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The HPV Vaccine Story

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CERVICAL CANCER AS A SEXUALLY TRANSMITTED INFECTION

Research on the cause of cervical cancer first appeared in the scientific literature in the 1840s. As a common cancer, it was contrasted with breast cancer in a study demonstrating a statistical method to show that the distribution of disease between two different communities (nuns and married women) was unlikely to be due to chance alone.[1] While these data suggested a link between cervical cancer and sexual activity, it was not until the 1960s that an association of cervical cancer with frequency of sexual intercourse was reconfirmed, including an observation that cervical cancer was more commonly linked with "first coitus on the ground than with first coitus in a hotel or motel"![2]

IDENTIFYING THE RESPONSIBLE INFECTION

Herpes simplex virus (HSV) was the sexually transmitted virus first considered a candidate causative agent for cervical cancer.[3] However, while serological evidence of increased antibody to HSV in the blood of cervical cancer patients confirmed an association of the infection with the cancer, it was unclear how a virus which kills the cells it infects might promote cancer in those cells. In the 1970s, recognizing that papillomaviruses caused cancer in animals and commonly produced warts in the genital tract, Harald zur Hausen hypothesized[4] that human papillomaviruses (HPVs) might be responsible for cervical cancer. Zur Hausen, with Gissmann, then demonstrated in 1983 that papillomavirus DNA related to but not identical with that of the papillomavirus responsible for genital warts could be found in a majority of tested cervical cancer specimens.[5] The novel papillomavirus, which was named HPV16, and a number of related α-papillomaviruses designated as "high-risk" were subsequently found in almost all cervical cancer cells, and these viruses were subsequently shown to be capable of capable of transforming and or immortalizing many cell types *in vitro*, resulting in HPV-16 becoming the first virus held as causative for a human cancer.

MY INTRODUCTION TO HUMAN PAPILLOMAVIRUS

In 1981, as a young clinician scientist with an interest in immunology, I came to Melbourne from Scotland. In 1984, after a visit to Harald zur Hausen in Germany, I became interested in the increased incidence of HPV infection in immunosuppressed patients with immune system dysfunction due to what was subsequently recognized as HIV infection. With Dr. Gabrielle Medley, I demonstrated a connection between HPV infection and anorectal dysplasia, a premalignant change in the epithelial cells lining the anal canal and a precursor of anal cancer,[6] thus defining a second cancer likely to be casually associated with HPV infection. At that time, little was known of the immunology of HPV infection; there were no blood serological tests for HPV infection and no *in vitro* system for propagating the virus in cell cultures, as would be used to isolate other viruses. Initial studies using HPV proteins derived from bacterial expression systems, using the then relatively new technology of genetic engineering, led to the realization that we needed an animal model of HPV infection to study whether and how it might cause cancer. This encouraged me to visit Cambridge and the lab of Dr. Margaret Stanley, ostensibly to make a mouse transgenic for the E6 and E7 oncoproteins of HPV as a model of persisting infection of skin by HPV. That effort failed, although the goal was subsequently achieved by others.[7] The visit to Cambridge led to a chance meeting in 1989 with Dr. Jian Zhou (Figure 1), a virologist visiting from China to a neighboring lab, whom I discovered was also interested in HPV. We put together a plan to make an infectious papillomavirus in the laboratory, using the mammalian cell gene expression techniques that had recently become available to produce the HPV structural proteins (L1 and L2) in cell culture. Jian was interested in the HPV mechanisms of cellular transformation, and I wanted to study the immune response to this oncogenic virus. Both of us needed intact virus to achieve our aims.

PARTNERING WITH JIAN ZHOU: THE KEY TO THE VACCINE

Jian and his partner Xiao-Yi Sun agreed to move to Brisbane and take up positions in my laboratory in 1990. Together, we worked on building the shell of the papillomavirus by expressing the viral capsid proteins in monkey kidney cells, using a recombinant vaccinia virus vector to express the proteins. The viral capsid consists of 360 copies of the L1 capsid protein together with a variable number of L2 proteins (Figure 2). We had available the HPV16 capsid genes from a precancer clinical lesion, which proved fortunate, because the clones of the L1 and L2 capsid genes then available from the Gissmann lab, which were derived from capsid genes from a cancer, were subsequently found to have a mutation that prevented capsid assembly from the expressed major capsid protein.[8,9] Nevertheless, it took a year of experimentation to find a method to express the two capsid proteins, L1 and L2, that allowed their assembly into something resembling the viral capsid. Along the way, we learned that it was necessary to express the L1 major capsid gene from the second translation initiation codon and that it was also necessary to change the vaccinia transcriptional promoter sequence and coexpress the L2 protein. Eventually, when we were nearly at the point of giving up, we produced and purified a few viral capsids, which

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Figure 1. Dr. Jian Zhou and Dr. Ian Frazer together at Wenzhou Medical University in 1993. Photo credit: unknown.

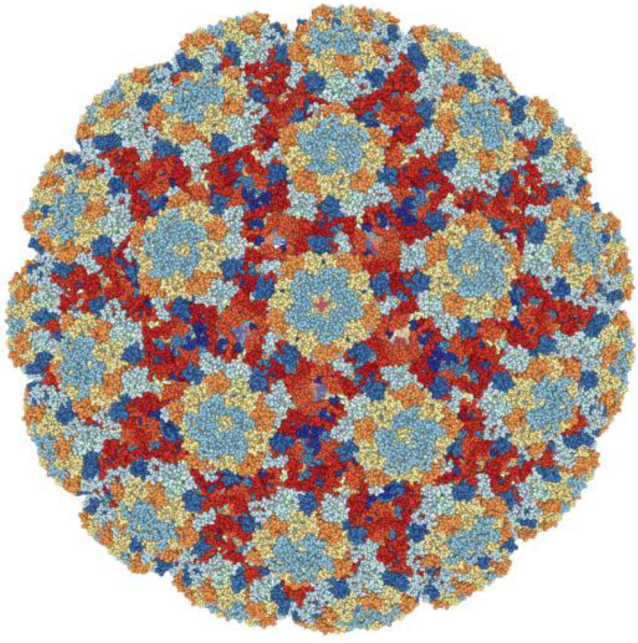

Figure 2. Human papillomavirus virus like particles, consisting of 72 pentamers of the L1 protein.

Dr. Deborah Stenzel was able to find on an electron micrograph.[10] Realizing that if there were ever to be a vaccine to prevent HPV infection it would be based on these viral capsids (or virus-like particles (VLPs) as they are now more commonly known), we filed a provisional patent protecting the means of their production in 1991.[11] We subsequently went on to make infectious papillomaviruses in the lab; however, the yield was small[12] and the studies on HPV immunity better pursued by other means.

FROM IDEA TO PRODUCT: PROTECTING THE INTELLECTUAL PROPERTY

Creation of the VLPs and demonstration of their immunogenicity in animals[13] were the practical outcome of the work that we undertook and became our contribution to the scientific basis of HPV vaccines. It was however the beginning of a long battle to produce a functional vaccine, which Jian was not to see the conclusion of, as he passed away in 1999. First, it was necessary to persuade the vaccine companies that a vaccine would be useful. It did not take too long to convince the research leaders about the science,[14] but it took rather longer to convince business developers to appreciate that there might be a market. To achieve a benefit for our University, we also had to prove that we were first to discover the technology, which took much longer than the invention process itself, and ended up in a four-way contest with three American universities. This was resolved in favor of the University of Queensland in 2006 in the United States patent court of appeal.[15] This success was largely dependent on our ability to show in 2004 that our original cloned gene of the L1 capsid protein was the correct native sequence,[16] rather than a mutated version of the gene as had been used initially by others.[8,9]

FINDING A COMMERCIAL PARTNER

Meanwhile, the vaccine companies, more specifically Merck and GSK, pursued the commercial development of HPV VLPs as a potential vaccine for preventing HPV infection. The commerciality of a vaccine depended on showing that infection with so-called high-risk HPVs, which were strongly associated with cervical cancer, was actually rather common. These studies, largely undertaken among college students, demonstrated that at least 50% of sexually active women would acquire a high risk HPV within 3 years of starting college[17] and that while the majority would clear the infection spontaneously a small percentage would go on to develop precancer. Trials of HPV VLP-based vaccines[18] showed that infection could be prevented but not cured by immunization, and in 2007, HPV

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vaccines based on VLPs were licensed in many countries including the United States and Australia as a means of preventing cervical cancer. Given the association of HPV infection with oropharyngeal cancer, the vaccines may also impact more widely on the cancer burden, though a study to prove this is unlikely as the time between infection and cancer development is 20−30 years.

GLOBAL ERADICATION OF CERVICAL CANCER: A GOAL FOR THE 21ST CENTURY

While initial public and government reaction to a vaccine designed to prevent a sexually transmitted infection was mixed, vaccine uptake has now been successful enough in developed countries to enable a prediction of the eradication of cervical cancer.[19] Although there are still barriers to be overcome for universal immunization,[20] the adoption by the WHO of a global campaign to eradicate cervical cancer based on HPV vaccination has led to a prediction that the 21st century may be the last during which we will experience HPV-associated cancer, currently responsible annually for over 300 000 deaths across the globe.

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Notes

The author declares the following competing financial interest(s): I.F. is employed by the University of Queensland which receives royalty payments from the sale of the vaccines described in this article.

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