PRO(N)

MASTERING IMMUNITY

Ebola
Could this be the deadliest weapon on earth?

Not if there is an effective vaccine that can be readily deployed. Developing one (using ProImmune's REVEAL™ platform) is a central goal for Dr. Gene Olinger's, research at the Virology Division, US Army Medical Research Institute of Infectious Diseases (USAMRIID).

Figure 1: Ebola virus transmission electron micrograph image colorized, taken at 160,000x magnification.

There is no specific treatment or vaccine available for either Ebola or Marburg virus. These deadly diseases are potential agents of biological warfare or terrorism and are also a global health threat. Since their emergence they have claimed hundreds of lives, with over 90% mortality in some outbreaks. A concern for military personnel is that they could be exposed during the course of humanitarian work, and the only treatment is rehydration and containment of a patient. An effective vaccine against Ebola virus would not only decrease the significant threat to human health, but also provide a substantial disincentive to its use as a biological weapon.

The very limited availability of human disease state and convalescent blood and serum samples, and the fact that it is not possible to carry out studies in humans when developing vaccines for these diseases, makes the need for technologies that characterize immune responses to such viruses *ex vivo* even more compelling. At USAMRIID, the lead medical research laboratory for the U.S. Biological Defense Research Program, Dr. Gene Olinger has used ProImmune's Antigen Characterization Platform to accelerate his research into a vaccine against Ebola and Marburg virus.
(*continued on page 2*)

Customer Research

Dr. Gene Olinger, Virology Division, US Army Medical Research Institute of Infectious Diseases (USAMRIID), USA
Advances Toward a Vaccine for Ebola and Marburg Viruses

Prof. Paul Moss, CRUK Institute for Cancer Studies, University of Birmingham, UK
Decreasing the Hazards of Bone Marrow Transplantation

Dr. Sanja Selak, Intercell AG, Austria
Lessons in Developing Effective Vaccine Strategies

Dr. Terrance O'Hanlon, National Institutes of Health, USA
Finding Targets for Treatment of Inflammatory Myopathy

Dr. Lena Israelsson, Karolinska Institute, Sweden
Throwing Light on the Mechanisms Causing Rheumatoid Arthritis

Advances Toward a Vaccine for Ebola Virus

ProImmune's Antigen Characterization Platform Accelerates Development of a Vaccine for Ebola and Marburg Viruses

Gene Olinger, of the US Army Medical Research Institute of Infectious Diseases (USAMRIID), used the ProImmune Antigen Characterization and Biomarker Discovery Summit to present the very latest thinking on strategy for developing a vaccine for those exposed to filoviruses. There are several challenges to working with Marburg and Ebola viruses: their high infectivity and lethality have obviously impeded basic research, so relatively little is known of their virology, and further, they are highly species-specific. Researchers commonly rely on rodent and macaque models for the diseases, but for filoviruses, bats are strongly implicated as a reservoir species, while higher primates, ungulates and humans will die from the same strain.

There are five strains of Ebola virus, and Gene Olinger is working with the two most lethal, Zaire and Sudan. Ebola belongs to the filovirus family (figure 1); it is a single-strand RNA virus that produces 7 mRNAs upon infection of a host, including one encoding glycoprotein (GP). GP presents an attractive vaccine target as it is a surface protein, and likely to be involved in cell entry.

In collaboration with Alphavax, scientists in the Olinger lab have been working with a Venezuelan equine encephalitis replicon (VRP) engineered to express filovirus GP. They believe this approach strikes a good balance between vaccine potency and safety. They want to evaluate the antigen components required to generate an immune response in humans. To identify potential T cell epitopes in Ebola Zaire GP, Dr. Olinger chose to use ProImmune's REVEAL & ProVE® Rapid Epitope Discovery System to analyze the MHC affinity and binding kinetics of a library of Ebola-derived peptides.

As a result of his project using the ProImmune REVEAL™ platform, Dr. Olinger has a picture of the regions of Ebola Zaire GP that are likely to cause an immune response in HLA-A*02:01–positive human subjects. Three peptides in particular look to be potentially immunogenic, displaying the strong MHC binding and slow off-rates that, in ProImmune's experience, are indicative of a good T cell epitope (figure 2).

Figure 2

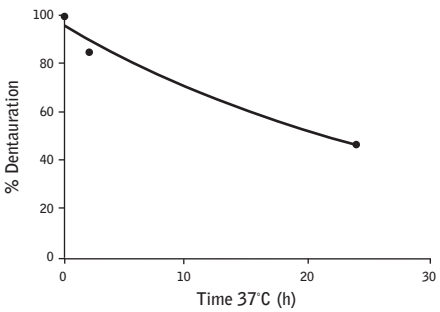
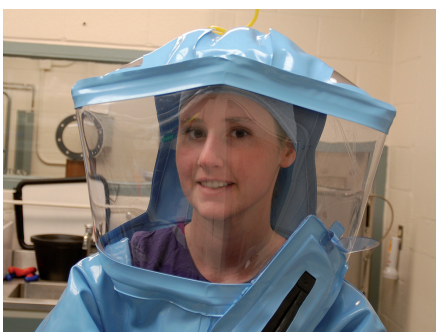


Figure 2: Denaturation curve for a complex of a single Ebola peptide with HLA-A*02:01, measured using the ProImmune REVEAL™ Rate Assay.

> "ProImmune accomplished in two months what would have taken three years in our laboratory."
>
> Dr. Gene Olinger



Laura Pruger, who carried out much of the epitope validation work in the Olinger Lab, photographed in USAMRIID's BSL4 testing facility in Frederick Maryland.

This information will allow the team to understand the fine detail of an immune response to their vaccine candidate when it comes to be tested. Since ultimately they need to correlate an immune response with protection, this is useful information indeed. The Olinger lab's next step will be a move into animal models with the newly identified epitopes. Ultimately they are working on developing immune assays to predict protection in vaccinated macaques that can be applied to human vaccine trials.

This work was carried out in the labs at USAMRIID www.usamriid.army.mil

Watch and listen to a slidecast of Gene Olinger's presentation at the Antigen Characterization and Biomarker Summit at www.proimmune.com/ecommerce/page.php?page=slidecasts

The information contained in this article does not necessarily reflect the position or the policy of the U.S. Government and no official endorsement should be inferred.

Decreasing the Hazards of Bone Marrow Transplantation

Single-Antigen-Specific Donor Lymphocyte Infusion: A Promising New Way to Address the Significant Risk of Opportunistic Infection Following Transplantation

Professor Paul Moss of the CRUK Institute for Cancer Studies, Birmingham, UK, used his presentation at the ProImmune conference to give an overview of more than a decade's work in his lab.

Professor Moss was part of the team who laid the foundations for research into antigen-specific T cells through developing the very first MHC multimer. Today, his work benefits from the huge range of Pro5® MHC Class I Pentamers available from ProImmune. Looking to the future, he is particularly excited by the therapeutic potential of adoptive transfer of antigen-specific T cells. He presented stories from two very different immune scenarios.

Much of the early work with MHC multimers was carried out in the cytomegalovirus (CMV) field, not least because CMV infection is very common and results in a large $CD8^+$ T cell response and in some cases later in life more than 50% of the total $CD8^+$ T cell repertoire. In some parts of the world, CMV infection is found in over 90% of the population, and is mostly latent, as the virus has evolved to co-exist in an immunocompetent host and under chronic immune control. Nonetheless, CMV infection has a dramatic effect on the $CD8^+$ T cell repertoire. Throughout the lifetime of a 'healthy' CMV-infected individual, the presence of CMV causes an increase in the number of memory T cells and also accelerates the decline in number of naïve $CD8^+$ T cells seen with age. The exact mechanisms of this switch are unclear, although they may well contribute to a clinically observable effect – evidence is emerging that elderly CMV carriers have a reduced life expectancy. Professor Moss and his team are characterizing CMV-specific $CD8^+$ T cells using multimer staining in conjunction with cytokine analysis to begin to understand how CMV infection modulates the T cell repertoire.

More severe problems arise when the host becomes immunocompromised. Opportunistic infections in stem cell transplantation still account for a mortality rate of up to 15% in stem cell transplant patients over five years following the transplant. In the early days of stem cell transplantation, CMV was a major cause of patient death, as patients have very few CMV-specific T cells following myeloablative treatment. Adoptive transfer of donor CMV-specific T cells to correct this becomes a very attractive option; the cells will not be rejected as the recipient remains largely immunocompromised, and the cells can expand and clear CMV viremia in their new host. Professor Moss demonstrated that MHC tetramers in conjunction with magnetic beads can be used to purify epitope-specific T cells for this style of treatment and cited a study where a patient who had been suffering from CMV for a few weeks, was resistant to drugs and had no T cells in their blood, was given CMV epitope-specific T cells. Nine days later T cells were present and the patient became CMV-negative (1).

Professor Moss has found that he obtains the cleanest separation of cells using ProImmune Pentamers, compared with tetramers for the same epitopes (figure 3).

Figure 3

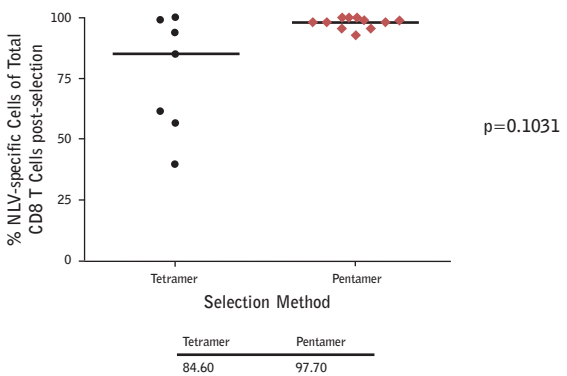


Figure 3: Anti-biotin magnetic bead-based separation of CMV epitope-specific T cells from donors using A*02:01/NLVPMVATV multimers. The purity of selection is much greater with ProImmune Pentamers versus tetramers. As a consequence cells prepared using Pentamers will be much more effective when used in adoptive transfer than cells separated using tetramers, which will contain many more non-specific T cells. The viability of the selected cells was also shown to be excellent.

The technique of epitope-specific T cell purification using Pentamers was followed more recently by Dr. Michael Uhlin at the Karolinska Institute in Sweden. He used ProImmune's Pro5® MHC Pentamers to save the life of an eighteen year old patient who had developed EBV linked post-transplant lymphoproliferative disease following cord blood transplantation (2). The patient did not respond to rituximab and with no other treatment option available, Dr. Uhlin decided to infuse the patient with Pentamer selected EBV-specific T cells obtained from the patient's mother. The reinfusion was followed by a clearly observable, rapid clinical response and viral clearance.

Similar work with tumor antigen-specific $CD8^+$ T cells has raised more questions than answers. Cancer Testis Antigens (CTAgs) are expressed solely by germline cells. The existence of a testis-blood barrier means that the testes remain immune-privileged and tolerance is not established against CTAgs. Some tumors acquire expression of CTAgs, and an immune response is sometimes mounted against them. However, CTAg-specific $CD8^+$ T cells are much more difficult to find than their CMV-specific counterparts. In a recent study, 15 out of 37 myeloma patients showed anti CTAg-specific T cell response which measured between just 0.01 and 0.7% of the $CD8^+$ T cell pool (3). If these cells could be expanded *ex vivo*, could they be adoptively transferred with therapeutic benefit? In a case reported several years ago, two patients receiving kidneys from the same donor both developed melanomas in those kidneys. Investigation found that the donor had had a small melanoma removed 16 years earlier (4). This suggests that the tumors were transferred with the transplant, but that a population of $CD8^+$ T cells that remained in the donor actively suppressed the expansion of sub-clinical tumors. It is a possibility that the $CD8^+$ T cells were targeting CTAgs. Presumably then, receipt of tumor-specific $CD8^+$ T cells from the same donor could send a tumor into remission. Professor Moss questions this idea because his work has shown that the CD8 response actually increases during terminal stages of cancer. He believes that more work is needed to correlate immune response with clinical outcome before adoptive transfer is applied to treat cancer and MHC multimers, such as Pro5® MHC Pentamers, will play a key role in investigating these questions further.

Watch and listen to a slidecast of Paul Moss's presentation at the Antigen Characterization and Biomarker Summit at www.proimmune.com/ecommerce/page.php?page=slidecasts

1. Cobbold, M., *et al.*, (2005) Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA–peptide tetramers. J. Exp Med 202: 379-386

2. Uhlin, M., *et al.*, (2009). A novel haplo-identical adoptive CTL therapy as a treatment for EBV-associated lymphoma after stem cell transplantation. Cancer Immunol Immunother. 59: 473-477

3 Goodyear, O., *et al.*, (2008) Differential pattern of $CD4^+$ and $CD8^+$ T-cell immunity to MAGE-A1/A2/A3 in patients with monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma. Blood 112: 3362-72

4 MacKie, R., *et al.*, (2003). Fatal melanoma transferred in a donated kidney 16 years after melanoma surgery. New England Journal of Medicine 348: 567-568

Forging New Relationships Across Academia and Industry

Experts Connect at ProImmune's Antigen Characterization and Biomarker Discovery Summit

Over the last decade, ProImmune has become a leader in the field of immune monitoring and antigen characterization through our many unique products and services. In January 2011 we invited our customers from academia and industry to a summit on antigen characterization and biomarker discovery at St. Anne's College, Oxford, UK. The theme for presentations and discussions was the state-of-the-art of current technologies in this area, and how they can be improved.

The meeting provided an ideal setting for speakers and delegates to engage in discussion. Scientists from both academia and industry presented their research to understand the mechanisms driving development of disease, and the best methods to combat and prevent it. Two major themes emerged – the importance of epitope discovery, and novel methods for T cell characterization.

There was consensus on the requirement for greater information sharing and best practise approaches to employing existing technology. Mirroring practise in other fields, 'minimal information' about assays and their validation could be agreed to allow standardization of such information. Bringing assays up to good clinical practise standards and working with regulatory authorities to develop appropriate immunological readouts for emerging therapeutics was highlighted as an important objective.

Since the summit in January, ProImmune has announced that it is taking part in a detailed study by the Cancer Immunotherapy Consortium's third Multimer Proficiency Panel, and donating Pro5® Pentamers for the laboratory work. The objective is to provide data to establish harmonized guidelines for research, helping to validate and accelerate the approval of effective cancer immunotherapies.

Biomarker discovery, antigen characterization and T cell immune monitoring technologies are developing fast and increasingly moving into preclinical and clinical settings. Debate and information sharing will remain crucial, so ProImmune will be holding further meetings in 2011 and 2012 to promote discussion and collaboration in order to help shape future developments in this fast moving field.

Join us at 'Mastering Immunogenicity', September 12-13, 2011, Boston, USA

ProImmune's Mastering Immunogenicity conference provides an exclusive forum to network with leaders in this important and dynamic field. Meet immunogenicity section heads from companies including Pfizer, Genentech and Roche, who will share their experiences, and discuss how the immunogenicity risk of biologics can be managed by design in biologics development programs by bringing together the best new technologies.

The program will focus on the current state-of-the-art for available technologies. Presentations by experts in the field will highlight the different approaches and methods currently in use, complemented by guidance from regulatory bodies on assay development. Breakout sessions will address the benefits and limitations of these technologies and the improvements that can be made to accelerate research and clinical studies. The aim of the summit is to help shape future developments in the way research programs are designed and implemented. The knowledge gained will help you to set the direction of your future research plans.

Full details and registration is available at www.proimmune.com/ecommerce/page.php?page=conference1

Dr. Sanja Selak (front row, second from left) and the Serology and Immune Assays team at Intercell AG, Austria.

Lessons in Developing Effective Vaccine Strategies

Insights into Vaccine Discovery at Intercell AG

Epitope discovery systems, such as ProImmune's Antigen Characterization Platform, often identify a large number of candidate epitope sequences. While many protein sequences can potentially be antigenic only a few will be relevant as key antigens with addressable immune responses *in vivo*. To complicate matters further, the available antigens from pathogens may alter over the course of an infection, as Dr. Sanja Selak of Intercell AG, Austria, outlined in her presentation at ProImmune's Antigen Characterization and Biomarker Discovery Summit in January 2011.

Dr. Selak is investigating the B cell epitopes of pathogenic bacteria, and their potential for use in vaccination strategies. Bacterial antigens are especially tricky to work with because bacteria do not express the same proteome consistently. They react to their environment, in particular the availability of micronutrients, and respond by expressing the cellular machinery they require to survive, so that a whole gamut of new antigens can suddenly become available. She has to consider not only the 'who' of candidate epitopes from her discovery strategies, but the 'when' 'where' and 'why', as bacteria adapt to survive in their local habitat.

The antigen identification strategy used at Intercell follows a multistep process, requiring many different assays and taking a sizable amount of time. Firstly, acute and convalescent serum pairs from patients who successfully recovered from infection are studied. High antibody titres suggest that the antigen was immunogenic, expressed during infection, and, most importantly, contributed to bacterial clearance. As comparators, the team use sera from healthy, disease-exposed individuals, and analyze the antibody repertoire of patients who fail to clear the infection.

Next, *in vitro* assays are brought in to play. The target antigen must be an accessible cell surface protein, and the antibodies from patient sera need to demonstrate high-affinity binding. The 'antigenome' is considered – are the candidates conserved between bacterial strains?

To validate their candidates, the research team at Intercell rely on knowledge of the immune evasion mechanisms specific to each pathogen to create custom assays. Human pathogens such as *S. pyogenes, S. pneumoniae* and *P. aeruginosa* survive in their hosts through expression of complement-neutralizing proteins, Fc binding proteins, extracellular matrix binding proteins, and host nutrient acquisition proteins. These adaptations to human hosts are not always apparent in straightforward *in vitro* cell culture experiments. In the example shown in figure 4, when *P. aeruginosa* is grown in normal cell culture conditions the D5 cell surface antigenic epitope is barely detectable, but adding 50% human serum causes up-regulation of D5 expression, validating it as a lead candidate.

Figure 4

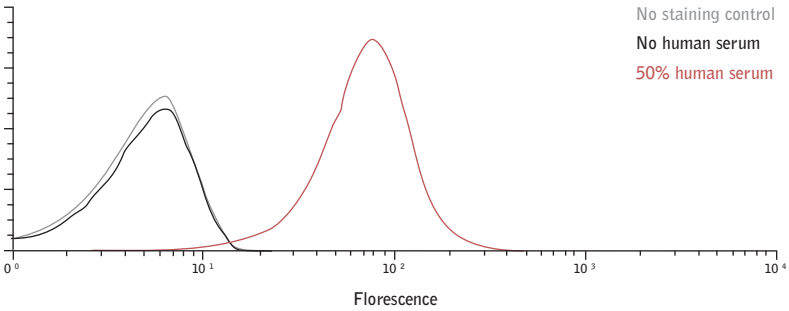


Figure 4: *P. aeruginosa* stained with an antibody to the DR5 antigen in the absence (black) and presence (red) of 50% human serum. When *P. aeruginosa* is grown in normal cell culture conditions the D5 cell surface antigenic epitope is barely detectable, but adding 50% human serum causes up-regulation of D5 expression, validating it as a lead candidate.

Other assay adaptations the team have explored include cell culture in iron-depleting conditions, and opsonization and cell killing assays performed with varied phagocytic cells in the presence of human or animal complement sources. Different combinations of phagocytic cells, complement sources and growth conditions are tested to identify the most optimal assay setup for testing bactericidal activity of antibodies against a particular pathogen; taking into account its immune evasion mechanisms.

Since animal models are of limited value with such well-adapted human pathogens, Dr. Selak and her colleagues are now beginning to consider the potential offered by tissue explant cultures for simulation of bacterial microenvironment during infection. Their knowledge–driven approaches to validating leads from each bacterium they investigate could yield effective vaccine strategies.

Intercell's approach is enabling them to develop a new generation of vaccines that include only essential components and that stimulate both T cells and B cells. Complementary technologies, such as ProImmune's Antigen Characterization Platform, can be used to enhance understanding of T and B cell responses and provide tools to track such responses at different stages of disease. In particular, the *in vitro* ProImmune REVEAL™ MHC-peptide binding and rate assays, offer a rapid method for T cell epitope discovery, accelerating research projects and consequently saving time and money.

Watch and listen to a slidecast of Sanja Selak's presentation at the Antigen Characterization and Biomarker Summit at www.proimmune.com/ecommerce/page.php?page=slidecasts

Furthering Advances in Autoimmune Disease Research

Autoimmune disease is the third largest category of illness in the industrialized world, and one of the leading causes of death for women under 65, behind only heart disease and cancer.

Despite investment of billions of dollars in autoimmune disease research, our understanding of what causes these debilitating conditions is still relatively poor today. However, recent advances in gene chip technology and molecular immunology have now led to first successes in picking apart the complex molecular cascades of events as they progress to cause these destructive and self-augmenting inflammatory diseases. These foundations will enable the identification of potential targets for treatment, and new therapies to prevent, modulate and ultimately cure autoimmune diseases.

Here we feature two studies that employed ProImmune's Antigen Characterization Platform, in research to further the development of sensitive tools for early and definitive diagnosis and identification of at-risk individuals, pushing the boundaries of our knowledge of autoimmunity.

Finding Targets for Treatment of Inflammatory Myopathy

Featured Specialist Editorial by Dr. Terrance O'Hanlon Biologist, Environmental Autoimmunity Group National Institute of Environmental Health Sciences, National Institutes of Health, Bethesda MD, USA

The idiopathic inflammatory myopathies (IIM) are heterogeneous autoimmune diseases that share the common features of muscle inflammation and weakness. In addition to muscle, these systemic connective tissue diseases may also affect the skin, joints, gastrointestinal tract, lungs and other organ systems. The IIM are the most common acquired myopathies in adults and result in incurable, progressively disabling disease with significant morbidity and mortality and high socioeconomic costs.

Polymyositis and Dermatomyositis (i.e., myositis with associated skin rashes) are the most frequent clinical subtypes of IIM and are thought to involve distinct etiopathogenic mechanisms. Polymyositis is characterized by oligoclonal antigen-restricted $CD8^+$ T cell muscle infiltrates that damage myocytes via perforin-mediated cytotoxicity. In contrast, dermatomyositis involves a primary vasculopathy, perivascular accumulations of B and $CD4^+$ T lymphocytes and dendritic cells, and complement-mediated vascular endothelial cell damage.

In addition to these immunohistopathologic differences, approximately one-third of myositis patients manufacture myositis-specific autoantibodies (MSA) directed against different components of the protein biosynthetic pathway including several amino-acyl tRNA synthetases, translational elongation factors, and signal recognition particles. Most prevalent among these are autoantibodies targeting the histidyl-tRNA synthetase protein (HRS); commonly referred to as anti-Jo-1 autoantibodies and detected in ~20% of all myositis patients.

While the pathologic significance of the MSAs remains unclear, considerable interest exists in defining immunodominant T cell epitopes of the anti-Jo-1 antigen. To this end, we have utilized ProImmune's REVEAL™ Class II Rapid Epitope Discovery System to investigate the potential binding of overlapping, synthetic peptides spanning the entire anti-Jo-1 antigen molecule (Module 1: PEPscreen® Custom Peptide Library Synthesis). To date, our studies with ProImmune have resulted in the successful identification of several candidate T cell epitopes in binding assays with the IIM associated genetic risk factor HLA-DRB1*03:01 (Module 2: Class II MHC-Peptide Binding Assay). Moreover, our T cell epitope discovery data were enhanced further by equilibrium binding assays measuring precise rates of kinetic association (Ka) and disassociation (Kd) of peptide MHC Class II complexes (Module 4: Class II Complete Rate and Stability Assays). Currently, our studies with ProImmune have progressed to the functional validation phase of the anti-Jo-1 T cell epitope discovery project. Herein we plan to use a combination of cellular assays (e.g., ELISpot and T cell proliferation) using myositis patient-derived peripheral blood cells to examine clonal expansion of antigen-specific, MHC Class II restricted T cells.

Cut the Time and Cost for Epitope Discovery Projects

The ProImmune REVEAL™ MHC-peptide binding and rate assays can significantly accelerate your T cell epitope discovery projects. The technology overcomes the major constraints of time and cost that can be incurred using traditional methods, making otherwise uneconomical projects possible. It specifically addresses the main challenges and limitations faced by researchers attempting epitope discovery today:

- **Identifies MHC restrictions:** unlike functional assays, the MHC-peptide binding assay determines MHC restriction for putative epitopes at the outset
- **Optimizes the use of limited patient samples:** the assays are carried out *in vitro* and refine the number of potential epitopes for validation with patient samples; this reduces the amount of cellular material required for the discovery process
- **Rapid and cost effective:** epitopes are identified in weeks, rather than months or years, and the customized service saves both time and money when compared with existing labor-intensive functional assays

For further information, visit www.proimmune.com/ecommerce/page.php?page=reveal

Throwing Light on the Mechanisms Causing Rheumatoid Arthritis

Defining Autoantibody Binding to Citrullinated Target Proteins Using Sensitive Peptide Microarrays

Lena Israelsson, Karolinska University Hospital, Karolinska Institute, Stockholm Sweden.

Professor Lars Klareskog and his team at the Karolinska Institute in Stockholm, Sweden, have a long-standing research interest in Rheumatoid Arthritis (RA) (1). Characterizing new protein based biomarkers of relevant autoantigens associated with RA is crucial for early diagnosis of the disease. Research to date strongly suggests that it is possible to predict the onset of RA by detecting autoantibody responses before clinical symptoms and joint damage occur in the patient. Since the damage caused by RA is a self - augmenting process once it has started, commencing treatment as early as possible is of paramount importance in managing the disease successfully in patients.

RA onset is associated with MHC class II–dependent activation of adaptive immunity. A variety of proteins (such as vimentin, alpha-enolase, type II collagen, and fibrinogen) undergo posttranslational modification of arginine to citrulline over time, and a working hypothesis is that the citrullination may be aggravated by smoking or bacterial infection. Autoantibodies towards these modified proteins may complex with their target citrullinated proteins as part of a multistep process for RA development.

Understanding the B cell epitopes on candidate autoantigens is central when dissecting this complex disease and with that intent researcher Lena Israelsson turned to ProImmune's B cell Epitope Mapping Service.

ProImmune synthesized a series of novel and previously identified peptides from RA-associated proteins in both their native and their citrullinated variants, then spotted them onto multiple ProArray™ slides for screening. Sera collected from RA patients were assayed for binding to the paired native and citrullinated peptides. Across the set of patient samples, differences were apparent between citrullinated peptides and their unmodified counterparts, as shown in figure 5.

Figure 5

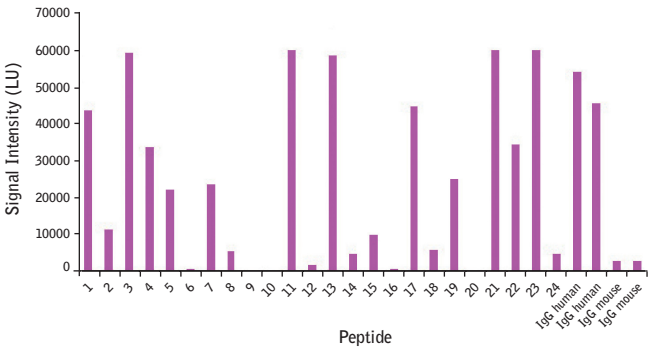


Figure 5: ProArray™ results for binding of a serum sample to 12 peptide pairs, citrullinated (odd numbers) and unmodified arginine containing sequences (even numbers), from RA-associated proteins. The results show that for each of the peptide pairs screened, the patient antibodies bound much more strongly to the citrullinated variant (odd numbered peptide) than the native peptide, supporting the theory that citrullinated epitopes could be important in RA progression.

Citrullination of proteins was suspected as a factor in the development of autoantibodies, and the results indicate that the immune responses seem to be biased very strongly towards citrullinated epitopes while their uncitrullinated equivalents are in many cases not recognized. ProImmune's peptide array based mapping is sufficiently sensitive to highlight these differences in binding. While further validation work is required, provisionally, these results support the hypothesis that citrullinated epitopes could be important for the development of RA. The data generated by ProImmune in a short space of time, helped shortlist some of the novel peptides for further studies at the Karolinska Institute.

1. Klareskog, L., *et al.* (2008) Immunity to Citrullinated Proteins in Rheumatoid Arthritis. Annual Review of Immunology 26: 651-675

This work was part funded by the European Commission under AUTOCURE: an integrated project of the framework programme 6. www.autocure.com

ProArray Ultra™

Scale | Flexibility | Precision | Power

Do you need to increase the throughput of your ELISA assays and do more with less sample at a lower cost? ProArray Ultra™ delivers a combined protein and peptide array platform with unrivalled scalability and flexibility, while exceeding the signal quality and performance of optimized ECL ELISA assays.

- More than 30,000 proteins or peptides per array
- As low as 1 µl sample required for analysis
- Signal performance better than ECL ELISA and comparable to MSD®
- Flexibility to immobilize any protein or peptide
- 1-24 replicate sub-array formats for multiple applications
- Delivered as a rapid turnkey service with comprehensive report

ProArray Ultra™ has been specifically developed as a combined peptide and protein array platform; combining the epitope/interaction site resolving power of scanning peptide arrays with that of high throughput and protein interaction screening.

ProArray Ultra™ achieves full compatibility for overlapping peptide libraries and proteins, and uses a flexible peptide synthesis platform to enable printing of arrays optimized for specific applications. Moreover, our design improvements result in greater uniformity of spot morphology between peptides and proteins, reduced background signal and improved assay performance in terms of sensitivity, dynamic range, and variance.

The result is a protein array platform that is comparable in analytical performance to MSD® technology and that outperforms ECL ELISA assays, while tens of thousands of interactions can be detected in a single experiment. More importantly only microliter amounts of sample are required for each analysis, enabling projects using limited amounts of sample that are simply impossible to carry out with 96-well plate-based assays.

The Turnkey Solution to Your Epitope Discovery Program

Flexibility, scalability and sensitivity are the hallmarks of the ProArrayUltra™ platform. Adding real value to your research projects is the additional element brought to you by our customer service team of PhD immunologists and biochemists. Discuss your project with us and we will design an assay and array format tailored to achieve your objectives. The ProArrayUltra™ technology is the turnkey solution to your epitope mapping and discovery programs.

Applicable Program Areas

The ProArray Ultra™ technology is appropriate for a range of programs and projects, including:

- Antigen discovery
  - Reverse immunology on proteins or entire pathogen proteomes
  - Screening sera from disease state or convalescent donors against overlapping peptides
- Mapping of monoclonal antibody binding sites
  - Mapping is linear in the first instance (unless conformational peptides are used), but strong conformational epitopes will come up in linear sequences too
  - The new ProArray Ultra™ platform also allows for the use of constrained peptides, e.g. cyclized peptides
- Understanding the epitopes presented and recognized
  - Disease progression cycle
  - Pathogen life cycle and recovery phases
- Batch release testing: vaccines, biologics
- Evaluate post-translational modifications on binding (large scale potential)
  - Highly flexible regarding peptide synthesis and available modifications
- Enzyme interaction site mapping: kinases, phosphatases
- Biomarker discovery and tracking
  - What to measure – potential biomarkers and/or surrogate endpoint markers
  - When to measure – patient sample progression over time
  - Multiple biomarker assays – simultaneous assessment of a range of identified markers of interest
- Cytokine detection in samples
- Iterative optimization of peptides

Flexible Array Design

Our printing technology confers complete flexibility for design around your requirements. Different printing layouts can be configured depending on the number of peptides/proteins and the number of samples to be analyzed. Figure 6 shows some examples. The slides can be overlaid with a separation gasket allowing each sub-array to be incubated with an individual antibody-sample, i.e. up to 24 separate samples can be analyzed per slide. The different print setups can accommodate a large range of custom printed features (peptides & proteins) alongside standard control spots.

Figure 6

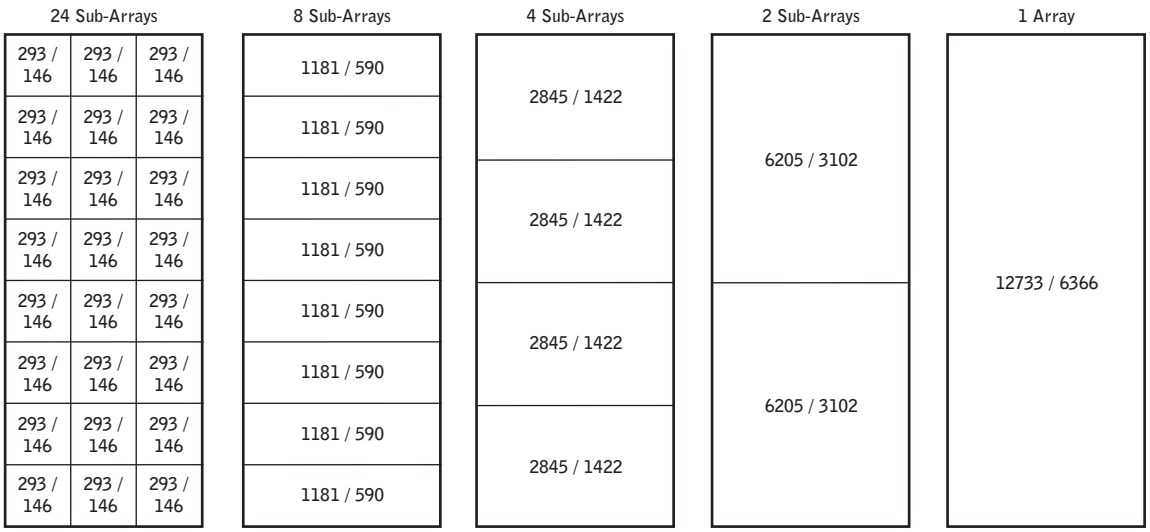


Figure 6: The diagram shows 5 example layouts of the microarray printing design. The first number in each pair indicates the number of samples that can be spotted in triplicate; the second number indicates the number of spots in sextuplicate. The sub-arrays can be physically separated from each other using specially fitted gaskets, allowing incubation of each discrete sub-array with a different sample.

Performance Data

Better Design Leads to Ultra Performance

The design of ProArray Ultra™ overcomes the compromise between analytical quality multi-well immunoassay platforms and the scalability of existing protein and peptide array technology. Evaluation of our new design features against other more conventional assay methodologies, such as optimized ECL ELISA and the Meso Scale Discovery (MSD®) technology shows that ProArray Ultra™ compares favourably to both plate-based platforms, and has lower background and better overall signal to noise performance (figure 7).

Figure 7

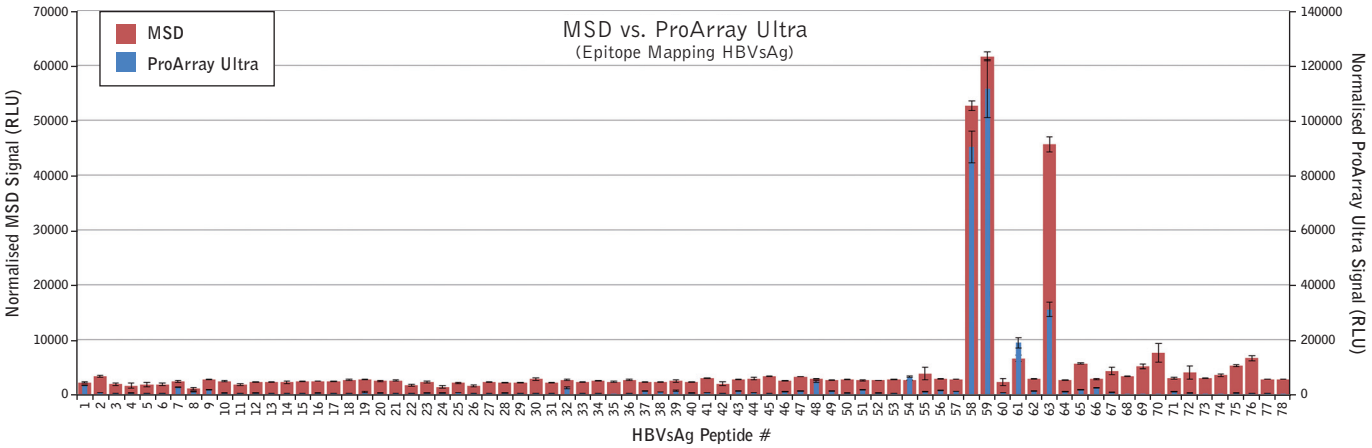


Figure 7: Results of a direct comparison of ProArrayUltra™ (blue bars) and high-performance 96-well MSD® assay technology (red bars) for identical peptide libraries derived from the well characterized Hepatitis B Virus surface Antigen (HBVsAg) incubated with the same anti-HBVsAg seropositive serum sample. All data include error bars reflecting standard error of the mean (SEM) values. ProArray Ultra™ compares favourably to the MSD® plate-based platform, and has lower background and better overall signal to noise performance. The peptides identified as epitopes by ProArray Ultra™ correlate with results from the MSD® assay.

Figure 8

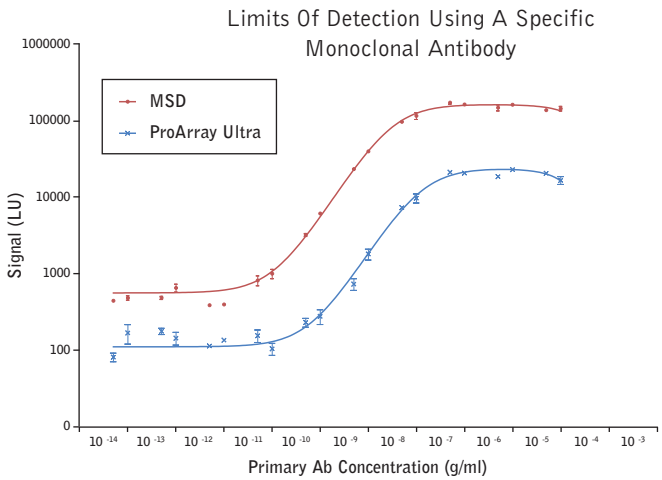


Figure 8: Limit of Detection (LOD) comparison for MSD® and ProArray Ultra™ using a specific monoclonal antibody. A saturation curve profile was obtained with a linear range of approximately 2.5 logs for both ProArray Ultra™ and MSD® assay. Very low signal variation was found for both technologies in terms of signal detected. The limit of physical detection can be seen as ~0.5ng/ml.

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