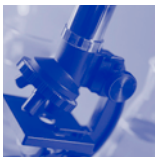

MEDICAL ONCOLOGY GROUP OF AUSTRALIA PIERRE FABRE CANCER ACHIEVEMENT AWARD*

Papillomavirus specific immunity as a means to prevent deaths from cervical cancer

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Cervical cancer was shown by Professor Harald zur Hausen in the early 1980s to be initiated by infection of cervical epithelium with certain human papillomaviruses, now designated as "high risk" papillomaviruses. While working with Dr Ian Mackay in Melbourne I showed, in collaboration with Dr Gabrielle Medley, that papillomavirus infection was also associated with pre-cancer of other anogenital epithelium.[1] The increased incidence of anal pre-cancer we observed in immunosuppressed males with HIV/AIDS inspired further studies on the immune responses to papillomaviruses and particularly to the antigens expressed in cervical cancers. Over a number of years, members of my research group, and of many other groups worldwide, have studied immune responses to human papillomavirus (HPV) encoded antigens expressed in infected cervical epithelium and cervical cancer cells, with a view to developing vaccines to prevent and to treat cervical cancer.

In the early 1990s, the late Dr Jian Zhou and I pursued the idea of making a vaccine to prevent cervical cancer based on papillomavirus like particles, comprising the coat proteins of HPV, produced in vitro using recombinant DNA technology. This approach was adopted because conventional vaccine strategies (killed virus, attenuated virus) were not feasible for papillomavirus, as papillomaviruses could not be propagated in vitro. Using papillomavirus genetic material from a clinical isolate and the then relatively novel and challenging technique of long PCR, Dr Zhou produced expression clones for the capsid proteins of the highest risk human papillomavirus (HPV16). We established that to produce self assembling virus like particles of HPV16 in vitro, it was necessary to express the viral capsid genes in a eukaryotic system. This was achieved by producing recombinant vaccinia virus encoding expression of the relevant proteins.[2]

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We further established that it was necessary to express the major capsid protein (L1) from the second initiation codon, as expression from the first initiation codon produced a protein that did not acquire the correct structure or self assemble into virus like particles. These findings were presented to the scientific community at a meeting of the International Papillomavirus Society in Seattle in September of 1991, and shortly thereafter published in the journal *Virology*. This disclosure was followed by reports of similar findings from many groups (Kirnbauer, Schiller and Lowy at the National Institute of Health,[3,4] Schlegel and Bennet Jensen[5] at Georgetown University and Bonnez and Rose[6] at the University of Rochester) at that time interested in HPV

vaccine research. We and the other mentioned groups went on over the next few years to demonstrate immunogenicity of the virus like particles produced using recombinant DNA technology in eukaryotic cells and their more efficient expression using a range of eukaryotic expression systems, particularly baculovirus and yeast. This global collaborative effort has led to the production of two commercial vaccines (Cervarix® and Gardasil®) based on virus like particle technology which have been shown near 100% effective at preventing HPV associated cervical pre-cancer associated with the relevant viruses. These vaccines, which have the potential to prevent at least 70% of cervical cancer and over 200,000 deaths worldwide, have recently been introduced into routine childhood vaccine schedules in Australia, the US and elsewhere.

Another major theme of the research effort of the scientists working in my group at the Princess Alexandra Hospital has been the development of effective immunotherapy for existing papillomavirus infections. Treatment of cancer and of chronic viral infection by induction of immune responses against specific antigens expressed by the tumour cell or virus infected cell has been extensively studied in animal models and in human subjects. In principle, induction of effector T-cells targeted at the relevant antigen by immunisation should result in specific elimination of the relevant cells. In practice, induction of effector T-cells of the relevant specificity can be achieved in animal models and in human subjects. However, while effectiveness of these cells can be demonstrated both in vitro and in some in vivo models based on transplantable tumours, clinically useful outcomes in spontaneously arising animal and human tumours, and in chronic viral infections in animals and humans have been much harder to demonstrate. Early animal studies undertaken by Tindle and various students in the group demonstrated the immunogenicity of the major papillomavirus encoded tumour specific antigens (E6 and E7) in animal models.[7,8] Clinical trials conducted in patients with cervical cancer as investigator initiated studies,[9] and more recently in cooperation with CSL in patients with cervical pre-cancer (CIN 2,3),[10] have shown that it is possible to induce specific immune responses against the relevant papillomavirus encoded tumour specific antigens (HPV16 E7 and E6). However, for a number of reasons the clinical trials to date have not been sufficient to address the question of whether such immune responses are clinically useful.

Animal models of cervical cancer are available, and transplantable tumours expressing the relevant papillomavirus specific antigens have been used to test potential vaccines.[11] These models are based on transplantable tumours induced to express HPV16 E7 protein, which grow continuously in immunocompetent mice and eventually kill the animal. A wide range of potential vaccines are at some level effective at eliminating such transplantable tumours in vivo.[12] However, the tumour protection assays are not well able to discriminate between potential vaccines, as the tumours are easily cured by vaccines shown non-effective in the clinic. To develop better models for studying cervical cancer immunotherapy we have worked, in collaboration with Lambert and Griep at the McArdle Institute, with mice which express the relevant cervical cancer associated antigens as transgenes in the skin, and specifically in keratinocytes expressed from the Keratin 14 promoter.[13,14] These mice are tumour prone, but are not ideal models for studies of immunotherapy, as the constitutive expression of E7 not only induces tolerance to this antigen, but also impairs general immune responses by altering thymus biology. Rather, we have used skin from transgenic animals expressing papillomavirus and other antigens[15] transplanted on to immunocompetent animals as a model for testing specific immunotherapy.

All tumour specific antigens are not equal in this transgenic grafting model. Some antigens are able to induce graft rejection without further immune manipulation. These include well recognised non-self antigens (ovalbumen) and neo-self antigen (human growth hormone). Other antigens, including the E7 and E6 proteins of HPV16, do not induce spontaneous graft rejection, though their presence does not impair rejection of grafts bearing "good" antigens. Thus these antigens are not well presented to induce an immune response, perhaps because they are expressed at low levels, or are non-secretory proteins. Such E7 expressing grafts therefore serve as models for testing E7 specific immunotherapy.

We have used transgenic skin grafts expressing E7 or other antigens to study many aspects of tumour immunotherapy over the last decade. The broad questions we have addressed are:

1) What are the immune effector mechanisms that can eliminate epithelium expressing E7 proteins?

2) How does the local environment influence the effectiveness of immune effector cells?

3) How can the effectiveness of antigen specific immunotherapy be enhanced through temporary resetting of the innate immune system?

While these studies are ongoing, several practical conclusions can be drawn from the work to date, which we are in the process of translating into the clinic. Perhaps unsurprisingly, antigen specific CD8 T-cells are necessary for elimination of grafts in this model – and are, for newly placed grafts, sufficient provided that they are administered or induced in sufficient numbers.[16] However, conventional immunisation strategies do not produce sufficient effector cells, or alternatively do not enable these cells to reach their target, in contrast to antigen specific CD8 T-cells transferred in large numbers to a recent graft. The reasons for this discrepancy are currently under investigation. Inflammation can be shown to play an important role in determining the local effectiveness of immune effector mechanisms induced by vaccination or prior priming, as antigen bearing grafts protected from effector mechanisms by their temporary depletion will heal in place and are then resistant to further effector cells induced by immunisation or grafting (Zhong et al submitted). Further, application of a pro-inflammatory stimulus to a healed graft in the form of a toll like receptor (TLR) agonist such as imiquimod, which

promotes local inflammation by activating bone marrow derived cells expressing TLR 7 and 8, enables their subsequent rejection. Thus, local regulation of the relatively anti-inflammatory environment of skin tumours may facilitate effective vaccine induced immunotherapy for epithelial tumours.

An alternative approach, which Dr Liu in my group is investigating, is to remove one of the anti-inflammatory cytokines, Interleukin 10. This cytokine plays a key part in regulating the induction and function of cytotoxic effector T-cells, particularly where there is chronic exposure to antigen.[17,18] Therapeutic use of IL-10 inhibitors, such as antibody to IL-10 and soluble IL-10 receptor, are under consideration as interventions for a number of autoimmune diseases and their temporary use might also be expected to enhance the effectiveness of immunotherapeutic interventions for tumours, particularly those of epithelial origin which are likely to secrete this cytokine.

Thus, the future for immunotherapy for cervical cancer and its precursor lesions is a little more complex than the future for prophylactic vaccines for HPV-associated cancer. An additional dimension noted by many researchers, including ourselves, is that fully transformed cervical cancers often exhibit defects in antigen presentation.[19] This is a mixed blessing – on the one hand it tends to confirm the idea, suggested by the increased incidence of these cancers in immunosuppressed patients,[20] that immune responses to tumour specific antigens are relevant to control of cervical cancer. On the other hand, these lesions in the antigen presenting machinery may ultimately limit the use of antigen specific immunotherapy targeted at HPV antigens to cervical cancer precursor lesions rather than invasive cancer.

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**The Medical Oncology Group of Australia Pierre Fabre Award is granted annually in recognition of an outstanding contribution to the scientific study of cancer and/or to the control of cancer in Australia by an Australian scientist, clinician or other healthcare professional. Professor Ian Frazer was the 2006 recipient of the award.*

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