In a different study
for demonstration of autoantibody signature in prostate
cancer, a 22-phage-peptide detector had 88.2% specificity
545 and 81.6% sensitivity with 95% confidence interval [134].
It was concluded that autoantibodies against peptide
derived from prostate cancer tissue could be used as the
basis for a screening test for prostate cancer, and that these
550 autoantibody signatures may improve the early detection of

cancer [134]. This approach, utilizing a mini-array of tumor
antigens, was also applied to other types of cancer with very
encouraging results [128]. This mini-array of TAAs was
composed of full-length recombinant proteins expressed
555 from cDNAs encoding c-myc, p53, cyclin B1, p62, Koc,
IMP1, and surviving. Enzyme immunoassay was used to
detect antibodies in 527 sera from 6 different types of
cancer. Antibody frequency to any individual TAA was variable
but rarely exceeded 15 – 20%. With the successive addition
of TAAs to a final total of 7 antigens, there was a stepwise
560 increase of positive antibody reactions up to a range of
44 – 68%. Breast, lung and prostate cancer patients showed
separate and distinct profiles of reactivity, suggesting that
uniquely constituted antigen mini-arrays might be developed
to distinguish between some types of cancer [135,136]. 565

5. Protective and pathogenic roles for antibodies

The presence of one or more types of autoantibody in
570 the blood is a common characteristic of autoimmune
diseases [1,16,53,137]. The production and presence of anti-
bodies by themselves are not sufficient for the development
of autoimmune diseases, because antibodies can be
protective, as in the case of infections and some autoimmune
575 diseases, or destructive, as they are in many other auto-
immune inflammatory disorders. The pathogenicity of
antibodies depends not only on the isotype (e.g., IgG or
IgA versus IgM), avidity and titer, but also other factors
such as general immune regulation, cytokines, chemokines,
580 neurotransmitters, matrix metalloproteases (MMPs),
generation of immune complexes and activation of the
complement system. Therefore, antibodies could be detected
in some individuals and persist for many years without
development of the disease, but in other cases detected
585 antibodies precede the development of full-blown auto-
immune disease. In the latter group these autoantibodies
have been used to study disease activity, determine the rate
of disease progression, and help classify and predict clinical
disease [1,16]. A recent study presented evidence for the
590 involvement of gliadin and transglutaminase antibodies in
the pathogenesis of celiac disease [41]. This was shown by the
ability of antibodies to increase epithelial cell permeability,
induction of monocyte activation, and enhanced production
of TNF- α and IL-6. After implementation of a gluten-free
595 diet, the antibodies disappeared and all other markers returned
to their normal levels [41].

Autoantibodies to various nuclear antigens, including
ANA, ssDNA, ss-A (Ro), ss-B, Sm and Sn-RNP, are
detected in patients with lupus. Owing to the production of
600 these multi-reactive antibodies, the disease often involves
inflammation and injury to the joint, skin, kidney, body
cavity membranes, lung, heart, gastrointestinal tract and
brain [53]. Patients with lupus experience progressive
cognitive loss without evidence of CNS vascular disease or
605

606 inflammation. Although autoantibodies are central features
of SLE, exactly how they mediate tissue damage remains an
area of active investigation [56]. It was demonstrated recently
610 is responsible for mediating neuronal excitotoxicity and
death [138]. However, it was not clear how these antibodies
could cause brain damage when present in the systemic
circulation. Neuropathology requires a breach in the integrity
of the blood–brain barrier (BBB). Infection is one such
615 circumstance that leads to the abrogation of the BBB. This
was shown by the administration of lipopolysaccharides
(LPS) to mice with high titers of antibodies; only after the
LPS injection did the antibodies gain access to the brain [138].
In the same study it was shown that antibodies binding to
620 the hippocampal neurons resulted in cognitive dysfunction, altered
hippocampal metabolism and neuronal cell death [138].
Therefore, a combination of antibodies produced against DNA
plus infection-enhanced BBB dysfunction may be responsible
for neuropathology and neuronal cell death in lupus.
625 Autoimmune demyelination is driven by pathogenic
immune response against myelin proteins and lipids.
Interestingly, in addition to neural cell antibody, organ-
specific IgM autoantibodies to liver, heart and kidney have
also been detected in the sera of patients with MS and other
630 neuroimmune disorders. To demonstrate the pathogenicity
of these antibodies, mice were injected with myelin
oligodendrocyte glycoprotein monoclonal antibody. This
resulted in immunoglobulin deposition in the kidney and
liver, indicating that transitional forms between CNS
635 organ-specific and systemic autoimmune disease exist [139].
Furthermore, intrathecal antibody production in mice
infected with Theiler's murine encephalomyocarditis virus
developed an immune-mediated demyelinating disease
characterized by weakness associated with disability [140].
640 Finally, evidence was presented that anti-MBP antibodies of
MS patients and EAE mice exhibited site-specific proteolytic
cleavage of the MBP molecules that may contribute to
pathological destruction of the myelin sheath [141,142].
Thus, the discovered epitope-specific antibody-mediated
645 degradation of MBP suggests a mechanistic explanation of
the slow development of neurodegeneration associated with
neuroimmune disorders.

6. Expert opinion

650 This manuscript was inspired by the pioneering work of
AL Notkins, who, in 'New predictors of disease', emphasized
that 'one day Y-shaped molecules called autoantibodies in a
patient's blood may tell doctors whether a patient is "brewin"
655 a certain disease and may even indicate roughly how soon
the individual will begin to feel symptoms' [1]. Furthermore,
the article states 'molecules called predictive autoantibodies
appear in the blood years before people show symptoms of
various disorders. Tests that detected these molecules could
660 warn of the need to take preventive action' [1].

661 Considering the fact that the evolution of autoimmune
response inducing neo-autoantigen and epitope formation
and immune response to these antigens over time is
sequential, more diverse autoreactive antibodies develop
665 over time. Therefore, only the inclusion of antibody assays
against a panel of antigens, some of which are tissue-specific
and others related to the etiologic agents, may enhance
clinical sensitivity, specificity and predictive value in
future studies [1,16,53,137].

670 Although agreeing whole-heartedly with Dr Notkins's
statements, without the identification of factors such as
infections, dietary proteins and xenobiotics as major
instigators in the development of autoantibodies, clinicians
will not be able to take preventive action [2-9,12-15]. Researchers
675 and clinicians should ask the question, why does the human
body react to its own antigens, which results in the production
of potentially harmful autoantibodies? The cause may be
due to environmental factors such as bacterial or viral
infections, or haptenic toxic chemicals binding to human
tissue, causing modification of self-antigens and the
680 subsequent production of autoantibodies.

685 Many examples of such antibodies against crossreactive
or modified antigens are mentioned throughout the
manuscript. Rotavirus antibody in celiac disease,
Chlamydia HSP-60 antibody in arthritis and cardiovascular
690 autoimmunity, coxsackievirus and milk antibodies in type 1
diabetes, oxidized LDL antibody in cardiovascular disease,
acinetobacter and milk butyrophilin antibody in MS,
streptococcal antibody in PANDAS/OCD, *C. jejuni*
antibody in GBS, aflatoxin and fumonisin antibodies in
695 complicated pregnancies, and heavy metal antibodies in
systemic autoimmunity, including scleroderma, are just a
few of many cited examples.

695 For instance, in celiac disease, in addition to the inclusion
of antibody measurements against transglutaminase,
measurements of antibodies against other enzymes, receptors
or regulators of the GI tract in future studies of PA
is recommended.

700 For this reason, antibodies against GAD, TG, TPO, HSP,
MBP, neurofilaments, cerebellar and many other antigens
may be measured in order to detect autoimmune and
neuroimmune disorders in patients with gluten sensitivity
and celiac disease. Antibody testing against the repertoire of
705 these antigens would not only address celiac disease beyond
the gut, but also may contribute to the clinical specificity
and sensitivity of detected antibodies.

710 With regards to cancer, autoantibodies against different
tumor-associated antigens and peptides could be useful in
early detection of autoimmune response in different types of
the disease [112-136,143]. Although detection of autoantibodies
715 against peptides derived from prostate, breast, colon and
lung cancer tissues could be used as the basis for screening,
further studies of patients in the early stages of cancer and
high-risk individuals and the design of unique antigen
panels for different cancers would help to determine whether

716 multiple antigen antibody arrays for the detection of auto-
antibodies might contribute a clinically useful non-invasive
approach to cancer detection and diagnosis.

720 Returning to Dr Notkins's article, it goes on to say that
'so far much of the work I have discussed has been confined
to a small number of academic laboratories related to a few
of the major autoimmune diseases' [1]. We must disagree
with this statement, because during the past 20 years, as is
borne out by the publication of numerous manuscripts in
725 scientific journals, we and several other specialty clinical
laboratories, upon their validation and according to govern-
ment regulations, have implemented and introduced to
clinical use many antibody assays not only for the detection
of autoimmune reactions, but also for cardiovascular diseases
730 and cancer [8,9,85,144-147].

In the absence of clinical sensitivity, clinical specificity
and clinical prediction values for some of these antibodies,
there would be many advantages in using a panel of
different autoantibodies, some of which are related to the
causative agents. For one, the sensitivity of these assays
735 would be increased. Also, based on these antibodies, a
modality that prescribes the removal of causative factors
from the patient's environment may allow the monitoring of
the disease's progression. The disappearance of these auto-
antibodies upon therapy might also indicate a beneficial
740 response. Further, simultaneous measurement of antibodies
against an array of 200 pure antigens performed in our
laboratory for research only makes possible a rapid screening
for dozens of diseases. Earlier work by Quintana *et al.* [148]

with animal models lends support to this approach. The
745 author's overall goal is to make this high-throughput assay a
routine part of medical examinations in the near future.
This way, patients visiting their doctors might have their
blood tested for multiple predictive autoantibodies in a
single test. Keeping this in mind, it should not be forgotten
750 that the presence of predictive antibodies would not mean
that a patient would definitely become sick but would give
a percentage of risk for numerous conditions that may
develop over future months or years.

Although the author believes that some of these antibodies
755 may be protective and others pathogenic, it is advisable for
patients or healthy controls with elevation in antibodies
against an array of tissue-specific antigens to be admitted as
subjects into therapeutic intervention trials if their antibodies
persist for more than 6 months. 760

In this regard, more studies are needed not only to
address antibody levels, but also to examine immune
regulatory cytokines (in particular Th17), intestinal
barrier function, MMPs, BBB integrity and neurotransmitter
level in order to expand the number of testable hypotheses
765 by some order of magnitude [149-155]. Such proteome-
wide applications to generate blood-driven disease
signatures have been suggested as future biomarkers in
clinical psychiatry [156]. 770

Declaration of interest

A Vojdani is the co-owner of Immunosciences Lab., Inc. 773

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as either of interest (*) or of considerable
interest (**) to readers.

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