The missing link for autoimmune disease

Researchers at McMaster University, Ontario, Canada, are developing protein microarrays for serum-based diagnostic testing. These microarrays, developed using a Tecan laser scanner, are able to combine autoimmunity, bacterial and viral antigens on a single array, and are helping researchers to investigate the link between infection and autoimmune disease.

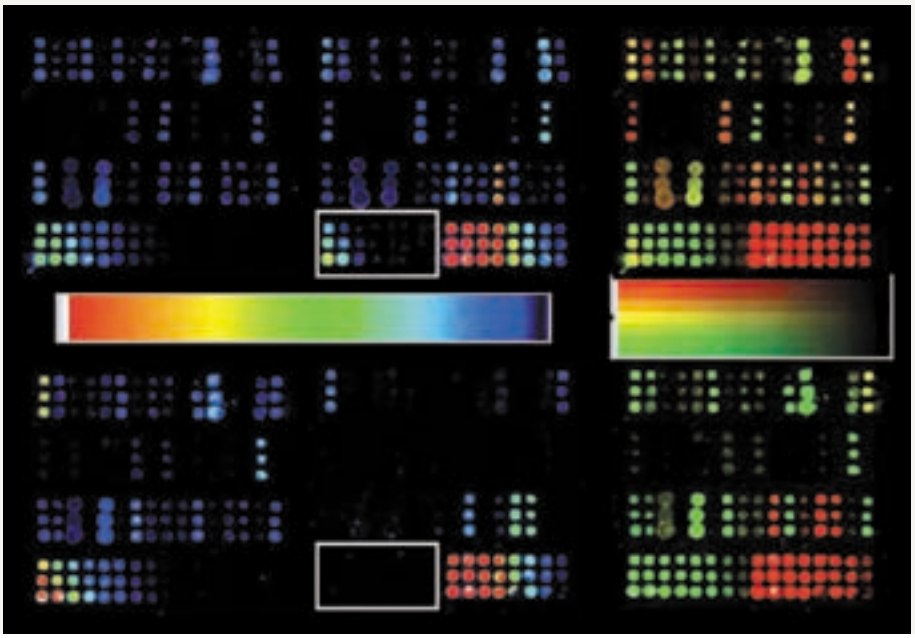
Muscle and joint pains commonly occur as side effects of common infectious diseases, such as colds and influenza. These symptoms are the result of the body's inflammatory response to infection, and are generally transitory. However, in some cases they appear to precede the onset of progressive autoimmune disease affecting joints, muscles, nerves, connective tissues and organs. Researchers at McMaster University in Ontario, Canada, are using protein microarrays to explore this potential link between infection and autoimmunity in adults.

Dr Sandeep Raha, an Assistant Professor in the Department of Pediatrics, explained the focus of the project: "Although we are primarily involved in pediatric research, we are very interested in the development of clinical diagnostic tests based on microarray technology, due to the minimal specimen requirements for serodiagnostic testing. Use of microarrays allows parallel testing of a patient's serum sample for a wide variety of pathogen and autoimmunity antigens from less than a milliliter of serum, significantly reducing costs and allowing 'finger prick' testing in a primary care setting."

"The link between autoimmunity and infection offers a good demonstration of the versatility of protein microarrays," continued Dr Tom Ewart, a Visiting Professor at McMaster University who is spearheading the development of this technology. "Analysis of this relationship requires a high degree of parallel screening, generating large quantities of complex data for each individual. This type of analysis would be virtually impossible using traditional ELISA techniques, due to the high costs, labor-intensive processing requirements and need for large amounts of patient material. By contrast, a patient's serum can be screened for a broad spectrum of target antibodies using a protein microarray."

"Tecan's LS Reloaded™ laser scanner is perfect for analysis of these microarrays, offering rapid data acquisition with minimal user input. It has excellent autofocusing and is a very fast scanner, with a typical scan taking just two minutes at 10 µm resolution. This allows complete analysis of an autoimmunity array in under eight minutes, ideal for use in a primary care setting. The scanner is also very good for microarray development, as it is able to handle both transparent and opaque substrates, and offers the flexibility to work with a variety of microarray formats. We used the scanner extensively for assessing silicon substrates during development and optimization of the array, assessing various binding chemistries for both peptides and DNA."

Neil Winegarden, Head of Operations at The Microarray Centre, University Healthy Network in Toronto (right), shows their new Tecan LS Reloaded scanner to Tom Ewart, McMaster University, Hamilton (left). The two long time colleagues have a mutual interest in proteomic microarrays. "We are interested in the 4 color laser scanning of the LS Reloaded since it would double our array multiplexing capability. We could quantitatively capture all four IgA, D, G and M class antibody responses to infectious and autoimmune diseases in the same sample image," said Dr Ewart. The Microarray Centre (www.microarrays.ca) is the major shared resource for microarray studies serving the University of Toronto and six core area university-affiliated hospitals of Toronto. It is located in the expansive new MaRS (Medical and Related Sciences) research complex on the original Toronto General Hospital grounds.



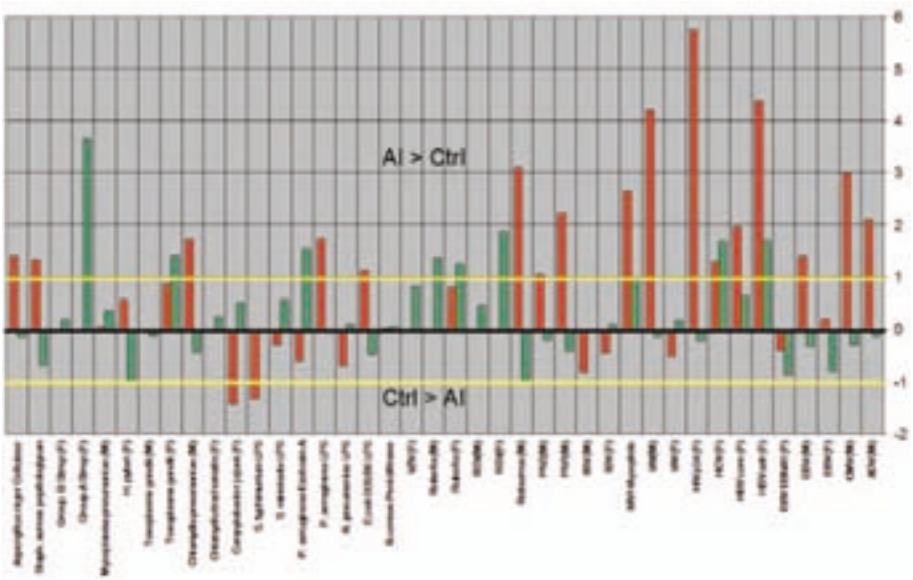
The LS Reloaded 200 scanner generates concurrent color scaled IgG (left) and IgM (center), and combined response images (right) for an autoimmune patient (top) and a control subject (bottom). The autoimmune patient shows generally higher IgM acute phase responses to the bacterial and viral pathogens printed in the array as well as high rheumatoid factor (white boxes in the printed IgG calibration area).

Tom continued: "Robust data analysis and interpretation is a major factor of transferring this technology to a clinical environment, and the Array-Pro® Analyzer software Tecan supplies is very easy to use. We currently have around 80 different antigens complexed onto the microarray, split approximately equally between pathogen and autoimmunity targets. In addition, the microarray features internal calibration standards to compensate for changes in dilution, and the presence of interfering factors such as rheumatoid factor or immnuosuppresive therapeutic agents. Inclusion of these standards, in addition to accurate gridding and quantitation of the array, is crucial for meaningful interpretation of results, and the software certainly provides this."

"We have already validated the array using a relatively small number of samples from 'control' patients confirmed not to have autoimmune deficiencies, and have begun to analyze samples from patients with diagnosed autoimmune disorders. Our preliminary findings have been very interesting – indicating that patients with autoimmune disease have a much higher reactivity to infectious disease antigens – and we are now recruiting more patients from both populations to allow us to verify these results."

"Although development of this technology has so far focused on exploring the links between infection and autoimmunity, due to the local clinical interest and the availability of a relevant patient population, this platform has a wide range of potential applications. Microarrays offer a low cost alternative to ELISA techniques, with approximately 100 assays in microarray format costing the same as a single ELISA assay. This, combined with the minimal requirements for patient material, makes microarrays an attractive prospect for future point-of-care diagnostic tests," Tom concluded.

Array Pro Analyzer is a registered trademark of Media Cybernetics, Inc.



By dividing the average autoimmune group responses to each pathogen by those of the control group, we obtain the fold ratio bar chart. This is a measure of the extent to which the autoimmune patient group is more reactive to each infectious disease. IgM (red) responses outside the ± 1 range (yellow lines) are significantly higher, suggesting active or unresolved infections.