Interview

Cancer vaccines and immunotherapy of autoimmune diseases

Vasso Apostolopoulos heads the Immunology and Vaccine Laboratory of Burnet Institute (Australia). She completed her PhD in 1995 at the Austin Research Institute (Australia). Her work on cancer vaccine development has been in clinical trial since 1994. In the last 16 years, she has received more than 100 awards/honors for her achievements. Most notable are the Premiers Award for Medical Research, Victorian Young Australian of the Year, Channel 10/Herald Sun Young Achiever of the Year, Victorian Tall Poppy, inductee into the Victorian Honour Roll of Women, a torchbearer in the International Athens 2004 Olympic Torch Relay and was named Woman of the Year. In 1998, she received the prestigious NHMRC CJ Martin Fellowship and undertook research at the Scripps Research Institute (CA, USA) until 2001. In 2001, she returned to the Austin Research Institute (now the Burnet Institute) and was a NHMRC RD Wright Fellow until 2007. She is currently a Professor, Principle Research Fellow, Sir Zelman Cowen Cancer Research Fellow, Australia Day Ambassador and a Patron of the Womens Health Network. She has published more than 120 papers and books and is an inventor on 12 patents. She is on the board and a regular reviewer for a number of journals. Her current interests are in the development of new improved cancer vaccines and new modes of antigen delivery for immune stimulation. She is also interested in the 3D x-ray crystal structures of peptide–MHC complexes.

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■ Your current interests involve the development of new improved cancer vaccines and new modes of antigen delivery for immune stimulation. What led you to your interest in these areas?

During my PhD, I worked on mucin (MUC)1 and its potential as a target for tumor immunotherapeutic applications. In the early–mid-1990s, not much was known about its immunogenicity with regard to eliciting T-cell responses following immunization in mice or humans. In addition, I had worked on a number of methods of delivering MUC1 to antigen-presenting cells (APCs) in order to enhance cellular immunity. Mannan was one method that was developed and it was used to conjugate to MUC1. Mannan–MUC1 conjugate was able to efficiently deliver MUC1 to APCs to induce cellular and/or humoral responses in mice. This work quickly moved into studies in monkeys and then in human clinical trials. Since then, I have been interested in developing new, improved cancer vaccines and new modes of antigen delivery for enhanced immune stimulation.

■ You are also interested in the 3D x-ray crystal structures of noncanonical peptide–MHC complexes. What is its importance in immunotherapeutic applications?

Pool sequencing of the peptides isolated from particular class I alleles suggests that there are common features of peptides that bind particular alleles. These data have been pivotal in shaping our view of which peptides bind MHC molecules. However, the processes involved tend to isolate peptides with high affinity for class I. Moreover, these data can be skewed by particularly abundant species. As the algorithms designed to predict class I-binding peptides that are based on our current ‘rules’ are, at best, approximately 80% accurate, there are significant holes in our understanding of which peptides bind to MHC molecules (and hence the way they bind). I am interested in evaluating the binding of a range of different peptides (including noncanonical; peptides that do not contain canonical anchor motifs) to MHC molecules. A critical issue is whether these peptides are representative of a much larger and yet relatively undetected or unstudied pool of peptides or whether

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they represent a relatively rare cohort that normally do not compete well with peptides with canonical motifs. Hence, their identification on tumour cells will elucidate their relevance in vivo. Understanding the structural basis by which these peptides bind MHC should resolve some of this confusion and might provide useful information about alternative targets for peptide-based vaccine strategies.

■ You have published many articles on the use of the tumor-associated antigen MUC1 in cancer vaccines. Can this approach be applied to various types of cancer and what is the future prospect of MUC1-based cancer immunotherapy in your opinion?

Since the isolation and sequencing of MUC1 20 years ago, numerous papers have been published to describe its characterization, expression on tissues, mechanism and immune responses in animal models. Being used as a target for tumor immunotherapy studies, MUC1 quickly entered into human clinical trials in cancer patients. MUC1 is an attractive target for immunotherapy as is it overexpressed on adenocarcinoma cells, such as breast, ovary, prostate and kidney. In addition, MUC1 is expressed on a number of other carcinomas including, head/neck, esophageal, gallbladder, hepatocellular, urinary tract, thyroid, endometrial cancers and most of the blood malignancies. All these features have formed the basis of studies on MUC1 as a target for tumor immunotherapy. Thus, MUC1 immunotherapy could be applied to various types of cancers. In human clinical trials, the majority of patients used to test the efficacy of MUC1-based vaccines had advanced metastatic disease and, thus far, robust antitumor responses with long-term complete regression have yet to be seen. Only a couple of trials have involved patients with early disease or no evidence of disease, and MUC1 immunotherapy was beneficial. In our experience and after 13 clinical trials, early stage patients demonstrated protection against recurrences 8 years on after MUC1 immunotherapy. In the next 5 years, more results from Phase II and III clinical trials will become available, in addition, new strategies to immunize against MUC1 will be entering

into early Phase I trials. All this information will lead to a therapeutic cancer vaccine, which could be injected not only in early cancer patients but also in high-risk individuals.

■ Besides cancer vaccines, your research also involves immunotherapy of autoimmune diseases such as multiple sclerosis. In a recent publication, you have mentioned the therapeutic potential of cyclized antagonist peptides. Is multiple sclerosis a good model to study this class of therapeutic agents and what is its future application in other autoimmune diseases?

Altered peptide ligands (APLs) constituting mutations at T-cell receptor contact sites when bound to MHC, which can alter immune responses from Th1 to Th2, are a promising approach to the immunotherapy of autoimmune diseases including multiple sclerosis. Cyclization of peptides is of great interest, since the limited stability of linear peptides restricts their potential as immunotherapeutic agents. Together with my collaborators in Greece, Professor John Matsoukas and his group, we designed and synthesized a number of APLs of linear and cyclic analogs from MBP peptide, the immunodominant peptide epitope identified in multiple sclerosis. Mutations to the native (agonist) peptide are able to reduce Th1 responses; however, when conjugated to our novel carrier, reduced mannan, Th1 responses were diverted to Th2 responses. A number of studies have also demonstrated that the use of APLs are able to alter immune responses in a number of other autoimmune diseases, such as rheumatoid arthritis, allergic diseases, diabetes, myasthenia gravis and primary biliary cirrhosis. APLs show great promise as therapeutic agents in autoimmune diseases.

■ In your opinion, what are the main obstacles that researchers are facing in immunotherapy?

The most successful vaccine was the development of the smallpox vaccine in the 1700s by Edward Jenner, and smallpox eradication was declared in the late 1970s. This vaccine regime made use of cowpox vesicles to immunize against

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smallpox, today termed 'mimicry'. No immunodominant or protective antigen was known, no type of immune response generated was known and no difficulty in production procedures were apparent, yet this vaccine remains the most successful vaccine to date. With attempts to control more complex diseases and the need to improve vaccine safety, stability, efficacy and cost, there is pressure for more precisely defined vaccines. Public awareness of health and safety issues is now far higher than it was 70 or more years ago. Vaccines must now meet higher standards of safety and biochemical characterization than they did in the past. Thus, we need to come up with new molecular and biological technologies in the fields of genetics, chemistry and immunology in order to develop new and improved vaccines, in an attempt to move from traditional live virus/bacterial/attenuated vaccines to the 'theoretical' safer but 'less immunogenic' vaccines. To enhance immunogenicity of the 'safer' vaccines, researchers have to identify T- and B-cell epitopes, binding and presentation by MHC class I or class II alleles, x-ray crystallography to identify structure and regions of a protein/virus/bacteria, DNA and recombinant DNA approaches, peptide synthesis, protein production, identification of antigens expressed on a protein/virus/bacteria, develop safe adjuvants, identify which cytokines increase immunity and come up with methods to deliver the highly 'purified' antigen to APCs for stimulation of T cells. More recently, the role of Toll-like receptors have shown importance in enhancing immune responses of antigens/peptides. Once a vaccine candidate has been designed, then we must come up with techniques to assess its immunity. Such methods include: cytotoxic T-lymphocyte assays, proliferation assays, tetramers, ELISPOT and ELISA assays. If no immunity is shown, then the candidate will not be continued. But are

our methods of assessing immunity optimal? So, are we going backwards? Why are we developing all these methods? Why are we going round and round in circles, when the whole virus in the smallpox vaccine was protective? Why not use whole tumor cells in cancer immunotherapeutic approaches that include all antigens and have the potential to have epitopes that could be presented by a number of HLA types? Some laboratories do in fact use this approach, but they still have to show that this approach is immunogenic by demonstrating responses to certain antigens. Moreover, in tumor immunotherapeutic clinical trials, the patients studied are usually advanced metastatic cancer patients. Are these the correct cohort of patients to study? Despite this, the future does hold promise for new improved, safer vaccines to prevent, control and possibly eradicate diseases, including cancer and infectious diseases.

■ **Finally, where do you think your research focus will be over the next 5 years?**

I want to further our understanding of peptide-MHC interactions, which will aid in new approaches to peptide-based vaccines, in particular against cancer antigens. In addition, I would like to develop new delivery methods for peptide-based vaccines. Finally, I will pursue the use of APL in the immunotherapeutic applications to autoimmune diseases.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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