[Human Vaccines 4:5, 324-326; September/October 2008]; ©2008 Landes Bioscience

Portrait of a Leading Vaccinologist

The path of discovery

Denise L. Doolan

Queensland Institute of Medical Research; Brisbane, Queensland, Australia

Key words: malaria, vaccine, Plasmodium, immune mechanisms, target antigen, immunomics, molecular vaccinology

I was born in the highlands of Papua New Guinea (PNG) and lived there for the first decade of my life. My parents spent over 30 years in PNG; my father was initially a field officer with the PNG Administration venturing into the wilds of the PNG jungle and ended up as a District Commissioner responsible for law and order and just about everything else. Late each afternoon he would drive around "his" district checking the state of affairs, and I would invariably accompany him. I loved those trips, I loved living in PNG, and I loved the hot-headed sweet-natured Highlanders. Reflecting now on the course of my life, it is obvious that my childhood in PNG had a significant impact on my career choice, introducing me to the wonders of other cultures and providing an appreciation for the impact of disease on public health. My long interest in malaria and the development of an effective intervention to halt that particular plague of mankind was likely kindled by my dislike of the weekly Sunday morning ritual of those little bitter-tasting anti-malarial tablets. My path towards the sciences was also influenced by my grade 8 and 9 mathematics teacher (Ian Jennings), who ignited my interest in solving scientific and mathematical challenges, and my grade 10 science teacher (Craig Fleiter) who introduced me to the intricacies of colorful and aromatic chemical reactions. I was always intrigued with how things worked, how they were put together, and how they could be pulled apart. It was logical, therefore, that I pursue a career in science, despite the advice of my high school counsellor to go into the field of hospitality! When I enrolled at the University of Queensland for a Bachelor of Science degree, chemistry, physics, and mathematics dominated my first and second year courses. A turning point came during a second year physical chemistry tutorial when I realized that despite my curiosity regarding what electrons and protons were doing, I was far more interested in the biological sciences. I subsequently changed my major to biochemistry (at that time, there was no immunology core, and my formal training in immunology was limited to a three hour lecture series) and did an honors thesis on the protein metabolism of ascites tumor cells. With its relevance to cystic fibrosis, this was my first introduction to human health research, and my visit to the cystic fibrosis ward at the Royal Brisbane Hospital provided a powerful and moving example of the need for scientific research to make a significant impact on the health of humans.

After graduation, I accepted a position with the Australian government Commonwealth Scientific and Industrial Research Organization (CSIRO) and then took off for three months trekking in Nepal and India. This first real job was in the field of virology, with the long-term goal of developing a vaccine against Bovine Ephemeral Fever virus (a viral disease of cattle and water buffaloes with a high morbidity and significant economic impact). My duties ranged from shoving minced meat down the throats of cattle egrets to the optimization of growth and purification conditions of a family of Australian rhabdoviruses and the molecular immunological characterization of their viral proteins. The project was a joint project between the Australian and Chinese governments, and I was fortunate to spend six weeks in northern China communicating some of the knowledge gained in Australia to our Chinese counterparts. Given my age at the time (21 years old) and the value placed on age versus experience in the Chinese society, it was an interesting challenge to convey this information to scientists more than twice my age! I was awarded my Masters in Philosophy (Science and Technology) from Griffith University for that work.

In 1989, I moved from veterinary medicine to human health and tropical disease research when I accepted a job as a research technician with Dr. Michael Good who had just returned from a postdoctoral fellowship at the National Institutes of Health in the US to head the Molecular Immunology Laboratory at the Queensland Institute of Medical Research. I subsequently enrolled in a Ph.D. under Michael's mentorship (as his first Ph.D. student) and was awarded my doctorate in 1993 on the molecular immunological characterization of the *Plasmodium falciparum* circumsporozoite protein. Independent by nature, I appreciated being given primary responsibility for my research, including the radio advertisement and recruitment of individuals exposed to malaria as potential blood donors for my vaccine-oriented research. The support and enthusiasm for my research conveyed by those volunteers, who included a number of individuals who spevnt literally years suffering from malaria while in Prisoner of War camps during World War II, remains an inspiration to me. Michael taught me the value of independent thinking, hypothesis testing, and the importance of focusing on the key scientific questions and thinking "outside the box" of conventional scientific dogma. One other invaluable lesson learned from my Ph.D. research was the value of field research.

*Correspondence to: Denise Doolan; Queensland Institute of Medical Research; The Bancroft Centre; Post Office Royal Brisbane Hospital; Brisbane, Queensland 4029, Australia; Tel.: +61.7.3362.0382; Fax: +61.7.3362.0105; Email: Denise.Doolan@qimr.edu.au

Submitted: 07/22/07; Accepted: 07/28/08

Previously published online as a *Human Vaccines* E-publication: www.landesbioscience.com/journals/vaccines/article/6707

I spent three months in Thailand evaluating the immune responses of individuals living on the Thai-Burma border and to this day remain a firm believer that although much can be learnt in the laboratory and with model systems, there is no appropriate laboratory substitute for the natural host-pathogen interaction.

After my Ph.D., I was awarded a National Research Council Associateship (US National Academy of Science; 1993–1996) to undertake a postdoctoral fellowship with Dr. Stephen Hoffman at the Naval Medical Research Institute (NMRI/NMRC). Between the time of my Ph.D. award and my move to the US, I got married and my husband Kevin and I spent a total of 12 years in the United States where I remained affiliated with the US Navy. My original intention had been to go overseas for only 2–3 years! With his enthusiasm, optimism, persistence and dedication towards developing a malaria vaccine, Steve was an inspiring mentor. He provided a powerful example of the need for someone to "champion" a vaccine and of what could be achieved by never accepting failure as an option. During my time at NMRI/NMRC, I gained invaluable experience in all aspects of malaria vaccine development, spanning the areas of discovery research, preclinical research and development, and clinical trials. I learnt the importance of an integrated translational research portfolio or "pipeline" encompassing all areas of research such that the results from one area inform and direct other areas. That is, an iterative, recursive process in which results from clinical trials of first generation vaccines redirect and focus work on antigen discovery, immune mechanisms, and vaccine delivery systems, and in which promising results with new vaccine approaches in animal models are rapidly integrated into clinical trials. I also spent three months in Kenya working with the Kenya Medical Research Institute, CDC, and Walter Reed Army Institute of Research (WRAIR), which reinforced my belief in the value of field work. To see first hand the effect of the disease, particularly evident in a visit to a hospital in a malaria-endemic region where both children and adults were suffering from this disease, provides a powerful confirmation of the value of scientific research.

A consistent theme throughout my research has been the promise of rational vaccine design: the importance of understanding the mechanisms and antigenic targets of protective immunity, and of identifying the appropriate vaccine delivery system capable of inducing the required immune responses against the identified targets. Another underlying theme of my research has been that combating a pathogen as complex as Plasmodium will likely require a vaccine almost as complex as the parasite: a multi-stage multi-immune response vaccine based on more than one parasite antigen and/or their epitopes. Despite the potentially promising results with RTS,S in the field or gene-knockout parasites in the laboratory, I find it hard to believe that a parasite which expresses more than 5300 putative proteins and that is known to exert selective pressure on the evolution of its human host (as evidenced by the frequency and distribution of the sickle-cell gene in populations exposed to endemic malaria transmission) could be halted by a vaccine based on only one of those 5300 proteins. I also believe that the support of a team of talented and dedicated investigators and network of collaborators is a key ingredient for scientific success, and would like to recognize and thank the many friends and colleagues who have made invaluable contributions to my research over the years; when I say "I," I really mean "we."

ABOUT DR. DOOLAN

Dr. Denise Doolan received her undergraduate degree in Science majoring in Biochemistry in 1985 at the University of Queensland, Brisbane, Australia. She subsequently completed a Masters in Life Sciences at Griffith University, Brisbane, while working as an Experimental Scientist at Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO). She completed a Ph.D. in Molecular Immunology in 1993 at the University of Queensland, under the supervision of Dr. Michael F. Good at the Queensland Institute of Medical Research. She was awarded a postdoctoral fellowship from the United States National Research Council, The National Academies, to pursue studies on malaria vaccines with Dr. Stephen L. Hoffman at the Naval Medical Research Center (NMRC) in Rockville, MD USA. For more than ten years Dr. Doolan stayed affiliated with the NMRC's Malaria Program, beginning as a postdoctoral fellow, then being promoted to Director of Basic Research and later Director of Basic and Preclinical Research & Development and finally Scientific Director. During this time she also was affiliated with the Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD USA. Dr. Doolan was awarded a Pfizer Australia Research Fellowship in 2007 and returned to Australia to head the Molecular Vaccinology Laboratory, Division of Immunology, at The Queensland Institute of Medical Research. In 2008 she was appointed as a Credentialed Professor at the Griffith Medical Research College, Griffith University, and as an Adjunct Professor in the School of Medicine, University of Queensland.

Throughout her scientific career, Dr. Doolan has provided critical insights into multiple facets of malaria immunology and vaccine development, extending to the more general fields of immunology, vaccinology and the advancement of public health. Dr. Doolan has received numerous awards and honors, the most recent ones being *Who's Who in America 61st Edition* (2007) and *IBC Leading Scientists of the World* (2008). She served as Associate Editor of *The Journal of Immunology* and is an Academic Editor of *Public Library of Science* (PLoS) One. In the course of her scientific career Dr. Doolan has coauthored over 70 articles in well-established journals including Science and *Nature Medicine*. She also contributed several chapters to books in the field of malaria vaccine development and has edited a book on malaria methods and protocols. Dr. Doolan is inventor on a number of patents in the areas of antigen discovery, immune assays, or vaccination strategies.

During my postdoctoral fellowship, I performed preclinical studies in mice and nonhuman primates demonstrating that DNA vaccines against malaria are immunogenic, and protective in mice, providing the foundation for clinical assessment of this new vaccine technology. Subsequently, a colleague and I conducted the immunological studies that provided the first demonstration that DNA vaccines elicited cytotoxic (killer) T cells in normal, healthy humans. Unfortunately, although highly effective in rodents, and immunogenic for T cell responses in humans, DNA vaccines have largely failed to live up to their promise in larger species. After establishing the technological foundation for testing multi-stage multi-immune response vaccines in a variety of animal models, subsequent efforts have focused on other molecular-based vaccine technologies (recombinant adenovirus, recombinant poxvirus and viral replicon particles). Some of the most promising vaccine candidates have been now transitioned to clinical evaluation, and although efficacy in humans is yet to be demonstrated, first and second generation recombinant adenovirus vaccines are promising.

Other efforts throughout my career have been directed at the "discovery" or "basic research" areas which provide the foundation for the more downstream preclinical and clinical activities. I demonstrated in a rodent model of malaria that the protective immune response directed against liver-stage Plasmodium is extremely complex and varies depending upon both the host and the vaccine. Assuming a similar situation will exist in genetically diverse humans, those findings underscore the need for a vaccine that induces multiple immune responses against multiple target antigens, providing additional proof-of-principle for the concept of a multivalent vaccine. By evaluating a number of different mouse strains which together may be considered representative of a genetically diverse human population, I established that cytotoxic T cell responses, previously considered the optimal in vitro surrogate of protective immunity, do not predict protection in vivo and identified interferon-gamma as an appropriate in vitro marker of protection. Those studies established that vaccines designed to induce infection-blocking immunity (directed against the liver stage) should be designed to induce interferon-gamma responses rather than cytotoxic T cell responses.

With regard to antigenic targets, I completed the cloning, sequencing and characterization of a new malaria antigen expressed during the liver stage and blood stage of the Plasmodium parasite's life cycle and showed that it was protective, providing the first demonstration that an antigen that is first expressed during the liver stage of the parasite life cycle is a target of sterile infection-blocking immunity. For many years, researchers considered that pre-erythrocytic stage (sporozoite/liver stage) immunity was directed only at the sporozoite in circulation, and the liver-stage of the parasite was considered to be "immune privileged" and protected from host immune responses. I subsequently demonstrated that a DNA vaccine based on this antigen was effective in one strain of inbred mice but was ineffective in other strains differing in genetic background; and that a vaccine based on a second antigen had a different pattern of genetic restriction. Immunization with a combination of the two vaccines induced additive or synergistic protective immunity against pathogen challenge. This study established that a vaccine based on a single antigen could not be expected to protect a genetically diverse human population, providing experimental verification for the concept of a multivalent vaccine based on multiple antigens. This strategy negates one of the major obstacles to the development of vaccines that depend on cellular immune responses for efficacy, namely genetic restriction of the immune responses.

An extension of this concept was explored in the human system, in collaboration with Alex Sette and colleagues at Epimmune Inc. (San Diego, CA) who had identified "HLA superfamilies" composed of a number of HLA alleles that share similar peptide binding capabilities and that together represent almost all individuals worldwide regardless of their ethnicity. By identifying and characterizing CD8+ or CD4+ T cell epitopes on *P. falciparum* antigens that bind degenerately to HLA class I or class II molecules, we provided experimental verification of the HLA supertype concept. This research encompassed in vitro studies in the laboratory and in vivo studies in mice, human volunteers experimentally immunized with radiation-attenuated Plasmodium sporozoites, and individuals naturally exposed to malaria in Africa or Irian Jaya. These data demonstrated the feasibility of developing a universally effective vaccine by focusing on a limited number of peptide specificities. Our observations were extended to develop and demonstrate proof-of-principle for a peptide-epitope based screening strategy called ImmunoSenseTM, to identify target antigens and map T cell epitopes from large and complex genomes by integrating genomic and proteomic data with bioinformatic predictions, HLA supertype considerations, high-throughput binding assays, and cellular immune assays. Of particular significance, this strategy relies on screening using T cell responses, as opposed to the more typical readout of antibody responses. Using this approach, we identified novel *P. falciparum* antigens recognized by T cells from volunteers immunized with radiation-attenuated *P. falciparum* sporozoites, and demonstrated differential recognition by cells from volunteers who were protected versus not protected against sporozoite challenge. Importantly, some of those new antigens were more antigenic than well-characterized *P. falciparum* antigens currently in clinical evaluation suggesting that they may be better vaccine targets. Recent studies have established that one of the antigens identified using the ImmunoSenseTM strategy is a target of protective immune responses in the *P. yoelii* rodent model, and that vaccines based on this antigen can protect against both homologous and heterologous sporozoite challenge. This is a significant finding since cross-species protective immunity has not been demonstrated for those *P. falciparum* antigens currently in clinical evaluation.

More recently, with Phil Felgner and colleagues at the University of California Irvine and ImmPort Therapeutics Inc. (now Antigen Discovery Inc.), I have explored the potential of antigen identification using protein microarrays. Using *P. falciparum* as a model pathogen, we have demonstrated the potential of protein microarrays for determining antigen-specific serology on a whole proteome scale, comparing immunoreactivity profiles amongst clinically distinct populations, and identifying novel antigens from genomic sequence data for vaccine applications. Together, this protein microarray and ImmunoSenseTM research fall under the umbrella of an exciting new field called "immunomics" which essentially bridges the disciplines of genomics and proteomics by involving the immune system. With the advent of the era of genomics and the availability of the genomic sequence of the *P. falciparum* parasite and of its human host, we now have an invaluable opportunity for a new approach to malaria vaccine development. Although the translation of "omics" into the development of effective interventions against those pathogens

which have thus far proved elusive has not yet been demonstrated, that promise still exists. How to best achieve this translation is both exciting and challenging.

This is a challenge that I am continuing in Australia, finally returning home after many years overseas to have a baby and bring up my daughter Caitlin as a little Aussie. At 20 months of age she is already well travelled, having accompanied her mother to two international conferences and two national meetings. Her first meeting was at only 13 days old when I had to fly to Sydney to present and interview for a Pfizer Australia Research Fellowship. I was fortunate to be awarded this fellowship to support my return to Australian science, and am proud to hold this fellowship at the institute where I did my Ph.D.: the Queensland Institute of Medical Research in Brisbane, where I head the Molecular Vaccinology Laboratory.

Despite many decades of research by talented researchers, there is still no licensed malaria vaccine. Some scientists who have played an influential role in my career have gone back to the drawing board and are pursuing whole organism vaccine approaches, noting that such approaches have proved successful in other diseases. However, I still believe in the wonders of science and the powers of modern technologies, and remain committed to the rational development of a vaccine against a parasite which affects 40% of the world's population and has a staggering toll in terms of mortality, morbidity, and economic hardship.

"The future influences the present just as much as the past."
–Friedrich Nietzsche