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Immunomics: Brave new word

Innovators have always known that the marriage of a new technology with almost any field of inquiry is guaranteed to result in exciting new discoveries. That's why I used to run an “invention convention” workshop for my pre-teen science club (called Club DNA, of course). I handed out index cards with words on them and challenged the kids to create a new invention by combining at least two words. The inventions were fantastic and futuristic but one could imagine a day when they might become reality. In the same way, what once seemed fantastic and futuristic was the marriage of computers with large-scale genome sequencing. That combination of concepts lead to the development of many new fields of inquiry. Eventually, the field of “bioinformatics” emerged, with a host of tools that identify putative genes and align and compare gene sequences. But “immunoinformatics” (tools) and “immunomics” (the science) are still brave new words, associated with brand new approaches to vaccine science.

I was a research fellow at the National Institutes of Health, working with Michael Good and Russell Howard at the Laboratory of Parasitic Disease in the late 1980's, when I first heard the words “immunoinformatics” and “epitope-mapping algorithm” used. As a young researcher with a background in Fortran programming and an interest in infectious diseases, this new area of research, pioneered by Jay Berzofsky, Hannah Margalit and Charles Delisi involving computer programs that could be used to decipher signals that turned on the immune system, was one that captured my imagination. To most of my peers, the concept of applying *computers* to vaccine development was as strange a concept as the global shape of the earth was to early cartographers – something that could not yet be fully conceived. At the time, vaccine researchers preferred traditional approaches to mapping T cell epitopes – believing that epitopes identified using hands-on peptide-by-peptide *in vitro* assays were more substantial than epitopes selected by *in silico* methods.

I was convinced, however, that Jay, Charles and Hannah were right. I persuaded my mentors at the NIH to support me while I tested the hypothesis that we could predict malaria epitopes. We synthesized the peptides that the AMPHI algorithmi predicted, and I went off to The Gambia to work at the Medical Research Council to measure T cell responses to malaria. Although I had been to Africa before to work on a measles vaccination campaign as a medical student,[ii] the trip to MRC Fajara was my first exposure to research in the developing world. I met Hilton Whittle there, - a measles researcher who loved living in West Africa, and he taught me to love it just as much. The research was never published but the love for infectious disease vaccine research (and West Africa) remained, so I left Berzofsky's lab in 1989 to pursue further training in infectious disease under Gerald Keusch (at New England Medical Center, in 1989). A few years later, a casual conversation with another researcher at the New England Medical Center (Judy Lieberman) prompted me begin simple searches of protein sequences for patterns of amino acids that corresponded to

MHC (HLA) binding motifs- using Microsoft Word “Find” for this purpose – an idea that might seem as innovative as any of the inventions my pre-teens came up with- but it was from that point of departure, that I truly set out to discover the immunome.

Research in immunomics, by definition, involves risk- the field has never been mainstream. In the early years, it was nearly impossible to get funded, but I was given an opportunity to pursue my dream after winning a starter award from the NIH to use “computational immunology” and “epitope-mapping algorithms” (at this point, I was still using Microsoft Word “Find”) to develop a TB vaccine. I wasn't aware, at the time, that awards came with indirects and that University appointments usually came with startup funds, so I moved from Boston to Brown University with my “training award” into an empty lab that had no equipment other than the few desktop computers I had purchased with my own funds. I had to be innovative in expanding my funding base along with the usual method of grant writing, so I paid my first technician at the “TB/HIV Research Lab” at Brown directly from my own clinical salary. Brilliant Brown students like Gabe Meister and Bill Jesdale joined me, and we sold epitope mapping from our lab bench in order to support further research. My drive for funding led me to establish EpiVax, a biotech company, in order to take advantage of higher SBIR paylines. That initiative paid off, and EpiVax is now a thriving company with a portfolio of big pharma clients and SBIR research grants. I remain its CEO, charting the company's course while maintaining an active academic career.

My explorations have not been confined to the laboratory in Providence. Becoming an infectious disease specialist in the era of AIDS meant caring for HIV-infected women, a pursuit that led me inside prisons, became a passion and launched an online journal (Infectious Diseases in Corrections Report). Infectious disease research also meant travel for fieldwork - measuring immune response in far-flung regions of the world. My well-worn passport is proof that I have pursued this means of discovery - opening doors, in the process, for collaborations with developing world researchers who are interested in immunomics. My students also benefit, traveling with me to Mali, West Africa, where we have established a base for fieldwork and clinical HIV vaccine research (please see http://www.GAIAVaccine.org for more information on our work in Mali). That base has evolved into a full-fledged HIV prevention charity, complete with peer educators, HIV patients, a cutting edge HIV care clinic, and a great group of Malian collaborators.[iii]

The good news is that hard work does pay off. Immunoinformatics tools have improved over time, and T cell epitope-mapping algorithms are slowly being accepted and integrated into the scientific toolkit. Since the T cell epitopes are bound in a linear form to HLA, the interface between ligand and T cell can now be modeled with breathtaking accuracy. A large number of T cell epitope-mapping algorithms have consequently been developed for the purpose of exploring the immunome.[iv,v] Fearless exploration of the applications of immunoinformatics has ensued. For example, if we were to apply an accurate and rapid screen to the entire proteome of a pathogen, uncovering unique peptides that stimulate responses in the human host, the resulting data could be immensely valuable for the development of new diagnostics, new vaccines, and new comparisons with other pathogens.

And if it were possible to examine the human immunome – that is – the set of peptides derived from the human genome, that interact with the human immune system, then it would also be possible to reveal new truths about autoimmunity. My colleagues and I at EpiVax and at the new Institute for Immunology and Informatics (I'Cubed) at University of Rhode Island (http://www.immunome.org) are just setting out on that voyage of discovery – planning to apply immunomics tools to the discovery of the immunogenic components of human pathogens and (human) autologous proteins – we hope to do so in the context of the

"immunome project" which is a new initiative at the I'Cubed, this year. A few examples of the types of discoveries we expect to be making are provided here.

Fishing for antigens in the silicon sea

Consider, for a moment, the interface between the human host's immune system and the proteome of the tuberculosis (TB) pathogen. The dynamics of that interface have been puzzling researchers for years. They have been disassembling it, protein by protein, trying to find out which of the pieces allows human beings to control TB infection, and which of the pieces is associated with its virulence. But the proteome of *M. tuberculosis* (*Mtb*), the etiologic agent of TB, contains almost 4,000 proteins. Evaluating each one could take decades. We think it makes more sense to screen the whole proteome *in silico*, using epitope-mapping tools, followed by a finer focus on the resulting sets of peptides. The resulting peptides can then be used to test whether human subjects who have been infected with the TB pathogen have immune (T cell) responses to the peptides. Such a response would imply that the peptide belonged to a protein that was expressed in the course of infection, and was also processed and presented to the immune system in the course of a `natural' immune response to the pathogen. Thus, by identifying immune response to an epitope, we have revealed an antigen (immunogenic protein) that might not previously have been identified. We have called this approach "fishing for antigens using epitopes as bait".[vi]

With my team at EpiVax, we performed this process for *Mtb*,[vii] and found a remarkable diversity of human immune responses to proteins in the *Mtb* genome that have yet to be ascribed a function, suggesting that human immune response is omnivorous, not focused on single "immunodominant" proteins. The team has also begun to uncover a remarkable similarity between *Francisella tularensis* (the etiologic cause of Tularemia) and (human) self, at the epitope level.[viii] I believe that an immunome-driven approach may uncover differences in the absolute number of T cell epitopes, ranging from fewer epitopes in commensals to many in accidental pathogens. I believe that immunome research will also reveal potential antigenic relationships between unrelated but immunologically similar pathogens, which might pre-set individual immune response. In other words, prior exposure to pathogen A may tune immune response to pathogen B – as Ray Welsh and Liiisa Selisi have suggestedix – and we are collectivelypoised to start charting this new course.

New discoveries about the human immune response to self

Setting aside for a moment the well-established concept of tolerance (to self), we have also used immunomics tools to evaluate each of the autologous proteins that interface with the human immune system and rank them in order of epitope content. Indeed, we have discovered evidence that selected self proteins do have lower inherent epitope content, as compared to random proteins and to known antigens. Antigenic proteins from pathogens, in contrast, generally scored much higher on the scale for epitope content than some non-self proteins.[x] Based on this analysis, it would appear that some common human proteins contain significantly fewer T cell epitopes, when compared to random proteins and common antigens. These discoveries could dramatically change existing dogma.

While pursuing this line of inquiry, we discovered that C3 complement, one of the most abundant serum proteins, received an epitope score of +14.42. Collaborator Paul Knopf immediately pointed out that this observation makes sense, when one considers the role of C3 and, in particular, of a subcomponent of C3 complement, C3d. C3 is involved in both innate and adaptive immune responses, being activated directly by certain non-self antigens. Indeed, the C3d subcomponent of C3 is internalized along with the antigen for processing by the B cell, which would make C3d T cell epitopes available for presentation in MHC. Fearon *et al*. suggested that C3d lowers the threshold for B cell activation but did not

consider epitope content of C3d and T cell help.[xi] My team's current hypothesis is that C3d, which is packed with potential T helper epitopes, is an immunological “on switch” for immune response to antigens that have no other source of T-cell help.[xii]

Little did we know, when we opened our minds to the concept that autologous proteins could play active roles in immune response due to their T cell epitope content, that we would discover the “immunologic off switch” hiding in plain sight. This lead to what is probably the most significant discovery of my entire life. About two years ago, when mapping epitopes contained in IgG for clients who were interested in the potential immunogenicity of their monoclonal antibodies, Bill Martin and I realized that some strong signals for T cell help that were highly conserved in human immunoglobulin, were in fact triggers for regulatory T cells effectively shutting down immune response.[xiii] The discovery of T regulatory cell epitopes in IgG, known as “Tregitopes”, has set off a series of studies in my lab and with collaborators like Jeff Bluestone, David Scott and Mo Sayegh. The ramifications of this discovery are multitudinous – impacting basic immunology research and vaccine science, and potentially leading to new therapies for autoimmune disease and transplantation. These are some of the uncharted waters we have just begun to explore, using our immunomics tools and our knowledge of immunology and infectious diseases as compass and sextant.

New Horizons, Sailing forth

In the past 15 years of my research career, due to the innovative nature of the work we've been doing and the paradoxically difficult grant funding landscape, I have been primarily focused on the practical applications of immunome research (vaccines and therapeutic proteins).[xiv] The research I would like to pursue in the next decade of my life would be substantially different, in that I would develop and apply my immunomics tools and technologies to discover new information about the interaction between host and self, and between host and pathogen, at the epitope level. Those explorations will take place at the new Institute for Immunology and Informatics – where I am the principle investigator of a new $13M NIH grant to develop new vaccines for emerging infections and biodefense.xv Our research at the I'Cubed will be interdisciplinary by definition, using tools that we have carefully validated and accepted for the precision instruments they have become.

In the next decade, I plan to apply my immunomics tools to “immunome research” in the fields of infectious disease and auto-immunity. I will measure the breadth of several immunomes, which would be selected to be representative of commensal, opportunist and accidental human pathogens (such as *Mtb*, pneumococcus, and *Legionella pneumophila*, respectively). I will evaluate the interface between “self” proteins (such as abundant proteins, autoantigens, and proteins located in privileged sites - eye, brain, and testis) and the human immune system. I will match our *in silico* analyses with *in vitro* assays (binding studies, tetramers, and ELISpot assays). I will also pursue *in vivo* validation, using HLA transgenic mice. I plan to continue to teach this excitement to undergraduates at the University of Rhode Island and to explore immunomics collaborations with developing world researchers from Mali.

With time, what once seemed fantastic and innovative is finally becoming accepted research practice. Just as genomics emerged as a scientific field of inquiry, so too, has *immunomics*, the field of inquiry related to the interface between host immune system and proteins derived from pathogens or from self. With the aid of my many collaborators, I hope that we will continue to move immunome research into the mainstream. What was once revolutionary will become more commonplace. When that happens, I fully expect that my crew of scientific visionaries at the I'Cubed and at EpiVax will already be in search of new

horizons, revealing important truths and applying this newfound knowledge for the benefit of science and human health.

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iii. I am a fearless explorer of new horizons because I collaborate with a creative and extremely competent crew of researchers who share similar scientific interests and who have an interdisciplinary skillset. My business partner at EpiVax, William Martin, works primarily in immunoinformatics and runs the day-to-day operations of the company. My academic partners, Paul Knopf and Lenny Moise, have backgrounds in immunology and protein science, respectively. I collaborate in with academic and biotech researchers in many fields: DNA vaccines (David Weiner, Shan Lu), proteomics (David Sherman, Vladimir Brusic), protein drug-related adverse events (Gene Koren), vector vaccine research (Tom Mather) and auto-immunity (David Scott). At EpiVax, I direct the application of our immunomics tools and techniques to developing new products. And now at the University of Rhode Island, I have established a new laboratory for immunoinformatics research, the “Institute for Immunology and Informatics”, also known as the I'Cubed. My two teams of academic and commercial research scientists work in close collaboration and under strict oversight and intellectual property terms established by the University of Rhode Island.

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