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Antigen arrays for antibody profiling

William H Robinson

Antigen array technologies enable large-scale profiling of the specificity of antibody responses against autoantigens, tumor antigens and microbial antigens. Antibody profiling will provide insights into pathogenesis, and will enable development of novel tests for diagnosis and guiding therapy in the clinic. Recent advances in the field include development of antigen array-based approaches to examine immune responses against antigens encoded in genetic libraries, post-translationally modified proteins, and other biomolecules such as lipids. A promising application is the use of antibody profiling to guide development and selection of antigen-specific therapies to treat autoimmune disease. This review discusses these advances and the challenges ahead for development and refinement of antibody profiling technologies for use in the research laboratory and the clinic.

Addresses
Geriatric Research, Education and Clinical Center (GRECC), Palo Alto VA Health Care System, 3801 Miranda Ave, MC154R, Palo Alto, CA 94304, USA Division of Immunology and Rheumatology, Stanford University School of Medicine, Stanford, CA 94305, USA

Corresponding author: Robinson, William H (wrobins@stanford.edu)

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Introduction

Production of high-affinity, high-avidity antibodies is a hallmark of many autoimmune and infectious diseases. Further, detection of antibodies represents a mainstay in laboratory diagnostics for multiple autoimmune and infectious diseases. For example, detection of blood autoantibodies targeting immunoglobulin (rheumatoid factor) and/or citrullinated peptides contributes to the diagnosis of rheumatoid arthritis (RA) [1], whereas detection of autoantibodies targeting nuclear antigens (antinuclear antibodies [ANAs]) suggests the diagnosis of systemic lupus erythematosus (SLE) [2]. Microbial infections, including infections with Epstein Barr virus (EBV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV), can be diagnosed by detection of host antibody responses against the microbe.

Antigen arrays represent a powerful approach for large-scale characterization of antibody responses against candidate antigens. Antigen arrays provide the ability to identify and characterize antibodies targeting known and novel antigens, and to identify antibody profiles that provide insights into the pathogenesis of disease and provide diagnostic and prognostic utility. This review provides an overview of antigen arrays and highlights several recent applications of antigen array technologies in autoimmune disease, cancer and infectious disease.

Antigen array technologies

A variety of antigen array technologies for antibody profiling have been developed. Proteins and peptides representing candidate autoantigens can be attached to planar surfaces in ordered arrays to survey autoantibody binding [3–5]. Antibody characterization can also be performed using arrays of *in situ* synthesized peptides generated by photolithography [6] or synthesized on pins [7,8]. Arrays of mammalian cells [9] or yeast [10] expressing defined cDNAs, and arrays produced using *in situ* cell-free transcription and translation of cDNA [11], have been described.

To circumvent potential limitations of planar array systems, including drying and alteration of immunologic determinants on planar supports, several companies and academic laboratories are developing fluid-phase assay systems based on labeling antigens with addressable beads [12] (http://www.luminexcrop.com) or other tags [13]. It is anticipated that such fluid-phase systems will become a dominant technology in the coming years. We refer readers to several recent reviews describing these proteomics technologies in detail [4,14–16].

Applications for antigen arrays

Autoimmune disease

Autoimmune diseases affect an estimated 3% of the world population, and arise from aberrant activation of immune responses to target tissues or cells within the body. Examples of autoimmune diseases include RA, in which the synovial joints are targeted; autoimmune type I diabetes, in which β cells in the pancreatic islets of Langerhans are targeted; and SLE, in which a variety of nuclear components are targeted.

Despite knowledge of the specific tissues and cells targeted and the involvement of T and B lymphocytes, for many diseases the specific self-protein (autoantigen) targets remain elusive. Although CD4+ T cells coordinate the attack, multiplex analysis of T cell specificity is complicated by the low frequency of autoreactive

CD4+ T cells [17]. Examples of autoimmune diseases for which autoantigens have been identified and for which autoantibody reactivity provides diagnostic utility include myasthenia gravis, in which the acetylcholine receptor is targeted [18]; bolus skin diseases, in which desmogleins are targeted [19]; and type I diabetes, in which insulin, glutamic acid decarboxylase and IA-2 are targeted. Further, in type I diabetes, combinations of autoantibodies targeting multiple islet antigens are significantly more informative than reactivities against individual antigens [20,21]. Examples of prevalent diseases in which the autoantigens have not been conclusively defined include RA, psoriasis and inflammatory bowel disease.

Important future directions for antigen array profiling of autoantibodies in autoimmune disease include: (i) delineation of autoantibody profiles with diagnostic and prognostic utility; (ii) identification of molecular subtypes of disease based on differences in the specificity of the autoimmune response; (iii) identification of the pre-disease autoantigen targets, thereby providing insights into the mechanisms underlying the initiation of autoimmunity; and (iv) application of antibody profiles to develop and guide antigen-specific tolerizing therapy (discussed below).

Cancer

The observation that many patients with cancer produce antibodies against antigens expressed by their tumors suggests that such antibodies could provide diagnostic and prognostic utility [22–24]. For example, antibodies against the tumor suppressor protein p53 were first reported in 1982 [25] and were subsequently observed in multiple types of cancer [26]. Characterization of profiles of antibodies against panels of tumor antigens has proven more informative than detection of antibodies against individual specificities. For examples, Tan and colleagues [27••] performed enzyme linked immunosorbent assay (ELISA) analysis of antibodies targeting a panel of seven tumor-associated antigens, including c-myc, cyclin B1, IMP1, Koc, p53, p62 and survivin, and demonstrated that presence of antibodies targeting three or more of these antigens in any individual patient was predictive for cancer. Further, it has been observed that certain anti-tumor antibody responses can pre-date the development of clinically detectable disease [28].

Infectious disease

In addition to the detection of anti-pathogen antibodies for the diagnosis of microbial infection, several groups have demonstrated that profiling the breadth and specificity of the anti-pathogen responses can provide insights into immune mechanisms underlying the clearance of and protective immune responses against microbial pathogens. Multiple groups have utilized peptides synthesized on pins to survey the specificity of anti-viral immune responses [7], and we and others have developed antigen arrays for this application [29,30]. Antibody profiling can be applied to: (i) identify anti-microbial antibody specificities with diagnostic value for infection; (ii) characterize antibody responses associated with protective immune responses against and ability to clear the infectious agent; (iii) study antibody responses induced by vaccination; and (iv) map epitopes recognized by neutralizing antibodies.

Allergic disease

Allergic and atopic diseases are characterized by IgE antibodies targeting allergens. Several groups have described development of allergen arrays to profile the specificity of allergen-specific IgE antibodies [31,32].

Strategies for antigen discovery

A major limitation for most antigen array analyses is that the utilized antigens are limited to proteins and other biomolecules that are known to represent candidate targets, and for which synthetic or purified preparations are available. Further, for many autoimmune, malignant and infectious diseases the antigens remain poorly characterized. It is estimated that an individual cell expresses approximately 10 000 proteins [33]. The ability to produce or purify the constellation of polypeptide products representing the proteome of a cell or tissue represents a tremendous technical challenge. Great need exists to apply discovery approaches to identify novel antigen candidates, and a variety of approaches are now being utilized (Table 1).

Once candidate autoantigens are identified, protein array technologies can be used to screen candidate antigens to determine the sensitivity and specificity of individual, and combinations of, antibody reactivities against candidate antigens in cohorts of diseased and control patients.

Mass spectrometry

Mass spectrometry can be applied to identify antigens bound by antibodies present in blood, synovial fluid, spinal fluid or diseased tissues of patients with autoimmune, infectious or malignant diseases.

Genetic expression libraries

Phage, bacterial or mammalian cell-based cDNA and peptide expression libraries represent a strategy to identify novel autoantigens. An important limitation of this approach is the lack of post-translational modifications. Post-translational modifications including citrullination (deimination) and glycosylation are targets in RA [1,34], while epitopes generated by polypeptide cleavage and phosphorylation are targets in SLE [35]. Post-translational glycosylation of antigens also represents an important target of anti-viral responses against HIV and influenza [36]. As described above, several groups have developed methods for generating antigen

Table 1

Approaches to discover novel antigens.

Approach	Advantages	Disadvantages
Mass spectrometry	Identifies polypeptides Identifies post-translational modifications	Requires immunoblotting or immunoprecipitation of antigen Requires picomole quantities of antigen in samples
Genetic expression libraries	High complexity (up to 1013 elements) Can generate from cDNA isolated from diseased tissue or pathogen	Lack post-translational modifications Polypeptides are represented at varying frequencies
Tissue fractionation	Enables analysis of native proteins, including post-translational modifications	Fraction and antigen quantities are limited Must couple with MS or another antigen identification method

arrays representing the expressed products of genetic libraries.

Tissue fractionation

Column-based fractionation of diseased tissues for printing on antigen arrays also represents a powerful strategy for identification of novel autoantigens. A major advantage of this approach is that post-translational modifications are represented in the fractionated antigen pools. When antibody reactivity is detected against specific tissue fractions, then immunoprecipitation, immunoblotting and/or mass spectrometry studies are employed to identify the reactive antigen species.

Vignettes of antibody profiling to develop diagnostics and therapeutics for autoimmunity and cancer

Antibody and cytokine profiling identify molecular subsets of RA

We developed 'arthritis arrays' containing the putative autoantigens in RA. Arthritis microarrays revealed targeting of citrullinated proteins in a subpopulation of RA patients possessing clinical and laboratory features predictive of more severe arthritis [37•] (Figure 1). We also performed multiplex analysis of blood cytokines in RA using a bead array. Integration of blood autoantibody and cytokine profiles revealed distinct subtypes of RA (W Hueber and W Robinson, unpublished data). We identified a 'high-inflammatory' subtype characterized by anti-citrulline autoantibodies and elevated levels of blood cytokines, which is associated with clinical and laboratory features predictive of more severe arthritis. By contrast, a 'low-inflammatory' subtype is characterized by autoantibodies targeting native epitopes and low or undetectable blood cytokines, and is associated with features predictive of mild arthritis.

Antigen arrays guide antigen-specific therapy for autoimmune disease

One of our primary objectives is to use proteomic analysis of autoimmune responses to develop and select antigen-specific tolerizing therapies to treat autoimmune disease [4]. Antigen-specific therapies specifically inactivate the autoreactive lymphocytes mediating tissue injury, thereby preserving global immune function. We have developed and applied myelin antigen arrays to profile autoantibody responses in a rodent model for multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE). We further developed tolerizing DNA vaccines [38] based on the specificity of anti-myelin autoantibody responses identified with myelin arrays, and demonstrated that antigen-specific tolerizing vaccines encoding a greater number of myelin array-identified autoantigen targets provide superior efficacy in treating established autoimmune demyelination in mice [39••]. We termed this strategy 'reverse genomics'. These data suggest that autoantibody profiles can be used to guide development and selection of more efficacious tolerizing DNA vaccines. We anticipate that this strategy can also be applied to facilitate development and selection of other antigen-specific therapies, including peptide- and protein-based tolerizing therapies.

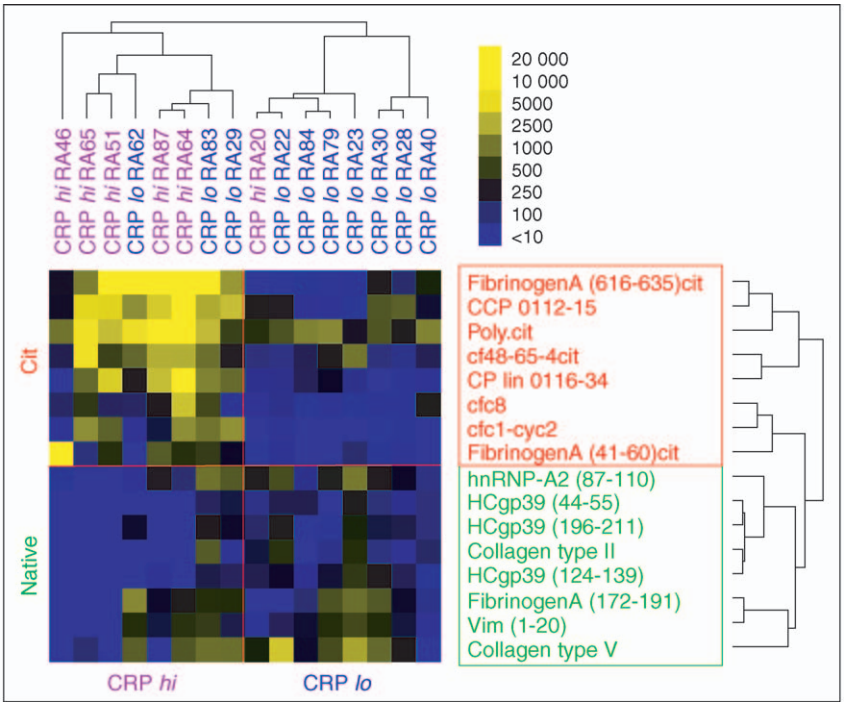
Diagnostic antibody profiles for prostate cancer

A phage display library derived from prostate cancer tissue was recently screened using an antigen array format to identify antigens targeted by antibody responses in prostate cancer patients [40••]. Twenty-two phage-encoded peptides, of which only four were derived from known in-frame coding sequences, were identified as targets of the antibody response in prostate cancer. Blood antibody reactivity against these 22 peptides provided 82% sensitivity and 88% specificity for the diagnosis of prostate cancer.

Lipid microarrays identify anti-lipid antibodies in multiple sclerosis

Recently, we and others have developed microarrays of lipids and carbohydrates for analysis of antibody responses in autoimmune and infectious diseases [41•,42]. SLE and Guillain Barré syndrome patients have autoantibodies to self-glycolipids and -gangliosides [43], demonstrating that autoimmune and autoantibody responses can be directed against lipids. Lipids comprise 70% of the myelin sheath, and several studies demonstrated increased anti-ganglioside antibody and T cell reactivity in MS patients versus controls [44]. We adapted a Camag ATS4 TLC (thin layer chromatography) Sampler to print myelin and other lipids in ordered arrays.

Figure 1



'Arthritis arrays' identify autoantibody profiles that stratify RA patients into low and high inflammatory subgroups. We developed 'arthritis arrays' containing the putative autoantigens in RA. Arthritis antigen microarrays revealed autoantibody targeting of citrullinated proteins in a subpopulation of RA patients possessing clinical and laboratory features predictive of more severe arthritis, including increased levels of the c-reactive protein (CRP) inflammatory marker. We previously published this figure in our manuscript describing development and application of arthritis arrays [37•]. Reproduced with permission. Copyright 2005, John Wiley and Sons, Inc.

We generated lipid microarrays containing 50 distinct brain and myelin lipids including cerebrosides, sulfatides, gangliosides, cholesterol, phosphatidylcholine, oxidized forms of cholesterol and phosphatidylcholine, and microbial lipids including lipopolysaccharide and lipoteichoic acid. We applied myelin arrays to profile anti-lipid antibody responses in cerebral spinal fluid (CSF) derived from a cohort of 20 MS and 10 other neurologic disease control patient samples [41•]. This MS cohort contains predominantly relapsing remitting MS (RRMS) patients, and a few primary progressive MS (PPMS) and secondary progressive MS (SPMS) patients. Our studies demonstrated that anti-lipid antibodies against sulfatides, 3b-hydroxy-5a-cholestan-15-one (an oxidized form of cholesterol), and oxidized phosphatidylcholine were increased in CSF derived from MS patients as compared to controls [41•].

Conclusions

Major progress is being made towards developing and refining antigen array technologies for profiling antibody responses in autoimmune, malignant and infectious diseases. Profiles of antibody reactivities are anticipated to provide superior diagnostic and predictive value as compared to individual specificities. Significant work remains to elucidate and define the antigen targets in a variety of autoimmune, infectious and malignant diseases. Major challenges also remain in the refinement, validation and establishment of good laboratory practice (GLP) antibody profiling assays for use in the clinic. It is anticipated that, in the coming decades, antibody profiling will become a mainstay for diagnosis, assessing prognosis, and guiding therapy for autoimmune and allergic diseases, and also possibly for infectious and malignant diseases.

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