VACCINES TO PREVENT AND TREAT CERVICAL CANCER

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Many epidemiologic studies now support a hypothesis, formulated 20 years ago by Gissmann and Zur Hausen, that papillomaviruses (PV) are a major cause of cervical cancer and other anogenital malignancies. PVs come in many varieties or genotypes. Persistent infection with one of a few high risk types of human papillomaviruses (HPV), of which HPV16, 18 and 33 are the most common examples, conveys significant risk of anogenital malignancy. There exists approximately a one in 20 lifetime risk of development of cervical cancer following persistent infection with a high risk type of HPV. The World Health Organization has determined that high risk HPV infection is therefore a "necessary" factor for development of cervical cancer[1], and the contribution of other identifiable genetic and environmental factors appears to be relatively small. Thus, prevention and control of cervical cancer might best be achieved through vaccine-mediated prevention of HPV infection, and/or elimination of persistent infection at a high risk for development of squamous malignancy. Initial work on therapeutic vaccines for cervical cancer allowed observations on production of PV virions which became the basis of current vaccines designed to prevent infection with HPV.

Natural immune responses to PV infection point the way to a vaccine

Generally, effective viral vaccines work through generation of neutralising antibody. Protection is proportional to the amount of antibody available at the virus entry site, and lasts as long as neutralising antibody persists. Larger scale longitudinal studies of papillomavirus seroepidemiology are available only for a limited subset of genital PV genotypes[2]. These demonstrated that papillomavirus infection naturally induces relatively low titres of neutralising antibody, and that some infected individuals seemingly acquire and clear infection without ever developing measurable antibody. Thus, serology would appear to have little role to play in screening for risk of cervical cancer. Following natural infection, serum antibodies to PV are largely directed against conformational epitopes displayed on the outer aspect of the virus capsid, and directed to the major capsid protein L1. Such antibodies are genotype specific, and mostly of IgG type, and are present only in low titre in mucosal secretions. The limited epidemiologic evidence available to date suggests that prior infection with a particular PV genotype is host protective against further infection with that genotype, though not with other types. Thus, vaccines to prevent PV infection will likely be designed to induce antibodies directed to conformational epitopes of the L1 capsid protein, and would be predicted to be type specific[3]. Papillomaviruses cannot be grown in tissue culture or purified in bulk from infected tissues, and these problems have slowed the development of a vaccine for this virus. We were fortunate to observe in 1990 that the L1 capsid protein of HPV16, when expressed in eukaryotic cells using recombinant DNA technology, assembled into virus-like particles (VLP)[4], and these VLPs have become the basis of the current efforts in PV vaccine development.

Vaccines to prevent PV infection and cervical cancer

VLP-based vaccines to prevent HPV infection are now in late phase clinical trials. One study reported at a recent international meeting included a post-hoc analysis of the results of a number of phase I and II studies of HPV16 specific PV vaccines based on recombinant L1 virus-like particles. While post-hoc analysis can be deceptive, the results, taken at face value, demonstrated absolute protection against new incident HPV infections of type 16 amongst individuals vaccinated with a range of doses and formulations of HPV16 VLPs (0 cases in 66 subjects), and several incident cases (nine in 129 subjects) amongst those given placebo vaccine. Similar numbers of incident cases of HPV infection with other genotypes in both groups confirmed that differences were unlikely to be due to chance variation in risk, and also confirmed the type specificity of vaccine-induced host protection. Several reported studies in human volunteers of VLP-based HPV vaccines of types 11 and 16 show good safety profiles and almost universal induction of high titres of virus-specific antibody, suggesting strongly that PV vaccines are likely to be at least partially effective in prevention of new infection with the high risk PV genotypes[3]. Modelling the decline of antibody titre following vaccination in the early phase human studies suggests that protection against infection will persist, like the protection following immunisation with the particle-based vaccine for Hepatitis B, for several years if not decades. Animal and human studies suggest that it should be possible to induce simultaneous protection against many types of PV with multivalent vaccines, though the limits to this have yet to be tested, and priming through past infection with one genotype may limit the ability of the immune system to respond adequately to other types incorporated into a multivalent vaccine, an issue not easily resolvable in animal trials. Mucosal antibody seems to be induced by systemic delivery of VLPs and can also be induced or boosted by mucosal delivery. This mode of delivery, however, would need to be demonstrated to be of comparable duration and protection as systemic delivery before it could be considered a preferred delivery route for vaccine in developing countries. Confidence that VLP-based vaccines have the potential to prevent PV infection has focused attention on both the cost-effectiveness and the feasibility of how these vaccines could be delivered to the developing world to have an impact in preventing cervical cancer. A potential advantage to local, cheap and simple production of VLPs has led to exploration of production of VLPs in plants and other simple expression systems.

Other means of inducing protection against PV infection have been trialled in animals. Polynucleotide vaccines are cheap to produce and heat stable, and may overcome some of the difficulties of delivering VLP vaccines to the developing world – where currently no vaccine program accesses women immediately prior to the onset of sexual activity. Polynucleotide vaccines incorporating the L1 gene of PV induce neutralising antibody in beagle dogs, and we have recently demonstrated that codon modification to allow better expression in eukaryotic systems improves immunogenicity of such polynucleotide vaccines[5]. The L2 protein of the PV capsid, while not as effective at inducing immune responses during natural infection as the L1 protein, has been shown to induce immune responses as a part of a vaccine which virus neutralising in vitro, and may therefore prove useful if a significant number of

subjects are proven unable to respond to an L1 vaccine delivered either as VLPs or as a polynucleotide. Therefore, there has been considerable progress in vaccine development for the prevention of PV infection. However, developing vaccines for the treatment of existing infections and particularly treatment of malignancies due to infection present a different set of problems to researchers.

Vaccines to treat PV infection and cervical cancer

It can be estimated that, globally, about 100 million women have already been infected with high risk genital PVs, and that about five million of these will have persistent infections that will in due course give rise to anogenital cancer if untreated. For this large group, there is no evidence that capsid protein-based vaccines, designed to produce virus-neutralising antibody, have much to offer for eradication of existing infection. Rather, therapy will be targeted at eliminating epithelial cells in the anogenital tract that are already infected with PV[6]. Specific antiviral immunotherapy, either given alone or in conjunction with specific antiviral drugs, might achieve this goal. Papillomaviruses generally encode six non-structural proteins (termed E1, E2, E4, E5 E6, E7) and two structural proteins (L1 and L2) which are expressed differentially across the maturing epithelium, though all are expressed at low abundance in the infected self-renewing stem cell populations at which immunotherapy would have to be targeted to eliminate clones of infected epithelial cells. Natural immune responses to PV-encoded antigens are generally weak and unpredictable, although a humoral immune response is observed to E7 in most cases of invasive cervical carcinoma[7]. Some evidence suggests that cell mediated immune response to the E2 and E6 proteins may be predictors of regression of PV-associated disease. Further, immunocompromised individuals due to HIV infection or following transplantation is a well-characterised risk factor for progression of PV infection to premalignancy and malignancy. Thus, targeting immunotherapy to some or all of these PV-encoded proteins is held to have potential for treatment of PV infection. However, in general, effective active immunotherapy is still a goal that has not been realised for any human disorder, despite some early successes of tumour antigen-specific immunotherapy in subsets of patients with cancer. Further, there are extra problems in targeting immunotherapy to PV-associated skin lesions, which lack the inflammation necessary to recruit innate immune responses.

Against this background, what has been achieved so far by ourselves and others – recently reviewed by Breitburd[8] – is to demonstrate firstly that the PV non-structural proteins are adequately immunogenic, inducing responses which can be used to prevent the grafting of transplantable tumours expressing these antigens, and in some cases to cause partial regression of existing tumours. For cottontail rabbit papillomavirus, partial therapeutic efficacy against natural infection has also been demonstrated. The optimal choice of antigen, means of production, dose, route of delivery, and frequency of immunisation has yet to be established, though many such delivery systems have been proposed and have been shown to be of benefit in at least one animal model[9]. Patients with cervical or other HPV-associated cancer or pre-cancer have been immunised with E6 and E7, and these studies have demonstrated that these proteins are immunogenic, and that there are hints of potential efficacy for cervical cancer and pre-cancer. One recent study undertaken by the centre demonstrated immunogenicity of HPV6 VLPs without adjuvant in patients with existing warts, and hinted at possible therapeutic efficacy[10].

A major effort will be needed to develop laboratory assays that predict vaccine efficacy that might be used to allow cost-effective dose-ranging studies of potential therapeutic vaccines in man. Therefore there is great interest in the epidemiologic studies currently being undertaken, to evaluate whether viral load is predictive of clinical outcome for PV, which has been the case for other viruses. Similarly, studies of cellular immune responses to vaccine proteins in man are being undertaken, though the constraint of only being able to access blood, and in limited quantity, creates practical problems which even the newer techniques of tetramer technology, ELISPOT, and intracellular cytokine staining have not yet overcome.

Conclusions

Vaccines to prevent papillomavirus infection, using papillomavirus virus-like particles to induce neutralising antibody, are in clinical trial and show all the characteristics likely to be associated with success. Results warrant global planning for the deployment of these vaccines within a decade, as part of a program to prevent cervical cancer.

Vaccines designed to treat existing papillomavirus infection, by inducing therapeutic cellular immunity targeted to viral proteins, are at a much earlier stage of development. The wide choice of potential and proposed antigens, routes and mechanisms of delivery, and possible treatment regimens suggest that, to move the field forward, surrogate assays for the relative efficacy of different vaccine approaches are required. These assays might be based on reduction in the load of virus infection following immunisation, and need to be validated in animal models and in man.

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