The Use of Microarrays to Study Autoimmunity

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As with the development of any novel and potentially powerful technology, the prospect of revealing new information that may dramatically change our understanding of biological processes can generate much excitement. Such is true for the emerging genomic approaches that make possible high-density assays using microarray platforms. Indeed, it is difficult, if not impossible, to imagine any area of biology that could not be affected by the wide range of potential applications of microarray technology. Numerous examples, such as those from the field of oncology, provide striking evidence of the power of microarrays to bring about extraordinary advances in molecularly defining important disease phenotypes that were otherwise unrecognized using conventional approaches such as histology. However, only a few studies in autoimmunity are available to date. Very recent work in alopecia areata, multiple sclerosis, systemic lupus erythematosus, and Sjögren's syndrome illustrates the potential for gaining new insights into the pathophysiology of these complex autoimmune disorders on a global, molecular scale. These new insights are likely to significantly improve our understanding of disease processes, diagnosis, identification of new therapeutic targets, and identification of patients most likely to benefit from specific and tailored therapies. ***Key words: microarrays, autoimmunity, autoimmune disease, gene expression. J Investig Dermatol Symp Proc 9: 18–22, 2004***

MICROARRAY TECHNOLOGY

Microarray technology provides an unprecedented and uniquely comprehensive probe into the coordinated workings of entire biological pathways and genomic-level processes. In general terms, microarrays refer to a variety of platforms in which high-density assays are performed in parallel on a solid support. Thousands to tens of thousands of datapoints may be generated in each experiment. The growth of scientific literature since the mid-1990s may provide some indication of the potential impact of this technology in the biomedical sciences (**Fig 1**). Of the approximately 1900 articles published through 2002 with reference to microarrays, however, less than two dozen include applications directly investigating autoimmune disease phenotypes. A majority of applications have been in oncology, although many examples from other fields are rapidly emerging and include examination of host response to pathogens, examination of drug responses, identification of temporal changes in gene expression, and comparisons of various experimental conditions.

Three major types of microarrays exist—tissue, protein, and DNA. Tissue microarrays immobilize small amounts of tissue from biopsies of multiple subjects on glass slides for immunohistochemical processing; protein arrays immobilize peptides or intact proteins for detection by antibodies or other means (see below). For the last several years, much excitement and attention has focused on DNA microarrays. Regardless of the specific platform used, these approaches offer new opportunities to address biologic questions in a way never before possible. **Table 1** provides just a few examples of the potential ways in which microarray technology can be utilized.

We provide here a general discussion of the most common microarray technologies and highlight some selected examples of how this approach is being applied to gain insight into immunologic and autoimmune disease processes. For those just entering the microarray arena or interested in more details, a series of particularly useful reviews have recently been published that take stock of the latest developments and discuss the most pressing challenges of this technology (Trent and Baxevanis, 2002).

Autoantigen and cytokine microarrays Applications of protein microarrays include assessment of enzyme–substrate, protein–protein, and DNA–protein interactions. Although efforts to develop these proteomic tools predate the first descriptions of DNA microarrays (MacBeath, 2002), progress has been relatively slower, in part because of challenges posed by natural inherent differences in proteins compared with DNA. As examples, proteins consist of highly diverse conformational structures that result from 20 amino acids versus the 4 nucleic acid building blocks that generate a relatively uniform structure in DNA. Proteins may exist as large complexes; they may be hydrophilic or hydrophobic, acidic or basic; and they may contain post-translational modifications such as acetylation, glycosylation, or phosphorylation. Functional and conformation properties of proteins must often remain intact when immobilized onto a microarray in order to retain the desired binding properties for detection of target ligands.

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Abbreviations: DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; cDNA, complimentary DNA; NIH, National Institutes of Health; RNA, ribonucleic acid; SLE, systemic lupus erythematosus; SS, sjogrens syndrome; MCTD, mixed connective tissue disease; RA, rheumatoid arthritis; AA, alopecia areata; MS, multiple sclerosis.

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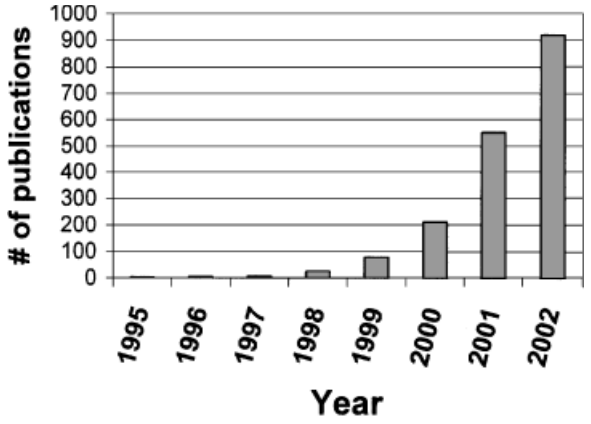

Figure 1. Growth in number of microarray publications. Resulting number of articles retrieved using PubMed searches with the term "microarray" and limited to English are shown for each year.

Table 1. Potential objectives of studies utilizing microarray technology

1. Distinguish patients from normal controls
2. Identify subsets of patients
3. Characterize host responses
4. Examine cellular pathways
5. Compare alternative experimental conditions
6. Examine drug response
7. Follow temporal changes in gene expression
8. Identify candidate genes for genetic studies

The development of protein microarrays to detect immunologic targets such as cytokines or autoantibodies has enormous potential for research and diagnostic applications in autoimmune diseases. Several groups, including Joos and colleagues in Germany (Joos *et al*, 2000) and Robinson and colleagues at Stanford University (Robinson *et al*, 2002), have made important strides in developing autoantigen microarrays for multiplex characterization of autoimmune serum. Joos and colleagues spotted 18 common autoantigens onto silane-treated glass slides and nitrocellulose at serial dilutions. Bound antibodies from minimal amounts of 25 characterized autoimmune serum samples and 10 normal blood donors were titered by using variable amounts of autoantigen. The autoimmune serum samples were obtained from patients with autoimmune thyroiditis (Hashimoto's thyroiditis and Graves' disease), systemic lupus erythematosus (SLE), Sjögren's Syndrome (SS), mixed connective tissue disease (MCTD), scleroderma, polymyositis, systemic vasculitis, and antiphospholipid syndrome. These assays proved to be highly specific and similar in sensitivity when compared to a standard ELISA format. Further developments will include optimizing the nature of the autoantigen material to minimize possible loss of antigenicity and expanding the representation of autoantigens on the array.

Robinson and colleagues developed a 1152-feature array containing 196 distinct biomolecules that represent major autoantigens targeted by antibodies produced by rheumatic autoimmune disease patients (Robinson *et al*, 2002). The autoantigens include hundreds of proteins, peptides, DNA, enzymatic complexes, and ribonucleoprotein complexes. Examples of autoantigens spotted include Ro52, Ro60, La, jo-1, Sm-B/B′, U1-70 kDa, U1 snRNP-C, topoisomerase 1, pyruvate dehydrogenase (PDH), and histone H2A. The arrays were characterized using multiple sera from eight human autoimmune diseases and included SLE, SS, MCTD, polymyositis, primary biliary cirrhosis, rheumatoid arthritis (RA), and both limited and diffuse forms of scleroderma. This work demonstrates the feasibility of using large-scale, fluorescence-based autoantigen microarrays to detect human autoantibodies with simple protocols and widely available equipment in a low-cost and low-sample volume format. Some of the potential applications for this technology include (1) rapid screening for autoantibody specificities to facilitate diagnosis and treatment; (2) characterization of the specificity, diversity, and epitope spreading of autoantibody responses; (3) determination of the isotype subclass of specific autoantibodies; (4) guiding the development and selection of antigen-specific therapies; and (5) use as a discovery tool to identify novel autoantigens or epitopes.

Microarrays that simultaneously detect multiple cytokines have been developed by Huang and colleagues at Emory University (Huang *et al*, 2001). Their method utilizes captured antibodies spotted onto membranes, incubation with biological samples such as patient serum, and detection by biotin-conjugated antibodies and enzymatic-coupled enhanced chemiluminescence. Twenty-eight cytokines have been detected using this method, including interleukins-1a, 2, 3, 5, 6, 7, 8, 10, 13, and 15; tumor necrosis factors α, β, and $\beta 1$; interferon-γ, and others. In addition to detecting multiple cytokines simultaneously, these assays were shown to be more sensitive than conventional ELISAs, with broader detection ranges. The ability to readily scale up this approach to include much larger numbers of cytokines and other proteins will undoubtedly fuel further development of this powerful tool for studying complex and dynamic cellular processes such as immune reactions, apoptosis, cell proliferation, and differentiation.

DNA and oligonucleotide microarrays DNA microarrays were first introduced in the mid-1990s (Schena *et al*, 1995) and have been the most widely utilized application of microarray technology. There are two commonly available DNA microarray types. First are the cDNA microarrays fabricated by robotic spotting onto glass slides of PCR products derived primarily from the 3′ end of genes and expressed sequence tags (EST). This is the method popularized by, among others, Patrick Brown at Stanford and Louis Staudt at the NIH (DeRisi *et al*, 1997; Alizadeh *et al*, 1998). The second type uses *in situ* synthesized oligonucleotide arrays fabricated using photolithographic chemistry on silicon chips. This is the method used in the proprietary Affymetrix system (Pease *et al*, 1994). The data generated using these two systems are highly concordant, as demonstrated in parallel studies of the yeast cell cycle (Cho *et al*, 1998; Spellman *et al*, 1998). In the spotted cDNA microarray system, two probes with different fluorescent tags are hybridized to the same array, one serving as the experimental condition and the other as a control. The ratio of hybridization between the two probes is calculated, allowing a quantitation of the hybridization signal for each spot on the array. In this system, the probe is first-strand cDNA generated by oligo-dT primed reverse transcription from an RNA sample (for additional details see http://cmgm.stanford.edu/pbrown/). In the Affymetrix™ system, only a single labeled probe is used and each gene on the chip is represented by 8 to 10 wild-type 25-mer oligonucletides and the same number of single-base mutant 25-mer oligonucleotides synthesized next to one another on the array. Signal intensity and the ratio of specific to nonspecific hybridization allows the generation of quantitative data regarding gene expression in the sample (for more details see http://www.affymetrix.com/technology/tech.probe.html).

Data analysis Microarray analysis is often considered discovery based rather than hypothesis driven (Albelda and Sheppard, 2000; Staudt and Brown, 2000), largely because of the potential for discovering altered expression of novel genes for which little or no prior information is available to suggest a role in the disease or experimental condition examined. High-quality experiments, however, are driven by addressing a scientific question (even if it

is simply "Are there genes that are differentially expressed between a group of patients and controls?"), consistency in execution of experimental protocols, use of sample sizes with as many replicates as is feasible, and a plan for statistical analysis and interpretation of the data. Including statistical expertise during the early phase of experimental design (i.e., prior to any data collection) is critical, particularly in the setting of microarray analysis, where each experiment can carry significant cost.

There are generally three approaches to data analysis: prognostic prediction, class discovery, and class comparison (Simon *et al*, 2002). Prognostic prediction methods are used when two or more groups of samples are analyzed and the goal is to develop a model for prediction of class membership for new, additional samples. This approach is common in studies designed to predict various clinical outcomes, such as treatment effectiveness or duration of survival in patients with cancer. Class discovery is often applied to datasets with a heterogeneous sample and the objective is to identify novel subsets of samples that may not otherwise be discernable using conventional techniques such as histological or morphological criteria. Class comparison involves two or more predefined groups, such as patients and controls, for which a function is determined that finds the genes that best discriminate between the groups. For each of these approaches, numerous statistical methods exist, and there is no "one size fits all" for the analysis and interpretation of the complex datasets generated by microarray studies (Slonim, 2002). Data analysis and interpretation are very active areas of research that require synergistic efforts from biologists, computer scientists, and statisticians in order to maximize the full potential of these powerful microarray technologies (Slonim, 2002).

APPLICATIONS OF MICROARRAYS IN AUTOIMMUNE DISEASES – LESSONS FROM CANCER

Over the last few years, there has been an understandably high level of excitement concerning the power of microarrays to radically change the level at which we understand biological systems (Staudt and Brown, 2000). This excitement has been bolstered by many successful applications in which important new insights have been gained, particularly in oncology. Microarrays have been used to identify new candidate genes for prostate cancer (Walker *et al*, 1999), to identify "sets" of genes that function in pathways such as cell proliferation or differentiation (Geiss *et al*, 2000; Ichikawa *et al*, 2000), and to distinguish previously unrecognized subtypes of clinical disease (St. Croix *et al*, 2000, Alizadeh *et al*, 2000). For example, underlying molecular heterogeneity in lymphoid malignancies has been revealed by Staudt and colleagues at NIH (Alizadeh *et al*, 1998; Eisen *et al*, 1998; Alizadeh *et al*, 2000). Gene expression profiles were identified that revealed two molecularly distinct forms of diffuse large B cell lymphoma (DLBCL) representative of different stages of B cell development. These tumors were clinically indistinguishable prior to microarray analyses. Importantly, the subset of patients bearing tumors with the germinal center B-like signature had a better overall survival than patients with the activated B-like DLBCL profile.

Other examples of improved or previously impossible classification of groups of heterogeneous tumors based on distinct gene expression profiles have been described for central nervous system embryonal tumors (Pomeroy *et al*, 2002), primary breast cancer (Bertucci *et al*, 2000), and cutaneous malignant melanoma (Bittner *et al*, 2000). In addition, analysis of gene expression patterns for 60 cancer cell lines available through the National Cancer Institute demonstrated consistent relationships between the profiles observed and the tissue of origin (Schuler, 1997), further supporting the utility of this approach for classification of disease at the molecular level. These studies provide convincing evidence that microarrays can be used to identify previously undetected but clinically relevant gene expression signatures in human disease.

Gene expression profiling in autoimmune diseases

Autoimmune diseases affect 3%–5% of the population and are mediated by an immune response to self antigens that may range from relatively organ or tissue specific to systemic in nature. Several lines of evidence support the suggestion that certain genes that contribute to autoimmunity may be shared by multiple disease phenotypes. Multiple autoimmune diseases are often found in families or individuals; they share overlapping susceptibility loci, as demonstrated by genome wide scans; and apparently healthy relatives of autoimmune disease patients often exhibit immune system abnormalities. What predisposes some individuals to develop an autoimmune disease and the factors that impart specificity of the disease phenotype are not clear.

Relatively few studies of gene expression profiling in autoimmune disease have been published to date; however, this will undoubtedly improve in the near future as efforts in our laboratories and others' progress. As with examples from other fields, the diversity of experimental designs in autoimmune disease studies published to date offers hints of the powerful nature of microarray approaches. A few specific examples of results from several autoimmune diseases follow.

Alopecia areata (AA) is considered to be a nonscarring, inflammatory, cell-mediated autoimmune disease characterized by spontaneous reversible hair loss that most frequently affects the scalp. In studies by Carroll and colleagues, gene expression profiles were examined in a mouse model of AA and in humans with AA (Carroll *et al*, 2002). Total RNA was extracted at multiple time points from skin of the C3H/HeJ inbred mouse strain with AA induced by AA-affected skin grafts, as well as from C3H/HeJ mice with spontaneous, chronic, extensive AA. The kinetic progression of gene expression in the induced model of AA was consistent with an autoimmune mechanism of disease progression. The earliest markers suggested that onset of disease involves tissue inflammation and vasodilation, proceeding to activation of macrophages and T helper 1 (TH1) lymphocytes, followed by alterations in expression levels of genes regulating immunoglobulin responses during later disease development. Downregulation of hair keratins and hair follicle–associated genes was coincident with activation of macrophages and T cells, and suggested that disintegration or collapse of hair follicle integrity is initiated by immune system attack, rather than by an immune response being invoked by hair follicle damage. Genes that were upregulated in studies of human skin biopsies from individuals with chronic, nonresponsive AA were suggestive of changes associated with chronic, innate immunity and infiltration of TH1 T cells. Genes involved in a variety of metabolic, adhesion, and signaling processes that previously had not been associated with AA were also upregulated. Of the 64 genes that were defined as downregulated, 15 are associated with human keratins or hair follicles. These results demonstrate the importance of cell-mediated immunological disease in AA and provide an important example of how new insights into the pathogenesis of autoimmune disease may be obtained through microarray technology.

An important and common goal for autoimmune disease research is the identification of new targets for the development of more specific and effective therapies. A powerful illustration of the potential value of microarray technology in making progress toward this goal was recently reported in studies of multiple sclerosis (MS) (Lock *et al*, 2002; Tompkins and Miller, 2002). MS is a chronic autoimmune disease involving demyelination of the central nervous system (CNS) white matter. Lock and colleagues described gene expression profiles for two distinct types of brain lesions in autopsy material obtained from MS patients: acute/active lesions characterized by inflammation and chronic/silent lesions with extensive scarring and demyelination (Lock *et al*, 2002). The authors described numerous genes that are differentially expressed between MS lesions and normal tissue, as well as genes that differentiate the acute and chronic lesions. From the genes identified, two were then chosen as potential

therapeutic targets and tested in a common murine model of MS, experimental autoimmune encephalomyelitis (EAE). The immunoglobulin Fc receptor I and IgE receptor genes were upregulated in chronic/silent lesions. Comparison of the severity of disease in Fc receptor-deficient and wild-type mice showed that acute disease was less severe and chronic disease was absent in mice lacking Fc receptor expression. The second gene targeted as a potential target was granulocyte-colony stimulating factor (G-CSF), which was elevated in the acute/active patients. Treatment with G-CSF was shown to decrease disease severity in the early stages of EAE when given before disease onset. Studies such as these provide important insight into distinct stages of disease and may facilitate development of tailored therapies for different forms of MS.

Genome scans have been the workhorse in linkage studies aimed at identification of susceptibility loci in complex autoimmune diseases such as systemic lupus erythematosus (SLE). SLE is often touted as the prototype systemic autoimmune disease. Gene-mapping studies in both mice and humans have identified numerous genomic regions that are currently under intense scrutiny in efforts to identify specific SLE genes (Gaffney *et al*, 2002; Nguyen *et al*, 2002). Once linkage is identified, the chromosomal region may be found to contain several hundred candidate genes for further evaluation. Microarrays provide an important complementary tool for identifying genes within linked regions that may display altered expression, perhaps as a result of polymorphisms that affect mRNA transcription. In the (NZB × NZW)F_1 mouse model of lupus, Nba2 is a major contributor to disease susceptibility and is thought to represent a quantitative trait locus important in production of IgG antibodies to nearly all of the lupus autoantibodies commonly studied (Rozzo *et al*, 2001). By using oligonucleotide arrays, Rozzo and colleagues identified two genes with differential expression that localized to the Nba2 interval (Rozzo *et al*, 2001). Through additional studies, these authors provide convincing evidence that the interferon-inducible gene, ifi202, represents a strong candidate gene for SLE. This work thus exemplifies the potential power of microarray analysis to greatly facilitate identification of a susceptibility gene in a linked region.

Microarray studies in human SLE have recently identified gene expression profiles that distinguish most patients from controls (Baechler *et al*, 2003). Baechler and colleagues examined 48 patients and 42 healthy controls using oligonucleotide array analysis of peripheral blood mononuclear cells. Interestingly, the arrays identified a subset of patients that show dysregulated expression of genes in the interferon (IFN) pathway. This IFN signature was associated with a more severe form of the disease, particularly involving complications related to kidney and/or central nervous system inflammation. Therapeutic targeting of the IFN pathway may thus be particularly beneficial in the subset of SLE patients with severe disease that demonstrate the IFN signature.

The underlying mechanisms that contribute to autoimmune disease may in part be shared among related phenotypes. This is thought to be likely for Sjögren's syndrome (SS) and related diseases such as SLE or RA. SS is a chronic autoimmune disease that may occur as the primary phenotype or as a secondary syndrome when present in the context of an additional autoimmune disease. It is characterized by focal lymphocytic infiltration of lacrimal and salivary glands, leading to dry eyes and dry mouth, and is frequently accompanied by a variety of extraglandular manifestations. Immunological features of disease that overlap among SS, SLE, and RA include production of anti-Ro/SSA and/or anti-La/SSB autoantibodies produced by many patients with either SLE or SS, and the production of rheumatoid factor antibodies, which are often found in RA or SS patients. Ongoing microarray studies of SS in our laboratory using methods very similar to those described for SLE by Baechler and colleagues have shown that genes inducible by interferons are upregulated in SS patients compared with healthy controls (Moser KL, unpublished observations). Both SLE and SS may thus share a common etiologic mechanism mediated through dysregulation of genes involved in the IFN pathway. Further application of microarray analyses should facilitate identification of both pathways that are common to SLE and SS, as well as pathways that uniquely distinguish patients with these disease phenotypes.

Studies comparing gene expression profiles among patients with SLE, RA, Type I diabetes, and MS also suggest that aberrant gene expression profiles may be similar among clinically distinct autoimmune disease phenotypes (Maas *et al*, 2002). Using cDNA microarrays with >4000 genes, Maas and colleagues first measured gene expression in nine healthy control subjects before and after immunization with influenza vaccine, and defined profiles associated with normal immune responses. Comparisons of normal immune response profiles and autoimmune disease profiles indicated that patterns in gene expression among RA and SLE patients and in MS and Type 1 diabetes patients are relatively similar. Genes that are overexpressed in all four autoimmune diseases represented various receptors, inflammatory mediators, signaling/secor messenger molecules, and autoantigens. Many of the downregulated genes identified in these studies are involved in apoptosis and ubiquitin/proteasome function and are inhibitors of cell cycle progression.

FUTURE DIRECTIONS

The development of autoimmune disease undoubtedly involves the complex interplay of many genes. Although the number and type of genes are not yet known, global assessment of gene expression is a very powerful approach for gaining insight into these processes. Gene identification will certainly contribute to advancing our understanding of the molecular basis for autoimmune disease and to our identification of novel therapeutic targets. Within a relatively short period of time, the information learned from the application of microarray technology to address complicated biological questions has not only met but often exceeded expectations. Despite their success, however, microarray studies are not without their challenges. Continued refinement of these techniques, including development of improved statistical methods for extracting information from large datasets and software tools for data processing, management, and storage, will likely increase the applicability and general use of these technologies. Additionally, establishing common standards for the publishing and sharing of microarray-generated data will be important. The applicability of this technology to the study of autoimmunity is only beginning to be appreciated. It is likely that microarray technologies will have a substantial impact on our understanding of autoimmune disease.

REFERENCES

Albelda SM, Sheppard D: Functional genomics and expression profiling: Be there or be square. *Am J Respir Cell Mol Biol* 23:265–269, 2000

Alizadeh A, Eisen M, Botstein D, *et al*: Probing lymphocyte biology by genomic-scale gene expression analysis. *J Clin Immunol* 18:373–379, 1998

Alizadeh AA, Eisen MB, Davis RE, *et al*: Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403:503–511, 2000

Baechler EC, Batliwalla FM, Karypis G, *et al*: Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci USA* 100:2610–2615, 2003

Bertucci F, Houlgatte R, Benziane A, *et al*: Gene expression profiling of primary breast carcinomas using arrays of candidate genes. *Hum Mol Genet* 9:2981–2991, 2000

Bittner M, Meltzer P, Chen Y, *et al*: Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature* 406:536–540, 2000

Carroll JM, McElwee KJ, King LE Jr, *et al*: Gene array profiling and immunomodulation studies define a cell-mediated immune response underlying the

pathogenesis of alopecia areata in a mouse model and humans. *J Invest Dematol* 119:392–402, 2002

Cho RJ, Campbell MJ, Winzeler EA, *et al*: A genome-wide transcriptional analysis of the mitotic cell cycle. *Mol Cell* 2:65–73, 1998

DeRisi JL, Iyer VR, Brown PO: Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278:680–686, 1997

Eisen MB, Spellman PT, Brown PO, *et al*: Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 95:14863–14868, 1998

Gaffney PM, Moser KL, Graham RR, Behrens TW: Recent advances in the genetics of systemic lupus erythematosus. *Rheum Dis Clin North Am* 28:111–126, 2002

Geiss GK, Bumgarner RE, An MC, *et al*: Large-scale monitoring of host cell gene expression during HIV-1 infection using cDNA microarrays. *Virology* 266:8–16, 2000

Huang RP, Huang R, Fan Y, Lin Y: Simultaneous detection of multiple cytokines from conditioned media and patient's sera by an antibody-based protein array system. *Anal Biochem* 294:55–62, 2001

Ichikawa JK, Norris A, Bangera MG, *et al*: Interaction of pseudomonas aeruginosa with epithelial cells: identification of differentially regulated genes by expression microarray analysis of human cDNAs. *Proc Natl Acad Sci USA* 97:9659–9664, 2000

Joos TO, Schrenk M, Hopfl P, *et al*: A microarray enzyme-linked immunosorbent assay for autoimmune diagnostics. *Electrophoresis* 21:2641–2650, 2000

Lock C, Hermans G, Pedotti R, *et al*: Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nature Med* 8:500–508, 2002

Maas K, Chan S, Parker J, *et al*: Cutting edge. Molecular portrait of human autoimmune disease. *J Immunol* 169:5–9, 2002

MacBeath G: Protein microarrays and proteomics. *Nat Genet Supplement* 32:526–532, 2002

Nguyen C, Limaye N, Wakeland EK: Susceptibility genes in the pathogenesis of murine lupus. *Arthritis Res* 4:S255–S263, 2002

Pease AC, Solas D, Sullivan EJ, *et al*: Light-generated oligonucleotide arrays for rapid DNA sequence analysis. *Proc Natl Acad Sci USA* 91:5022–5026, 1994

Pomeroy SL, Tamayo P, Gaasenbeek M, *et al*: Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature* 415:436–442, 2002

Robinson WH, DiGennaro C, Hueber W, *et al*: Autoantigen microarrays for multiplex characterization of autoantibody responses. *Nature Med* 8:295–301, 2002

Rozzo SJ, Allard JD, Choubey D, *et al*: Evidence for an interferon-inducible gene, *Ifi202*, in the susceptibility to systemic lupus. *Immunity* 15:435–443, 2001

Schena M, Shalon D, Davis RW, *et al*: Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270:467–470, 1995

Schuler GD: Pieces of the puzzle: Expressed sequence tags and the catalog of human genes. *J Mol Med* 75:694–698, 1997

Simon R, Radmacher MD, Dobbin K: Design of studies using DNA microarrays. *Genet Epi* 23:21–36, 2002

Slonim DK: From patterns to pathways. Gene expression data analysis comes of age. *Nat Genet Supplement* 32:502–508, 2002

Spellman PT, Sherlock G, Zhang MQ, *et al*: Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. *Mol Biol Cell* 9:3273–3297, 1998

St Croix B, Rago C, Velculescu V, *et al*: Genes expressed in human tumor endothelium. *Science* 289:1197–1202, 2000

Staudt LM, Brown PO: Genomic views of the immune system. *Annu Rev Immunol* 18:829–859, 2000

Tompkins SM, Miller SD: An array of possibilities for multiple sclerosis. *Nature Med* 8:451–453, 2002

Trent JM, Baxevanis AD: Chipping away at genomic medicine. *Nat Genet Supplement* 32:462, 2002

Walker MG, Volkmuth W, Sprinzak E, *et al*: Prediction of gene function by genome-scale expression analysis: Prostate cancer-associated genes. *Genome Res* 9:1198–1203, 1999