ARTHRITIS & RHEUMATISM
Vol. 46, No. 4, April 2002, pp 885–893
DOI 10.1002/art.10129

REVIEW

Proteomics Technologies for the Study of Autoimmune Disease

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Introduction

Autoimmune disease affects 3% of the US population, and likely a similar percentage of the population of the industrialized world (1). Although remarkable progress toward understanding immune function has been made over the last 4 decades in terms of the role of the major histocompatibility complex and the nature of lymphocyte antigen receptors that confer specificity to autoimmune responses, understanding of the underlying dysregulation and autoimmune response specificity remains limited. For certain autoimmune diseases, including Sjögren's syndrome and systemic lupus erythematosus (SLE), candidate autoantigens have been identified but their exact roles in the initiation, perpetuation, and pathophysiology are not well understood. For other autoimmune diseases, including rheumatoid arthritis (RA) and psoriasis, the targeted autoantigens remain unidentified despite extensive experimental efforts. Array and other multiplex screening technologies represent powerful tools for studying the pathophysiology and specificity of autoimmune responses.

The advent of DNA microarray technology during the last decade has led to an explosion of studies aimed at identifying novel messenger RNA (mRNA) transcripts, or patterns of transcripts, that are transcriptionally up- or down-regulated in association with a particular disease or phenotype. As the availability and costs of such "DNA chips" improve, it is anticipated that transcriptional profiling will gain even greater prominence in autoimmune disease research. "Spotted" DNA microarrays are now available at many university and industry laboratories and are providing a wealth of information regarding the underlying pathophysiology of autoimmune disease (2). However, use of RNA transcriptional profiling has important limitations and is likely unable to provide the comprehensive understanding of autoimmune processes that would be necessary to develop next-generation therapies.

RNA transcriptional profiling alone is an inadequate method for studying human autoimmune disease, for several reasons. First, diseases manifest not at the level of RNA transcription, but rather at the level of the protein. Second, there is a frequently nonpredictive correlation between RNA expression and protein expression and function (3,4). Messenger RNA undergoes a variety of processing events that can profoundly affect cell phenotype yet are not revealed in current transcriptional profiles. For example, mRNA encoding certain apoptosis-regulatory molecules exists in 2 or more alternative splice forms encoding proteins with opposing functions (e.g., proapoptotic isoforms such as Bcl-x_S and protective isoforms such as Bcl-x_L) (5). Translation of mRNA into protein is also regulated by translational regulatory elements such as 3′ mRNA AU–rich elements and by addition of poly(A) tails of various lengths (6–8). Third, protein function can be regulated by posttranslational modifications by enzymes such as kinases or proteases. Finally, autoimmune responses are regulated by autoantigen-specific T and B lymphocytes expressing distinct and heterogeneous antigen receptors that are not easily examined by transcriptional profiling.

Many of these limitations can be circumvented by direct study of the expression and function of proteins encoded by these RNA transcripts. The large-scale study of the expression, function, and interactions of proteins expressed in a tissue or organism is termed "proteomics" (9). With our entrance into the "post-genomics era," it is essential to develop novel tools with which protein

Dr. Robinson's work was supported by a Howard Hughes Postdoctoral Research Fellowship for Physicians Award, NIH grant K08-AR-02133, and an Arthritis Foundation chapter grant. Dr. Steinman's work was supported by NIH grants 5-R01-NS-18235 from the National Institute of Neurological Disorders and Stroke and U19-DK-61934. Dr. Utz's work was supported by NIH grants K08-AI-01521 and U19-DK-61934, an Arthritis Foundation investigator award, a Bio-X grant, and a Baxter Foundation career development award.

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Submitted for publication June 20, 2001; accepted in revised form September 24, 2001.

expression and protein–protein interactions can be explored. This report will review proteomics technologies under development, with a focus on human autoimmune disease. Readers interested in more comprehensive descriptions of proteomics are referred to several excellent reviews by other authors (9–13).

Recent advances in the detection of protein—protein interactions

The ideal assay for detecting proteins and their interactions should be sensitive, specific, and reproducible. Many such assays are already available, including Western blotting, 2-dimensional gel electrophoresis, and enzyme-linked immunosorbent assay (ELISA). However, these assays are not amenable to "multiplex analysis" whereby one can simultaneously screen thousands of individual proteins for their ability to interact with other molecules. Other techniques, including microfluidics- and mass spectroscopy–based approaches, are under active development but are unlikely to be widely available to academic and industry researchers in the near future. We will briefly describe these techniques before focusing the remainder of the review on spotted antigen microarray technology, a technique that is relatively straightforward and utilizes simple protocols and widely available equipment. We will specifically discuss the application of antigen arrays for the study of autoantibodies as well as for development and selection of antigen-specific therapies for autoimmune disease.

A commonly utilized method in proteomics is the separation of complex mixtures of proteins (e.g., cell lysates prepared from cell lines, primary cell cultures, or tissue) by 2-dimensional gel electrophoresis. Individual proteins are visualized by staining (silver or other staining methods) or by Western blot analysis using an antibody or autoimmune serum sample. This approach is readily amenable to identification of novel reactivities, such as the discovery of new autoantigens recognized by serum from patients with autoimmune disease, and it is fairly easy to perform by laboratory personnel familiar with basic biochemistry. Moreover, the technique is now highly standardized and facilitates purification of reactive proteins by excising a particular reactive spot and subjecting it to amino acid sequence analysis, mass spectroscopy, or tandem mass spectroscopy. A major drawback is that the identity of a reactive protein is not immediately known based on its position on the gel (see below), and the limits of detection are below those governing the utility of other techniques (14).

Genetic plaque- and colony-based strategies provide a powerful approach but frequently require large quantities of patient samples and cannot be used to identify posttranslational protein modifications. A variation of this strategy involves "living arrays," utilizing a modified yeast 2-hybrid system (15), to identify protein–protein interactions (16). Such "living arrays" can detect protein–protein interactions in living eukaryotic cells, using a genetic screen that produces a functional readout (16). This method has the distinct advantage of being more sensitive than traditional library screening methods, allowing dissection of biochemical pathways such as signal transduction and enzymatic pathways, and identification of components of multi-subunit complexes. Application of this approach to identify components of autoantigens, many of which exist in complex with other proteins or RNA molecules (17), will undoubtedly be attempted.

Microfluidics approaches have also been developed, to enable high-throughput screening of biomolecular interactions. Microfluidics utilizes microchannels etched in a solid support to mix solutions containing distinct biologic and/or other molecules, in order to study the interactions of these molecules. Small volumes of reactants (e.g., proteins, drug candidates, nucleic acids, biologic fluids) are subjected to electrokinetic flow in a network of channels such that small "plugs" of individual reactants are brought into contact with one another within the network (18,19). Binding events are observed as changes in the electrophoretic mobility of the reactants (e.g., retarded mobility of an interacting pair), which is usually observed by ultraviolet absorption or fluorescent detection (20). Microfluidics channels can be fabricated in plastic, silicon, or other materials, using lasers or photolithographic masks. A "microfluidic tectonics" platform that takes advantage of many recent advances in polymer chemistry, fluidics, and hydrogel development has recently been described (21).

Fluidics approaches have several advantages over spotted antigen array technology and other multiplex proteomics techniques. With fluidics techniques, interacting species are in solution phase at all times, eliminating the binding of antigen to a surface (as part of a well, matrix, or planar surface) that may disrupt important immunologic epitopes. Fluidics approaches enable real-time millisecond quantitation of binding kinetics. In addition, they have the potential to enable sensitive detection of low-affinity biomolecular interactions that is not possible with other proteomics technologies including surface plasmon resonance technologies, such as Biacore. Fluidics methodologies are amenable to multiplex analysis through design of complex channel networks and use of microfabricated electrokinetic channels to direct flow and mixing of all possible

Table 1. Protein and antigen array technologies

Array type	Arrayed elements	Surfaces	Detection	Applications	Refs.
Proteins, polypeptides, and peptides in spatially addressable arrays	Proteins, polypeptides, peptides, antibodies	Derivatized glass, membranes	Fluorescence, chemiluminescence, radioactivity	Detection of protein–protein, protein–macromolecule, protein–small molecule, antigen–antibody, and enzyme–substrate interactions	32,35–38,65
In situ–generated spatially addressable arrays: 1) expressed polypeptides 2) photolithography-generated	Polypeptides, peptides	Derivatized glass, membranes	Fluorescence, chemiluminescence	Detection of protein–protein, protein–macromolecule, protein–small molecule, antigen–antibody, and enzyme–substrate interactions	29,66
Living cell spatially addressable arrays	Live cells expressing polypeptides encoded by transfected expression vectors	Gelatin-coated glass, agar, membranes	Fluorescence, colorimetric	Detection of protein–protein, protein–macromolecule, protein-small molecule, antigen-antibody, and enzyme-substrate interactions	16,67
Particle arrays	Antigens, antibodies	Beads and nanoparticles	Fluorescence, microscopic	Detection of protein–protein, protein–macromolecule, protein–small molecule, and antigen–antibody interactions	22
Microfluidics	Micro-channels for analysis using electrokinetic flow	Channels etched in glass, acrylic, or other solid supports	Fluorescence, ultraviolet absorption	Multiplexed analysis of binding interactions	18,19

combinations of molecules introduced into the network. Several companies are developing "lab-on-a-chip" microfluidics technologies for multiplex proteomic analysis (see www.aclara.com and www.calipertech.com), which may become available to academic and industry laboratories in the future. A demonstration of this technology can be found on the World Wide Web at http://www.stanford.edu/group/microfluidics/.

One particularly attractive fluid-phase platform was developed by Aclara, Inc. (Mountain View, CA). Cleavable fluorescent reporters with unique electrophoretic mobilities are used to identify and quantify binding interactions between molecules, including proteins, using widely available capillary electrophoresis equipment (see www.aclara.com).

Several groups are developing addressable nanoparticle-based assays for multiplex analysis of RNA transcripts, proteins, and antibodies. The Luminex system involves use of 64 distinct sets of spectrally resolvable fluorescent beads (22). Each fluorescently addressable set of beads is conjugated to a different antigen, antibody, or oligonucleotide, incubated with the test sample, and then analyzed using a benchtop flow cytometer (22). Nanoparticles or beads can also be uniquely tagged using cylindrical metal nanoparticles that serve as "nanobarcode identification tags" (see www.surromed.com) and other addressable tag–based systems. Others have developed bead-based fiber-optic arrays for transcriptional profiling (23). Bead-based approaches have the potential to enable arrays of significantly greater complexity than the 10^5 order of complexity that is possible when using centimeter-size planar surfaces. Bead-based methodologies are being refined and developed for use as large-scale peptide and protein arrays. Protein and antigen array technologies currently under development are listed in Table 1.

Antigen microarray technologies

The remaining discussion will focus on methods for partitioning proteins at spatially resolvable positions, either in wells, within 3-dimensional matrices, or on a planar surface, unlike the methods described above. The position and identity of each immobilized protein is known, and the miniaturized format enables parallel detection of thousands of unique proteins using submicroliter quantities of human serum. This provides a distinct advantage over other techniques, such as phage display or 2-dimensional gel electrophoresis, in which the protein or peptide that is detected is unknown and thus requires further analysis for identification (14,24). In the late 1980s and early 1990s, Ekins and colleagues

described a "multi-microspot multianalyte immunoassay" (25–27). Recently, several groups have reported significant advances in the development and application of protein and antigen array technologies, as described below.

One simple method for performing parallel detection of protein–protein interactions is with the use of microtiter plates (e.g., by ELISA). Several groups have begun using smaller and smaller wells (e.g., 384 or 1,536 well plates) for spatial resolution, while others have developed arrays of microwells etched into silicon wafers (13,28). Such technologies enable reactions to occur in fluid phase within individual wells.

Various methods have been developed to fabricate arrays by depositing samples at distinct and addressable locations on solid surfaces, including "stamping," ink jet application, capillary spotting, and synthesis of molecules directly on the array using photolithographic masks or maskless fabrication with digital micromirrors (13,29–31). Fodor and colleagues described use of patterned, light-directed combinatorial chemical synthesis for the generation of peptide and oligonucleotide arrays (29). Ge developed a "Universal Protein Array" (UPA) by applying proteins to nitrocellulose membranes in order to detect molecular interactions between proteins, as well as interaction of proteins with chemical ligands, DNA, or RNA (32). Disadvantages of this UPA system include its requirements for relatively large quantities of purified antigen for spotting and samples to probe the arrays, as well as use of radioisotope-based detection. Others have developed and evaluated a "line immunoassay" in which antigens are deposited at discrete locations on strips of membrane, enabling consistent and simultaneous detection of multiple autoantibody species (33).

Figure 1 depicts a robotic capillary arrayer similar to the one developed in the laboratory of Dr. P. O. Brown to fabricate DNA microarrays (34,35). Several groups, including MacBeath and Schreiber at Harvard University (36) and Haab, Dunham, and Brown at Stanford University (35), developed methods utilizing such robotic arrayers to deposit proteins onto microscope slides in order to fabricate high-density protein arrays. The protein arrays described by these investigators contain hundreds of different proteins attached to the surface of microscope slides, where they are analyzed for interactions with other proteins, enzymes, or drugs.

MacBeath and Schreiber demonstrated that simple protein arrays can be used to detect interactions of immobilized proteins with antibodies, with cellular proteins such as p50 nuclear factor κB, and with protein–drug complexes such as FK-BP12 and rapamycin (36). They also demonstrated use of protein arrays to detect the enzymatic activity of kinases, with a panel of specific immobilized protein substrates. Haab et al characterized the reactivities of 115 antibody–antigen pairs, demonstrating that 50% of arrayed antigens and 20% of immobilized antibodies could be robustly detected in this format (35). A small subset of antibodies was capable of detecting antigens in complex solutions at

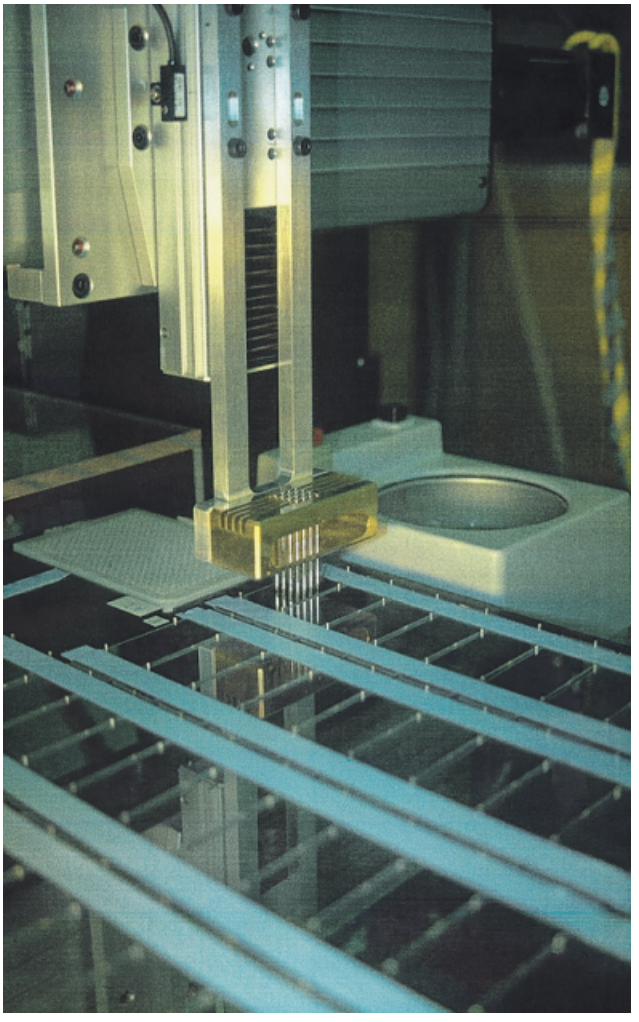

Figure 1. Robotic microarrayer used to fabricate antigen microarrays. A robotic microarrayer, similar to the one pictured, was developed by Brown and colleagues to fabricate DNA microarrays and, more recently, protein microarrays (34,35) (see http://cmgm.stanford.edu/pbrown). Microcapillary spotting pins draw up antigen solutions contained in microtiter plates and deposit each solution at a distinct location on successive microscope slides. The spotted antigens attach to the microscope slides electrostatically or covalently, or are physically entrapped based on the surface chemistry of the slides. Commonly used slide surface chemical materials include poly-L-lysine coatings, gelatin coatings, acrylamide coatings, and silane-treated glass slides.

levels that would allow their use in certain clinical applications, including detection of human serum proteins present at ng/ml quantities. Examples include markers of ischemic injury (troponin, creatine kinase MB), malignancy (prostate-specific antigen), and certain cytokines (tumor necrosis factor α, interleukin-1 [IL-1]) present at relatively high concentrations. Unfortunately, most cytokines are present at pg/ml or lower concentrations (IL-4, IL-10) and are thus not amenable to detection with current spotted antibody array systems. The spotted antigen arrays developed by Haab et al enabled sensitive detection of purified antibodies and antigens diluted into complex solutions of proteins.

Joos et al described the construction of autoantigen microarrays containing 18 prominent autoantigens spotted onto surfaces including silane-treated glass slides and nitrocellulose (37). Their arrays proved to be sensitive and specific for detection of autoantibodies to many of the spotted antigens, with as little as 40 fg spotted protein still detectable for one of the protein standards used. Bound antibodies were visualized using a secondary antibody conjugated to horseradish peroxidase prior to addition of a luminescent substrate, and imaged using a charge-coupled device chemiluminescence camera.

To date, comprehensive autoantigen microarrays that are capable of fluorescence detection of human autoantibodies present in biologic fluids such as serum, cerebrospinal fluid (CSF), or synovial fluid have not been described. We therefore developed and refined protein microarray technology to study the specificity of the autoantibody response in murine and human autoimmune diseases (38). Using a protocol modified from those recently described by Haab et al (35) and MacBeath and Schreiber (36), we used the robotic arrayer depicted in Figure 1 to attach peptides, proteins, nucleic acids, and protein complexes to distinct and addressable locations on microscope slides. At each antigen feature ~1 nl of antigen solution is deposited, producing a relatively uniform feature measuring 150 μm in diameter. Individual arrays are incubated with serum from autoimmune disease patients and controls, washed, and incubated with secondary antibodies covalently conjugated to spectrally resolvable fluorescent labels (such as Cy3 or Cy5). Alternatively, comparative analysis can be performed by incubating arrays simultaneously with a reference and disease serum sample, each directly labeled with a distinct, spectrally resolvable fluorochrome as described by Haab et al (35). The slides are analyzed using a fluorescence-based digital scanning system. Antigen microarrays use simple protocols and basic spotted DNA array equipment available at many academic medical centers and industry laboratories. Detailed protocols to fabricate and conduct antigen microarray studies are presented in our report (38) and can be found on the World Wide Web at http://www.stanford.edu/group/antigenarrays.

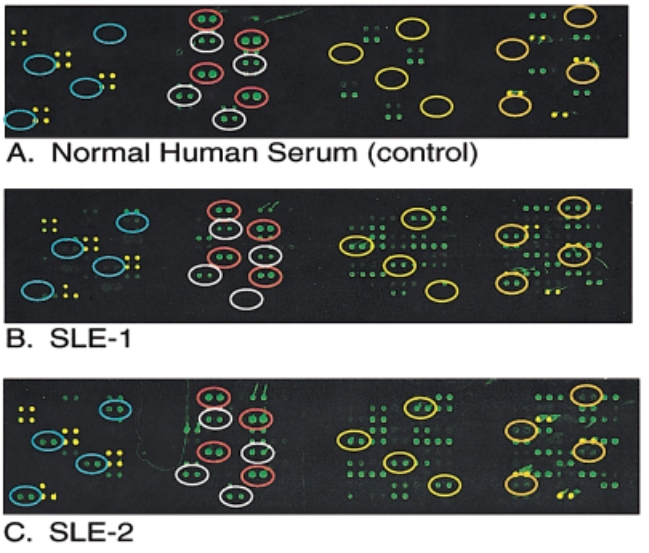

Figure 2. Antigen array detection of autoantibodies specific for common autoantigens in systemic lupus erythematosus (SLE). Protein arrays were fabricated by spotting common SLE antigens including DNA, histone 2-A protein, and serine/arginine (SR) proteins onto poly-L-lysine–coated glass microscope slides using a robotic microarrayer (34,35,38) (see Figure 1). Arrays were incubated with patient serum followed by secondary antibody covalently conjugated to a spectrally resolvable fluorescent label, and then scanned with a GenePix 4000B Array Scanner (Axon Instruments, Union City, CA). Shown are scanned images of arrays incubated with serum from a healthy control subject (**A**) and 2 SLE patients (**B** and **C**), followed by Cy3-labeled anti-human Ig secondary antibody. In SLE patient 1, autoantibodies specific for DNA (yellow circles) and SR proteins (orange circles) are detected. SLE patient 2 has autoantibody reactivity primarily against DNA (yellow circles) and histone 2-A protein (blue circles). The array-determined autoantibody profiles in these 2 SLE serum samples and the control sample are in exact concordance with the specificities previously determined using traditional methods including Western blot, enzyme-linked immunosorbent assay, and immunoprecipitation analysis (68). Antibodies specific for influenza A virus vaccine are detected in all samples (white circles). Spotted antibodies specific for human IgG detect the presence of IgG in each sample (red circles). Full details of the methods used to fabricate, probe, and analyze these antigen microarrays are presented in ref. 38 and on the World Wide Web at http://www.stanford.edu/group/antigenarrays and http://cmgm.stanford.edu/pbrown.

An example of use of our first-generation antigen arrays to characterize the specificity of the autoantibody response in human SLE patients is presented in Figure 2. More recently, we have expanded our antigen arrays

to include ~200 distinct protein, peptide, nucleic acid, and protein complex antigens targeted to humans with various autoimmune diseases including RA, SLE, polymyositis, limited and diffuse scleroderma, primary biliary sclerosis, and Sjögren's syndrome (38). We have also generated "myelin proteome" arrays containing >500 proteins and peptides derived from the myelin sheath that are targeted in multiple sclerosis (MS) and its animal model (experimental autoimmune encephalomyelitis [EAE]). We are using these arrays to characterize specificity and epitope spreading and to guide selection of antigen-specific therapies in EAE (Robinson WH, et al: unpublished observations). We are also using our myelin proteome arrays to characterize the specificity of the autoantibody response in CSF and brain plaque–eluted antibody from humans with MS.

A distinct advantage of our antigen array approach is that posttranslational modifications can be readily detected. Casciola-Rosen et al and others have proposed that such modifications may be important for initiating autoantibody formation, and this technique may be of use in further exploring this possibility (39–42). Furthermore, antigen arrays enable repeat multiplex analysis of the specificity of the autoantibody response against the same panel of putative autoantigens in many consecutive patients.

The human genome encodes between 26,000 and 120,000 proteins, not counting multiple isoforms or posttranslationally modified proteins (43). To produce, purify, and spot every protein on individual arrays is a daunting task. An interesting solution to this problem has been proposed by Walter et al (13) and was presented at the February 2001 meeting of the Association for Biomolecular Resource Facilities. These investigators have taken advantage of preexisting complementary DNA (cDNA) libraries used for transcriptional profiling, to produce polypeptides for microarray analysis (44,45). In one format, His-tagged proteins are expressed in liquid bacterial cultures, purified using nickel chromatography, and applied to a polyvinylidene difluoride surface using ink-jet technology prior to probing the array with an antibody solution. The investigators report robust detection of as little as 10 pg of a test protein (GAPDH), demonstrating the potential feasibility of this technology for large-scale production of protein microarrays using existing cDNA libraries (13). They are applying this technology as an antibody specificity screen to identify novel autoantigens targeted by autoantibodies in the sera of patients with Crohn's disease and ulcerative colitis (13).

Future directions

Application of proteomics technologies for diagnosis. One potential application of protein array technology is in clinical diagnostic testing. Most current rheumatologic tests rely on ELISA-based methodology in which individual tests (e.g., for autoantibodies against Sm, Ro, or RNP) are performed on multiple different serum samples within individual wells in the same microtiter plate (17). Although the transition may take years to decades, antigen microarrays, whether on planar surfaces or in other formats that are yet to be described, will enable multiplex parallel testing that will most likely replace currently utilized autoantibody testing methods. As with any new diagnostic test, more extensive validation using thousands of serum samples and demonstration of consistent and reproducible results will be necessary before this technology reaches the clinic. Moreover, certain autoantigens are not amenable to this detection format (Robinson WH, et al: unpublished observations), suggesting that continued efforts to optimize antigen production and purification, planar surface chemistry, fluorescence detection and quantitation, and analysis of complex data sets are needed.

Use of proteomics technologies to study the specificity and pathophysiology of autoimmune responses. The greatest immediate impact of proteomics technology in the field of rheumatology will be in the study of autoantibodies. This technology allows the rapid, simultaneous detection of thousands of autoantibody reactivities using <1 μl of serum per array. We are currently using protein and peptide microarrays to study inter- and intramolecular epitope spreading of the humoral immune response over time, both in animal models of autoimmune disease and in cohorts of humans with "early arthritis" (46,47). We are expanding our arrays to include autoantigens from many additional autoimmune diseases, including RA, diabetes mellitus, and autoimmune diseases of the skin. We are using this technology to determine the isotype subclass of antibodies bound to individual antigen features. It is known that Th1 cytokines (such as IL-12 and interferon-γ) favor the production of pathogenic, complement-fixing antibodies such as IgG1 and IgG3 (in humans), while Th2 cytokines (such as IL-4 and IL-10) promote class switching to generate non–complement-fixing IgG4 and IgE antibodies (48,49). Because most studies address global changes in antibody isotype levels and not reactivity to individual antigens or epitopes, this technology represents a powerful approach to elucidating the roles of Th1 and Th2 cells (and newly discovered Be1 and Be2 B lymphocytes)

in the generation of antigen-specific B and T lymphocyte autoreactivity (50).

Several reports have described transcriptional profiles of tissue from patients with autoimmune diseases, including rheumatoid synovium and brain plaques from patients with MS (51–54). Interesting findings have been culled from these large data sets, and the stage is now set to correlate these findings with the results of proteomic analysis from the same tissues. Such an analysis will certainly validate the importance of a subset of such transcripts in disease pathogenesis, while also identifying many other proteins as having important pathogenic roles. These novel proteomics technologies will enable complex analysis of protein levels within and between cells (e.g., comparing transcriptional and protein profiles of distinct lymphocyte populations), as well as parallel analysis of cytokine and chemokine levels in biologic fluids. Such studies could identify and further define critical signaling pathways involved in disease pathogenesis, cell surface receptors that may serve as novel targets of drug discovery, and proteomic characteristics of lymphocytes that are anergic, autoreactive, or regulatory. The powerful combination of genomics and proteomics has the potential to change forever how we study the basic biology of autoimmunity.

Application of proteomics technologies to drive development and selection of antigen-specific therapies for autoimmune disease. We propose use of autoantigen arrays to drive development and selection of antigen-specific therapies for human autoimmune disease. Conventional methods for determining the specificity of autoimmune responses, including T cell proliferation, ELISA, and radioimmunoassay analysis, do not enable large-scale determination of the specificity of autoimmune responses in individual patients. Antigen arrays allow simple parallel, multiplex determination of the specificity of autoimmune responses in individuals and cohorts of patients. Thus, we propose administration of antigen-specific therapeutic agents, defined by the binding specificity of patient autoantibodies. This could be accomplished utilizing a wide variety of different antigen-specific therapies. Antigen-specific therapies involve administration of the targeted autoantigen in a manner that induces immune tolerance to treat autoimmunity. Such therapies include 1) oral administration of antigen to induce "oral tolerance" (55), 2) administration of native peptides via intravenous or other routes (56,57), 3) administration of altered peptide ligands (58), 4) administration of whole protein antigens (59), 5) administration of other biomolecules such as DNA, or proteins and peptides with posttranslational modifications (60), and 6) administration of DNA-tolerizing vaccines encoding the targeted self proteins (61–64).

We anticipate that future antigen-specific therapies based on this strategy will deliver multiple targeted epitopes or protein antigens as tolerizing agents. Such therapies could deliver a consensus dominant targeted epitope or antigen, and could also deliver a cocktail of 20 or more of the consensus targeted epitopes or antigens to treat patients with a specific disease or subset of that disease. We are using antigen arrays to guide development and selection of antigen-specific DNA-tolerizing vaccine therapy, a strategy we have termed "reverse genomics." We are applying our reverse genomics strategy to develop and select antigen-specific DNA-tolerizing vaccines to treat EAE (61–63) and collagen-induced arthritis, a model for RA.

Summary

Array-based autoantibody profiling may provide a long-awaited platform of technology to determine the specificity of autoimmune responses in individuals and cohorts of patients. We believe this technology will drive development and selection of antigen-specific therapies for use in the clinic. It is possible, in a manner parallel to the use of skin testing in the allergy clinic to select desensitization therapy, that antigen arrays could be applied to select customized antigen-specific therapy for individual patients with autoimmune disease. We anticipate that such an antigen array–based strategy will be initially utilized to develop therapies that could be used to treat patients with a specific autoimmune disease and to select patients to receive such therapies. Once antigen-specific therapies prove safe and effective, and if inclusion of the exact set of targeted autoantigens in the therapy proves crucial for efficacy in individual patients, then customized therapies based on selection of agents including the targeted autoantigens in individual patients could be developed in the coming decades.

ACKNOWLEDGMENTS

The authors thank C. DiGennaro and the staff members of Drs. Utz and Steinman's laboratories for critical review of the manuscript.

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