The role of vaccines in the control of STDs: HPV vaccines

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Prophylactic vaccines for genital human papillomavirus (HPV) infection have been shown to be feasible in animal models, and suitable vaccine material based on virus-like particles can be produced in bulk at reasonable cost. Initiation of phase III clinical trials will follow definition of trial outcome measures through further epidemiological studies, and development of assays of host protective immunity. Vaccines could in principle eliminate HPV-related disease, as the human race is the only natural host for the relevant papillomaviruses (PVs). Therapeutic vaccines for genital HPV infection are also possible, but have not yet been demonstrated as feasible in practice because the choice of vaccine antigens is difficult, the method of their optimal delivery is uncertain, and the nature of the relevant antiviral immunity is unknown. PV species specificity will require trials to be conducted in man, which will slow definition of an ideal vaccine.

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1 Epidemiology
Worldwide, infection with papillomaviruses (PVs) which can be transmitted sexually and give rise to genital disease is common. Prevalence of such infection is reported as 20–80% amongst sexually active adults and the majority of infections are asymptomatic. A vaccine to prevent sexually transmitted human papillomavirus (HPV) infection might be designed to prevent either genital warts, which are the commonest clinical manifestation of genital HPV infection, or to prevent cervical and other rarer HPV-associated anogenital cancers, including penile, vulval, and anal carcinomas. Genital warts are commonly associated with infection with two genotypes of HPV, designated HPV6 and HPV11, although at least 10 other types are less commonly associated. Genital warts have a prevalence, by naked eye examination, of 1–5% amongst adults, and are particularly found amongst younger people who have recently become sexually active. Cervical cancer, of which 90–95% is HPV-associated, is the second commonest cause of cancer death amongst women worldwide, affecting about 1% of women, and the commonest cause of cancer death under the age of 50 years. The latent period between PV exposure and cancer detection is estimated as 5–25 years. A PV vaccine targeted at preventing HPV infections of the anogenital tract which predispose to anogenital malignancy would be designed particularly to prevent infections with genotypes HPV16, HPV18, HPV45 and HPV56, although many other types are more rarely associated with disease. For further information on the epidemiology of HPV infection and its association with cancer the reader is referred to recent reviews.

Another class of papillomavirus vaccine could be therapeutic for HPV-associated lesions, including HPV-associated cervical cancer. Current therapies for warts and for late stage cervical cancer are sub-optimally effective. A novel approach to therapy is needed, particularly for PV infection involving substantial parts of the anogenital epithelium, and immunotherapy seems an obvious choice. Given the widespread prevalence of asymptomatic HPV infection, and the possibility of vertical transmission of at least some genital HPV types, combined prophylactic/therapeutic vaccines may be desirable for disease elimination.

2 Prophylactic vaccination
Studies with bovine PV have demonstrated the value of prophylactic killed virus vaccines; protection against disease was seen in 5 of 6 calves following intramuscular administration of viruses, and was genotype specific. The prospects for a conventional killed or live attenuated vaccine for human PV types are nevertheless poor. Papillomaviruses cannot be propagated in tissue culture, which precludes development of a conventional killed vaccine, while the recognised tumour promoting effects of PV infection, together with their complete species specificity, militate against production of a live attenuated human PV vaccine. Recombinant DNA technology has permitted expression of viral proteins in bacteria and in eukaryotic cells, and has allowed the preparation of a number of possible PV vaccine antigens. Expression of the major L1 capsid protein of HPV as recombinant protein in yeasts, insect cells, or tissue culture enables assembly of this protein into virus-like particles (VLPs). VLPs are immunogenic, and their injection into animals either without adjuvant, or together with a suitable adjuvant, will result in antibodies to PVs. Infection with PVs which are specific for the cottontail rabbit and the beagle dog can be prevented by immunisation with the appropriate PV.
VLPs, and in each case disease is prevented after challenge with the natural virus. The canine oral PV(COPV) infection is noteworthy because this virus, like human genital PV infections, infects a mucosal surface, and because vaccination with COPV has been shown to prevent transmission by the natural route, as well as to prevent clinical disease after deliberate challenge11 in young age. Protection following vaccination has so far been shown to be against PV induced disease rather than against PV infection, and it remains to be established that this will be sufficient to prevent PV associated tumours. Protection can be mediated by immune antibody alone and, for the cotton-tail rabbit PV vaccine, the duration of protection is at least a year.33 Neutralising antibody is directed to conformational epitopes of the VLPs, as antibody raised to linear or denatured L1 capsid protein is non neutralising in vitro and non protective in vivo.34

2.1 Milestones towards a clinical vaccine

The above data suggest that prophylactic vaccines for HPV infection based on VLPs should be straightforward to produce and introduce into clinical practice, as the prospects for large scale production of cheap stable vaccine material seem good. What are the problems to be overcome through further research before such vaccines become clinically available? These can be divided into three:

2.1.1 Virus variability Papillomaviruses are double stranded DNA viruses, and their genome is therefore relatively stable, but there are a large number of genotypes (> 50). While these can be grouped clinically into three major groups, and molecularly into broad clades within these groups,39 there is some sequence variation in the L1 capsid protein even amongst single genotypes.36-38 It is currently being established whether genotypes and subtypes are immunologically cross reactive,39 and whether antibody raised against one type will protect against another. For HPV6 and HPV11, which are closely related by sequence, there appears to be little cross protection.34 Currently, only a guess is possible of how many different types of PV VLP would be required in vaccine to give protection against all sexually transmitted HPV infection; if, as has been speculated,40 there are as many unknown PV types as known ones, 20 different L1 proteins might be needed. A study of the genotypes of HPV found in the cervix of prostitutes in Copenhagen, reported at the 14th International Papillomavirus workshop, suggested that repeated exposure results in sequential infection with firstly the commoner genotypes of PV, then the rarer genotypes and finally the untyped and unknown genotypes. While these data suggest that genotype specific immunity can occur, they also require a multivalent vaccine approach, as otherwise vaccination may shift the spectrum of HPV genotypes associated with clinical PV infection from the commoner to the rarer types.

2.1.2 Knowledge of the clinical disease Understanding of the natural history of HPV infection is relatively incomplete because most infections with HPV are subclinical and there is no non-invasive, robust, and validated serological or molecular test for infection. Most visible genital warts are caused by HPV11 or HPV6. Warts appear quickly after exposure (6–12 weeks), and > 80% last for at least 3 months before regression. A regression rate of 30% per 3 month period thereafter is to be expected11 in young age. Protection following vaccination has so far been shown to be against PV induced disease rather than against PV infection, and it remains to be established that this will be sufficient to prevent PV associated tumours. Protection can be mediated by immune antibody alone and, for the cotton-tail rabbit PV vaccine, the duration of protection is at least a year.33 Neutralising antibody is directed to conformational epitopes of the VLPs, as antibody raised to linear or denatured L1 capsid protein is non neutralising in vitro and non protective in vivo.34

There is a dose dependent several month latency after infection before acute clinical manifestations of the infection are visible (as CIN1 in the cervix for HPV, and as hepatitis for HBV), the acute lesion regresses after a further 6–12 months, with concomitant development of immunity. Immunity comprises humoral immunity to capsid proteins (HBsAg for HBV, L1 for HPV) which apparently prevents re-infection with the same genotype only, and cell mediated immunity to other viral gene products (HBCAg for HBV, E7 especially for HPV) which helps to eliminate infection. Humoral immunity persists for many years and is re-boosted by re-exposure. Cell mediated immune responses to viral proteins are transient and more difficult to measure. A small percentage of infected people become chronic carriers; this is more common in patients who are not able to mount an effective immune response. Chronic carriage is associated with ineffective immunity to some gene products (antibody to E7/HBc), and lack of immunity to the capsid proteins (L1/HBs). Vertical transmission occurs and is associated with immunological tolerance and persistent viral carriage. Chronic carriage is necessary for viral integration and the increased risk of tumours promoted by viral genes.
While this comparison suggests some possible vaccine trial designs, the applicability of the above model to HPV infection needs testing in cohort epidemiological studies. Such studies are in progress at a number of sites and their outcome will help facilitate field trials of PV vaccines.

2.1.3 Assays for virus neutralisation As HPV cannot be propagated in vitro or in animals there are no easy tests for virus neutralising, as opposed to virus binding, antibodies. This implies that there will be no simple surrogate in vitro assay to replace clinical efficacy as an endpoint in early clinical trials, and such trials will therefore be large and expensive. This will slow optimisation of vaccines, and add to the cost. A validated surrogate system for testing neutralising antibody in vitro would greatly assist vaccine development. Antibody mediated blocking of virus binding to cells has been proposed, and some assays have been developed.46 47 However for neutralisation of HPV, only one labour intensive assay46 involving the transplantation of keratinocytes and virus under the renal capsule of nude mice is available, and this would not be practical for quantitative assay of large numbers of sera. Better neutralising assays are urgently required, and their validation in the course of clinical trials will be a high priority.

3 Study design
Because of the problems outlined above, design of studies to demonstrate vaccine efficacy is not self evident. In this section some possible vaccine trials will be outlined to highlight the issues to be resolved.

3.1 Prophylactic vaccines—genital warts
Young adults prior to sexual activity can be presumed to comprise a largely HPV6 and HPV11 naive population, and will therefore be the target population of efficacy studies. In contrast, by the age 20 years, one in five sexually active women will have HPV DNA in their cervix as judged by PCR DNA testing.2 One in ten will also have visible genital warts, although males particularly will often not be aware that they have them.49 Thus, a window of opportunity exists for testing vaccines in adolescents which is lost by age 20, and the need to test vaccines for efficacy in people under 18 years may cause some ethical concerns. A randomised placebo controlled double blind study will be required, probably involving a cohort of pre-college students, male and female. Exclusion of those subjects who give a history of genital warts, with external wart disease on examination, or with known immune compromise or systemic disease will be required. The volunteer process should self select for those who are likely to become sexually active. However, subjects motivated to participate in a vaccine trial may also be motivated to practice safer sex, which may reduce the risk of HPV related warts.

A successful vaccine might aim to prevent 80% of wart disease. Given an incidence of visible warts in the target population of 5% per annum, and a latency period after infection of up to six months during which a vaccine would be unlikely to be successful, an 18 month trial, with warts appearing during the first 6 months regarded as likely to include those that were incubating anyway, would be required. A 50% dropout rate might be expected, and a doubling of the sample estimate will therefore be required: to give a reasonable power, the study would therefore require about 3000 students!

3.2 A vaccine to prevent HPV cervical dysplasia
Cervical dysplasia is asymptomatic. For a study to be valid, the disease to be prevented must be the one which current opinion holds must be treated as part of a prevention programme for cervical cancer (LSIL or CIN1 in most countries). The histological appearance of CIN1 may represent two different processes: LSIL occurring immediately after any HPV or other viral exposure in the cervix as part of a repair process is relatively likely to regress, while LSIL occurring later in the course of HPV16 infection is a dysplastic process, and presumably more likely to progress. Reliable diagnosis of CIN requires a biopsy, which is prone to sampling and interpretation error. This error at recruitment will increase the risk that people with prior exposure are incorrectly included in a study. Reliable data on the natural history of CIN1 cannot become available in countries where discovery of cervical dysplasia on a Papanicoulou smear leads to biopsy, and a biopsy showing CIN1 mandates treatment. The study will be limited to women who are already sexually active, and to ensure a reasonable risk for acquisition of new HPV infection, subjects in the study will have to be HPV negative at recruitment and likely to change sexual partners in the next year. Recruitment will therefore be targeted at young unmarried women who have a normal cervix at entry, which tests negative for HPV DNA. The development of HPV 16 DNA +ve CIN 1 between 3 months after vaccination, and the vaccine study has been negative so far. No HPV 16 +ve CIN 1 over the duration of the study would be a success. Current cohort studies will give us an estimate of the new HPV 16 infection acquisition rate per year in the target population, and thus allow determination of sample size.

4 Therapeutic vaccines
For prophylactic PV vaccines, the underlying science is relatively clear, and there is some consensus that a vaccine based on VLPs, with an adjuvant designed to raise antibody to conformational epitopes, will be the basis of the vaccine. For therapeutic papillomavirus vaccines, in contrast, the scientific basis of a vaccine is not so well defined. The issues to be resolved are:
What are the viral proteins against which an immune response might be targeted, and are viral antigens presented at the cell surface?
What type of immune response is required to eliminate infected epithelial cells?
How long must this immune response be sustained and how might this be achieved?

How will latency be addressed and does it matter clinically?

Despite these issues, at least three Phase 1 clinical trials of HPV E7 specific immunotherapy for cervical cancer are under way, one in Australia based on a recombinant E7 protein, one in the Netherlands based on synthetic peptides, and one in the United Kingdom based on recombinant vaccinia virus, and each of these studies may help clarify the underlying science.

4.1 Scientific challenges for a therapeutic vaccine

4.1.1 Choice of immune response To eliminate infection with a non-lytic virus such as papillomavirus, a therapeutic response is required which will kill the infected cell. This might be achieved by a cytotoxic effector T cell specific for a viral antigen, which could in principle deliver a lethal hit via a contact dependent perforin/granzyme mediated pathway or by activating fas ligand or the TNF-receptor on the target cell.\(^{50,51}\) In practice the vulnerability of epithelial cells to lysis by these means is uncertain.\(^{52}\) Differentiated epithelial cells are doomed to die anyway and while their premature lysis might prevent maturation of virions and thus infection of others by the immunised person (an “altruistic” vaccine), such lysis would not remove the lesion. Thus the target of the immunotherapeutic assault would preferably be undifferentiated replicating basal and suprabasal stem cells.

4.1.2 Choice of target antigen Papillomaviruses express 6 or 7 non-structural and 2 structural proteins in infected cells and each of these might in principle be a target for an immunotherapeutic effector cell. However, to kill cells from which latent virus might repopulate the epithelium, it is necessary to target the proteins expressed in these cells, or to allow a bystander effect to kill the targets. The available antigens are:

E1 and E2, which are expressed in stem cells and facilitate HPV plasmid replication, but appear to be low abundance proteins.\(^{53,54}\)

E5, E6 and E7, which are expressed in replicating suprabasal cells. E6 and E7 are the only PV proteins consistently expressed in cervical cancers.\(^{55,56}\) Abundance of HPV16 E7 seems higher than E1 and E2 and some natural immune response to this protein\(^{57}\) confirms its relative immunogenicity.

E1 and E4, L1 and L2 proteins, which are abundant only in terminally differentiated epithelial cells.

Data reviewed elsewhere\(^{58}\) support the use of E2 in the rabbit\(^{59}\) and E7 in the cow\(^{60}\) as immunotherapeutic vaccines for PV induced warts, and E7 as immunotherapy for PV associated tumours.\(^{61}\)

4.1.3 The necessary immune response, and sustaining it No immunotherapeutic vaccine has been accepted by regulatory authorities as effective in man, or been shown to cure existing papillomavirus induced warts in an animal model. Answers as to what will be effective must therefore be modelled from animal studies designed to prevent PV infection by immunising with PV non-structural proteins, and from animal studies of immunotherapy for other viruses and tumours associated with papillomavirus proteins. These latter studies suggest that a cytotoxic T cell response specific for a viral protein may be sufficient to eliminate infection with a non-lytic virus.\(^{62}\) They suggest also that the wrong sort of immune response or a response to the wrong antigen may prevent effective immunity. One problem is that cytotoxic T cell effector responses tend to be short lived following infection or immunisation (after 3 weeks) and even in the face of persisting antigen the response will fade.\(^{50}\) As dividing cells are particularly prone to T cell mediated lysis,\(^{50}\) and the epithelial stem cell population carrying episomal PV DNA might not divide within this period, a vaccine may therefore not be effective unless some way can be found to overcome this problem.

4.1.4 The issue of latency One possible consequence of vaccination may be to render PV infection latent, as mentioned above, by eliminating cells in which vegetative PV reproduction is taking place. If this were true, it might eliminate visible disease but not the risk of future malignancy. This issue can be resolved to some extent in the cottontail rabbit model of carcinogenesis.

4.2 Trial design

The natural history of HPV6/HPV11 associated visible warts that patients are prepared to subject to experimental treatment is fairly well described from the placebo arms of a number of intervention studies.\(^{65}\) Approximately 20–30% will regress spontaneously in each 3 month period. The spontaneous regression rate is higher in younger lesions, and in patients with single lesions. No treatment modality produces a better outcome at 6 months than no intervention at all, if freedom from wart disease is the sought after outcome. However, most destructive or anti-proliferative therapies will help patients who are destined to be clear of visible warts at 6 months without intervention to achieve this outcome sooner, which from the patient’s point of view is highly desirable.

A therapeutic vaccine for HPV infection is predicated on the belief that it can do significantly better than current treatment modalities. A 50% better outcome would be clinically useful. Immunotherapy might be given as adjuvant therapy to prevent disease recurrence after ablative therapy, or as a single primary therapy. The former is preferable for early clinical studies because patients know they will receive best available therapy, and the time to primary study end point is shorter. HPV immunotherapy as primary therapy for extensive HPV associated wart disease might subsequently be trialed. As half of subjects will have recurrent disease by 3 months in the placebo group, a recruitment cohort of 300–500 subjects would be necessary for a reasonable power.
In conclusion, the prospects for prophylactic vaccines for PV infection look encouraging despite the ongoing clinical trial hurdles, and prophylactic PV vaccines have moved from a research to a development phase. Immunotherapy for PV associated cervical cancer and genital wart disease is more complex because of a lack of suitable animal models, but the prospects also look encouraging.

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