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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

Expert Opin. Med. Diagn. (2008) 2(6):1-13

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

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

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 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.

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. 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 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.

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 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.

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 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 autoimmune disease and then examine their stored blood for the presence of predictive autoantibodies [1,16-18].

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 specifications, including: accuracy; precision; analytical sensitivity and specificity if applicable; the reportable range of patient test results; the reference range(s) (normal values); 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 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

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 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.

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.

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.

2.1 Gastrointestinal autoimmunity

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 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.

Association between parietal cell and intrinsic factor antibody has been reported previously in patients with pernicious anemia (PA), which is an organ-specific autoimmune disease. These autoantibodies are detected in ~ 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-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 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 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 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 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 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 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 transglutaminase, heat-shock protein 60 (HSP-60), desmoglein 1 and Toll-like receptor 4. VP-7 is an outer capsid protein of 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 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 and gluten intolerance not only should include antibodies against gliadin and transglutaminase, which limits their findings only to the gut, but also should emphasize the importance of extraintestinal consequences. In IBD, although antibodies against *Saccharomyces cerevisiae* (ASCA) and 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 development of Crohn's and ulcerative colitis [11-13,44-46].

2.2 Thyroiditis, lupus, arthritis and osteoarthritis

Thyroid autoantibodies thyroglobulin (TG) and thyroid 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 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 in 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.

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are autoimmune diseases affecting different tissues and organs accompanied by the production of antibodies against modified nucleic acids, nucleoproteins and other self-proteins, which are typically present many years 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 autoantibodies 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, antiphospholipid 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 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

different organs, including skin, lungs, heart and peripheral 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 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 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.

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 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 $HgCl_2$ can induce systemic autoimmunity including scleroderma in 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 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.

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. 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].

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 (GAD), islet cell antigen-2 (IA2) and islet-specific glucose-6-phosphate catalytic-subunit-related protein (IGRP) 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), Coxsackievirus-B and milk proteins has also been 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 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 Coxsackievirus-B 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 Coxsackievirus could contribute to type 1 diabetes in genetically susceptible individuals [75,76]. 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 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].

3. Biomarkers for neuroautoimmune disorders

Response to injury is often accompanied by protecting 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 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 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 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].

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 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 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 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 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 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, MAG, ganglioside GM_1 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 GM_1, are also detected in patients with Guillain-Barre syndrome (GBS) [97-99]. Molecular mimicry 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 peripheral neuropathies [102].

Amyotropic 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 underlying motor neuron degeneration [106].

The unique clinical characteristics of the pediatric autoimmune neuropsychiatric disorder associated with the group A streptococcal infection (PANDAS) subgroup are the presence of volumetric changes in the basal ganglia, increased titers of 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 lysoglanglioside, and intracellular antigens such as tubulin, which have been described in a subgroup of patients with PANDAS [107-110]. The detection of antibodies against a panel of antigens in a subgroup of patients with PANDAS may justify antibiotic 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 antibodies 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 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 treatment modalities from the ones discussed above.

An example of environmental factors inducing neuroautoimmunity 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 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 simultaneous detection of antibodies against folate receptor and mycotoxins may further clarify their roles in complicated pregnancies.

4. Search for antibodies as peripheral disease 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 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 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].

The tumor suppressor p53 is a phosphoprotein barley 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 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

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 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 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].

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 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%) 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 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 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 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 autoantibodies in prostate cancer [133]. In one study a decision tree 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 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 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 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 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].

5. Protective and pathogenic roles for antibodies

The presence of one or more types of autoantibody in the blood is a common characteristic of autoimmune diseases [1,16,53,137]. The production and presence of antibodies 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 diseases, or destructive, as they are in many other autoimmune 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, 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 antibodies precede the development of full-blown autoimmune 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 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 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 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

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 that anti-DNA antibody crossreaction with NMDA receptors 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 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 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.

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 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 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]. 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 degradation of MBP suggests a mechanistic explanation of the slow development of neurodegeneration associated with neuroimmune disorders.

6. Expert opinion

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" 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 warn of the need to take preventive action' [1].

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 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].

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 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 subsequent production of autoantibodies.

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 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 complicated pregnancies, and heavy metal antibodies in systemic autoimmunity, including scleroderma, are just a few of many cited examples.

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.

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 these antigens would not only address celiac disease beyond the gut, but also may contribute to the clinical specificity and sensitivity of detected antibodies.

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 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

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

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 scientific journals, we and several other specialty clinical laboratories, upon their validation and according to government regulations, have implemented and introduced to clinical use many antibody assays not only for the detection of autoimmune reactions, but also for cardiovascular diseases 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 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 autoantibodies upon therapy might also indicate a beneficial 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 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 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 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.

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 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].

Declaration of interest

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

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