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High-throughput Methods for Measuring Autoantibodies in Systemic Lupus Erythematosus and other Autoimmune Diseases

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ABBREVIATIONS

GAD	glutamate decarboxylase
IA-2	insulinoma-associated protein-2
IDDM	insulin-dependent diabetes mellitus
MHC	major histocompatibility complex
MS	multiple sclerosis
PBMC	peripheral blood mononuclear cells
RA	rheumatoid arthritis
RNP	ribonucleoprotein
SLE	systemic lupus erythematosus
Sm	Smith antigen
TNF	tumor necrosis factor

BACKGROUND: CLINICAL DISEASE AND PATHOGENESIS

Systemic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease of unknown etiology that primarily affects women of childbearing age. As with most autoimmune diseases, the development of SLE is likely influenced by a combination of genetic, environmental, and hormonal factors.[1,2] Clinical manifestations of SLE are highly variable, as the disease can affect mutliple organs and organ systems. Common targets of damage in SLE include the skin, joints, blood elements, serosa, and nervous system. However, pathologic changes in the kidney are the most common cause of morbidity and mortality.[3]

AUTOANTIBODIES AS DIAGNOSTIC MARKERS

A hallmark of SLE is the presence of antinuclear autoantibodies. These autoantibodies are primarily directed against molecules that have roles in important cellular processes. Examples include the U1 small nuclear ribonucleoprotein/Smith (U1snRNP/Sm) complex, a mediator of pre-mRNA splicing in the nucleus of eukaryotic cells,[4,5] and the La antigen, which is involved in RNA polymerase III transcription.[6]

Some SLE serologic specificities, such as anti-histone and anti-DNA antibodies, are seen in a variety of autoimmune and inflammatory diseases. Conversely, anti-double-stranded DNA (dsDNA) and anti-Sm antibodies are highly specific for the diagnosis of SLE, and in fact are included as diagnostic criteria for SLE.[3,7] Specific autoantibody profiles are associated with disease subsets in SLE, and autoantibody levels can fluctuate with disease activity.[8,9] However, it remains unclear whether autoantibodies are directly pathogenic.

AUTOANTIBODIES AS PREDICTIVE MARKERS

Insulin-dependent diabetes mellitus (IDDM) is a striking illustration of a disease in which autoantibodies serve as predictive markers. This disease is characterized by lymphocyte-mediated destruction of the insulin-producing beta cells in the pancreatic islets of Langerhans. While tissue destruction in IDDM is primarily mediated by autoreactive $CD4^+$ T lymphocytes,[10] clinical onset of disease is often preceded by circulating autoantibodies against specific islet cell antigens such

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as insulin, glutamate decarboxylase (GAD)-65, or insulinoma-associated protein-2 protein tyrosine phosphatase (IA-2).[11] In addition, the presence of autoantibodies directed against any 2 of these autoantigens predicts a significant risk of developing clinical diabetes within 10 years.[12]

The value of autoantibodies as predictive tools is not limited to Type I diabetes. Numerous spontaneous murine models of SLE are marked by the presence of autoantibodies prior to the development of lupus nephritis,[13] and anti-dsDNA antibodies in human serum prior to diagnosis with SLE have been reported.[14] However, the notion that autoantibodies may serve as predictive markers for development of human SLE was largely unexplored until recently. Harley and colleagues evaluated Department of Defense Serum Repository samples obtained from 130 persons prior to diagnosis with SLE. These sera were analyzed for antinuclear, anti-dsDNA, anti-Ro, anti-La, anti-Sm, anti-ribonucleoprotein (RNP), and antiphospholipid autoantibodies. Antibodies to at least one of these autoantigens were detectable before the onset of clinical disease in nearly 90% of these sera. Furthermore, the appearance of autoantibodies appeared to occur in a predictable fashion: antinuclear, anti-Ro, anti-La and antiphospholipid antibodies preceded the onset of SLE by several years, whereas anti-Sm and anti-RNP antibodies were detectable only months before diagnosis.[15] The notion that autoantibodies may be useful in prediction of clinical outcome has also been elegantly demonstrated in the autoimmune skin disease pemphigus foliaceus.[16]

"HIGH THROUGHPUT, HIGH REWARD"

The diverse clinical manifestations of SLE, coupled with often-significant variations in the response to therapy between patients, have underscored the need for drastic improvements in diagnostic criteria and techniques. The importance of autoantibodies in lupus—as markers of disease, as well as potential mediators of pathogenesis—suggests that enhanced understanding of autoantibody responses may prove to be vital in this regard. Traditional approaches to studying autoantibodies—Western blot analysis and enzyme-linked immunosorbent assays (ELISAs), for example—tend to be laborious, providing only a limited amount of information at considerable expense. However, studying autoimmune disease from a much broader perspective—by evaluating hundreds or even thousands of parameters simultaneously—offers considerable promise in the elucidation of underlying mechanisms of etiology and pathogenesis.

Genome-wide transcriptional profiling by DNA microarray has provided tremendous insight into the genetic and molecular basis of a variety of biological phenomena. DNA microarray technology allows the mRNA expression levels of several thousand genes to be measured simultaneously. This technique has been used successfully in the classification and prediction of outcome of human malignancies, as well as in the identification of novel molecular and genetic events in basic biology.[17]

DNA microarray technology has been extended to study various aspects of specific autoimmune diseases, including gene expression levels in lesions of multiple sclerosis patients[18] and synovial tissue in rheumatoid arthritis (RA).[19] In addition, DNA arrays have been used to analyze RNA transcript levels in peripheral blood mononuclear cells (PBMCs) derived from SLE patients. These studies suggested that interferon-inducible genes are overexpressed in lupus patients. Furthermore, the interferon "biosignature" may correlate with more severe kidney disease in individuals with SLE.[20,21]

These observations highlight the utility of transcriptional profiling in the identification of distinct subgroups of SLE patients. However, a major limitation of gene expression profiling is that mRNA expression levels often do not correlate with expression levels or function of the encoded protein. This is illustrated by the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α). Despite abundant levels of its RNA transcript, TNF-α protein is often not detectable *in vitro*.[22] As with many other genes and their products, TNF-α protein expression is tightly regulated by both post-transcriptional and post-translational mechanisms.[23] Protein structure and function may also be regulated by post-translational events, such as phosphorylation and ubiquitination.[24] Transcriptional profiling is not useful for examining such events. As a result, proteomics-based approaches to studying biological systems are beginning to emerge. Protein arrays have been used to analyze the yeast proteome,[25] as well as to profile signaling pathways in cancer.[26] Various platforms for multiplex, high-throughput measurement of autoantibodies have been described.[27,28]

More recently, autoantigen arrays have proven useful in the study of autoantibody responses in human and animal models of autoimmune disease. Autoantigen microarrays are a novel and powerful tool for evaluation of autoantibody responses in autoimmune disease, with sensitivity and multiplex capability that greatly exceeds standard methods such as ELISA.[29] Autoantibodies have long been associated with clinical subsets of SLE, suggesting that autoantibodies may also be useful in identifying potential responders to therapeutic intervention. Studies by our group in the murine experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS) are elegant proof of this concept.

Tissue destruction in both EAE and MS is driven by autoreactive myelin-specific $CD4^+$ T cells,[30] although autoantibodies appear to play a prominent role in demyelination.[31,32] Immunization of a mouse with a myelin antigen emulsified in complete Fruend's adjuvant can induce a disease that shares many clinical symptoms with human MS; however, the exact disease course varies with the genetic background of the animal.[30]

Robinson *et al.*, used autoantigen microarray technology to monitor production of autoantibodies directed

against myelin antigens in the EAE model. Parallel analysis of clinical and autoantigen array data revealed that increased inter- and intra-molecular autoantibody epitope spreading correlated with relapse rate in the EAE model. Arrays were then used to guide selection of, and to follow response to, antigen-specific DNA tolerizing therapy. Consistent with previous reports,[33] DNA vaccination was effective in treating established EAE. Furthermore, array analysis indicated that animals treated with DNA tolerizing vaccines displayed reduced epitope spreading of their autoreactive B cell responses.[34] Thus, even in diseases that are classically considered to be T-cell mediated, autoantibody profiling can offer significant diagnostic and prognostic insight. Studies are currently underway in our lab to examine the effects of antigen-specific DNA vaccination on autoantibody epitope spreading and disease activity in spontaneous and inducible murine models of SLE.

SUMMARY

Numerous groups have now validated high-throughput approaches to autoantibody profiling in a variety of systems. Recently, we have used autoantigen microarray technology to identify distinct autoantibody profiles in H-2 congenic MRL/lpr mice (Sekine *et al.*, manuscript in preparation), and we are expanding this platform to study human and mouse models of IDDM and RA. We are also developing protein arrays for multiplex analysis of serum antibody isotypes.

Multiplexed methods for autoantibody profiling will undoubtedly continue to uncover novel aspects of autoimmunity and B cell biology. It is now time to move these technologies beyond the proof-of-concept phase, and start addressing the next series of important questions. These include, but certainly are not limited to: identifying "autoantibody signatures" associated with disease state or outcome; profiling autoantibodies during the natural course of murine and human disease; and monitoring changes in autoantibody profiles of patients in response to therapeutic intervention. However, the next set of challenges is just right around the corner. As data and statistical analysis tools become more robust, it will be possible to generate and approach new hypotheses at an unprecedented pace.

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