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Can genital-tract human papillomavirus infection and cervical cancer be prevented with a vaccine?

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Can genital-tract human papillomavirus infection and cervical cancer be prevented with a vaccine?

Joakim Dillner and Darron R. Brown

Human papillomavirus (HPV) infection is the cause of squamous cell carcinoma of the uterine cervix. This causative relationship has provided the rationale and incentive for development of a prophylactic vaccine. Such a vaccine, if found to be effective, could reduce the need for cervical cancer screening and have a profound effect on the incidence of cervical and other anogenital cancers. This review begins by examining the basic biological and epidemiological principles relevant to the development of HPV preventative vaccines. It then summarises studies examining the use of vaccines to prevent HPV infection in animals and humans, and, finally, discusses some of the unanswered issues surrounding vaccine development against HPV infection and cervical cancer.

Human papillomavirus (HPV) is the most common sexually transmitted infection. Over 90 different types of HPVs have been identified and fully sequenced, and a further ~30 putative types exist that have been partially characterised (Refs 1, 2). Approximately 40 of these HPV types infect the genital tract. All HPV types are epitheliotropic, completing the growth cycle only in differentiating keratinocytes of the skin and the anogenital and oropharyngeal mucosa. Since the specific host cell receptor(s) for HPV has not been determined, it is not known whether the strict tropism is determined at the receptor level or by host factors required for replication (Ref. 3). HPV infection of the human genitalia causes a range of clinical states including asymptomatic infection, genital warts, cytological abnormalities of the cervix and invasive cervical cancer (Ref. 1).

HPV types are assigned numerical designations once the DNA sequence has been established and a comparison with previously known types has found less than 90% homology in the L1, E6 and E7 regions of the virus (Ref. 4); isolates with more than 90% homology to known

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HPV types are classified as subtypes. The different HPV types can be subdivided into two categories – 'high risk' and 'low risk' – originally assigned on the basis of whether the HPV type could or could not be found in cervical cancer specimens. Individuals infected with low-risk viruses have a low risk for the development of cervical cancer. The low-risk types such as HPV 6 and HPV 11 are associated with benign, hyperproliferative lesions, commonly referred to as genital warts or condyloma acuminata (Refs 5, 6, 7). High-risk types such as HPV 16, HPV 18 and others cause dysplastic lesions of the cervix, including invasive cancer (Ref. 8).

The causative relationship between HPV and cervical carcinoma has provided the rationale and incentive for development of a prophylactic vaccine. A completely effective vaccine would reduce the need for cervical cancer screening and could have a profound effect on the incidence of cervical and other anogenital cancers worldwide. HPV vaccine development is under way. Therapeutic vaccines for HPV infection have recently been extensively reviewed by Chu (Ref. 9), and this review instead examines the basis for preventative vaccines. This article first presents basic biological aspects of HPV in the context of preventative vaccine development, and then summarises the epidemiology of HPV infection and immune responses to HPV. Next, vaccine trials that are under way are discussed, as are specific issues related to vaccine formulations, adjuvants and administration schedules. Finally, social, economic and behavioural issues related to prophylactic vaccines for HPV are addressed.

Biology of HPV related to development of prophylactic vaccines

HPVs are icosahedral, nonenveloped, double-stranded DNA viruses of approximately 55 nm in diameter (Ref. 10). All of the coding information is contained in one of the two DNA strands (Fig. 1). There are seven or eight open reading frames (ORFs), encoding several known viral proteins, some of which are formed by splicing events. The five 'early' proteins are E1, E2, E5, E6 and E7. Transcripts encoding the early proteins are detected in the basal and suprabasal cells in the early portion of the viral replication cycle, and encode proteins required for viral replication and cellular transformation.

Expression of the 'late' structural L1 and L2 genes is restricted to the differentiating epithelium

Schematic drawing of the human papillomavirus 11 genome

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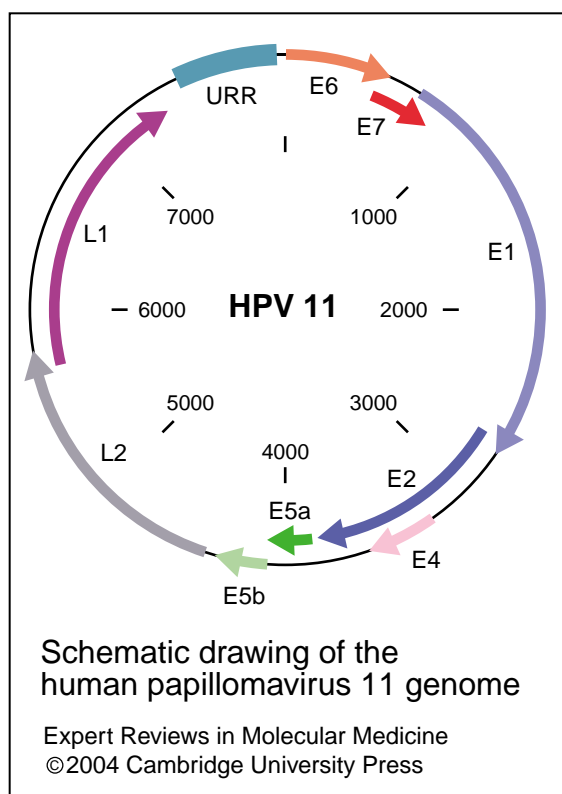
Figure 1. Schematic drawing of the human papillomavirus 11 genome. The open reading frames encoding the five 'early' proteins (E1, E2, E5, E6 and E7) and two 'late' structural proteins (L1 and L2) are indicated by arrows; the numbers inside the circular DNA molecule indicate approximate positions within the ~7900 base pair genome. Abbreviations: HPV, human papillomavirus; URR, upstream regulatory region.

where viral assembly occurs (Refs 11, 12, 13, 14). The L1 ORF encodes the major capsid protein of 55 kDa that makes up the majority of the virus shell. The L1 ORF is the most conserved gene between individual HPV types. The L2 ORF encodes a protein of 77 kDa known as the minor capsid protein because it contributes a smaller percentage of the capsid mass than does the L1 protein. The upstream regulatory region (URR), located between the L1 and E6 ORFs, does not encode HPV proteins but contains promoter and enhancer sequences necessary for viral replication and transcription (Refs 12, 13, 15).

HPV infects keratinocytes (Ref. 10), the predominant cell of epithelial surfaces. Presumably, only basal or undifferentiated keratinocytes are infected because the viral replication cycle is completed as the keratinocyte

undergoes the process of differentiation, before migrating towards the outer layers of the epithelium. Virus is assembled in the nuclei of the most differentiated keratinocytes, and can be detected in these cells as desquamation (shedding of epithelial elements) occurs from the epithelium.

Epidemiology of HPV and cervical cancer

Scope of the problem

Cervical cancer is the most important of the HPV-associated diseases, with approximately 370 000 cancer cases and about 150 000 deaths worldwide every year (Refs 16, 17). Developing countries have both a higher rate of cervical cancer and a poorer cancer-specific survival, and a major reason for this is the lack of access to cervical screening programs. There are both economic and logistic obstacles to setting up efficient screening programs in developing countries.

Other HPV-related malignancies include vulvar cancer, particularly the verrucous (warty) form (Ref. 18), vaginal cancer and anal cancer (Ref. 19). Anal cancer is HPV-positive in almost all cases (Ref. 19). Approximately 45% of cases of penile cancer are associated with HPV (Ref. 20). The most common type of HPV associated with noncervical genital-tract cancers is HPV 16, which causes greater than 50% of these cancers (Refs 18, 19, 20).

Oropharyngeal cancer, in particular epithelial tonsillar cancer, is now established as an HPV-associated disease (Refs 21, 22). The proportion of such cancers associated with HPV is less certain, with estimates varying from 20% to over 50%. Skin cancers of the fingertips, perineum or perianal area are also likely to be caused by HPV, as HPV DNA is regularly found in case series of these cancers and by analogy with the involvement of HPV in genital-tract malignancies (Refs 23, 24). Although each of these malignancies causes a smaller number of deaths than cervical cancer, the joint health impact of these cancers is comparable with that of cervical cancer. Worldwide cancer incidences and mortalities are not available for several of these cancers and the exact proportions of the cancers that are attributable to HPV and to each type of HPV are not well known.

Rate and determinants of acquisition of genital-tract HPV infection

The epidemiological studies described in this section were mainly performed using cervical specimens from young women. These studies indicate that HPV infection is characterised by a very high rate of acquisition. A two-year follow-up of previously uninfected female university students in the USA found a cumulative incidence of HPV infection (any type) of 32.3% (Ref. 25). The most important determinant of HPV acquisition is a change of sexual partner, where each change of sexual partner involves a substantial risk for HPV infection. In Sweden, the risk for seroconversion to HPV 16 increases linearly approximately 4% for each lifetime sexual partner up to a plateau of about 32% among women with an average of eight lifetime sexual partners (Ref. 26). A longitudinal cohort study of teenage girls found that no girls without sexual experience became seropositive for HPV 16 or HPV 33, whereas 54% of girls who had had five or more partners were positive at some point in time (Ref. 27). HPV infection in virginal women has been found to be rare or nonexistent in several studies (Ref. 25), and the very low prevalence of HPV infection that was found among virginal women was associated with nonpenetrative sexual contact (Ref. 25).

An interesting determinant of acquisition is how long the partners have known each other before having sex, with risks for HPV acquisition being substantially higher if sex occurs with a partner known for less than eight months (Ref. 25). This might be related to the fact that HPV infection usually has a self-limited course (see below). Having a male partner reporting a high lifetime number of sexual partners or other concomitant partners is also a risk factor for cervical cancer (Ref. 25). Finally, lack of circumcision appears to promote penile HPV infections that do not clear and instead become persistent, which also would tend to favour HPV transmission (Ref. 28).

Condom use has shown protection against HPV infection in some studies (Ref. 29), but most studies have failed to detect a significant protection. Several reasons for the apparent lack of protection have been proposed, including incorrect or inconsistent use, and the fact that HPV infection might exist in a wider area of genital epithelium than is covered by the condom (Ref. 25).

Neonatal HPV infection has been the subject of much discussion. Several studies have reported that HPV DNA can commonly be detected among children born to HPV-infected mothers. Since

HPV seropositivity is very rare among children of pre-adolescent ages (Ref. 30), it appears that neonatal HPV DNA contamination does not usually lead to infection. An extensive study that also included investigation of whether the children seroconverted for HPV (a classical criterion for infection) documented that neonatal HPV infection can occur, although it is quite rare (Ref. 31).

Rate of clearance and determinants of clearance/persistence

HPV infection is characterised by a very high rate of spontaneous clearance. Several cohort studies of young women positive for HPV DNA have been consistent in their estimates of a 70% clearance rate during a 12-month follow-up (Ref. 32). After 18 months, greater than 80% of infections have cleared (Ref. 33), and a five-year follow-up study found a clearance rate of 90% (Ref. 34). Clearance rates are lower in older women: HPV-positive women of approximately 35 years of age were followed for an average of 19 months and only 55% cleared their initial infection (Ref. 35)

Women with persistent type-specific positivity are at increased risk of developing invasive cervical cancer (Ref. 36). Indeed, women who have type-specific HPV persistence but have had normal results from cervical cytological screening (Pap smears) have commonly been found to have an undiagnosed high-grade cervical intraepithelial neoplasia (CIN) (in about 28% of cases) (Ref. 37). The determinants of HPV persistence and clearance are therefore also key determinants of cervical cancer risk. Several studies have found that HPV 16 (the most commonly detected type in cervical cancers) has a higher propensity to persist than other HPV types (Refs 32, 33, 38, 39).

Clinical aspects of cervical dysplasia related to vaccine trials

The term CIN has been used for approximately 25 years to describe a continuum of dysplastic abnormalities of the cervix, on the basis of histopathological criteria after examination of tissue specimens (Ref. 40). It has been customary to divide CIN into three grades. In CIN1, the diagnostic features are concentrated in the lower third of the cervical epithelium, mitotic figures are not seen, and koilocytes are generally present (koilocytes are cells with enlarged, irregularly shaped nuclei, and a prominent perinuclear halo). In CIN2, nuclear abnormalities are more pronounced, more abnormal cells are found in the lower two-thirds of the epithelium, and mitotic figures might be present. In CIN3, normal cellular maturation is generally absent, and nuclear abnormalities and mitotic figures are seen throughout the entire thickness of the epithelium. CIN1 therefore corresponds to mild dysplasia, CIN2 corresponds to moderate dysplasia, and CIN3 includes both severe dysplasia and carcinoma in situ.

On the basis of the 2001 Bethesda System, cervical cytological abnormalities have been divided into specific categories intended to provide clear guidance for management (Ref. 41). The basic categories for describing squamous cell abnormalities are: atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL). LSIL encompasses CIN1 lesions of the cervix, and HSIL encompasses CIN2, CIN3 and carcinoma in situ.

Risk factors for cervical cancer

HPV infection is by far the most significant risk factor for cervical cancer. Relative risks usually exceed 100 in case–control studies and are usually in the range of 10 to 20 in prospective studies (Refs 41, 42). The attributable proportion (fraction of cervical cancers predicted to disappear if the risk factor was eliminated) is in excess of 50% even when only counting HPV 16. If both HPV 16 and HPV 18 were eliminated, almost 70% of cervical cancers would be prevented. If the four most common oncogenic HPV types (HPV 16, 18, 31 and 45) were eliminated, a protective effect of approximately 80% is predicted.

A systematic monitoring of which HPV types actually cause cervical cancer in various parts of the world is important for the design of HPV vaccines and for predicting the effect of vaccination in different populations. A systematic, worldwide review by Clifford et al. gives a good overview of the type distribution in cervical cancers (Ref. 42). However, deviations from the typical distribution of HPV types in cervical cancer have been reported. For example, HPV types 52 and 58 have been reported to be quite common in studies from Taiwan, China, Japan, Mozambique (Ref. 43) and Costa Rica (Ref. 44). There is an interesting over-representation of three

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HPV types – HPV 16, 18 and 45 – in invasive cancer series as compared with high-grade CIN series, suggesting not only that these three types are able to cause high-grade CIN but also that high-grade CIN caused by these types might have a greater risk to progress to cervical cancer (Ref. 42).

Most of the other known risk factors for cervical cancer are the same as those that determine either HPV acquisition or persistence, with some exceptions, as described below. Studies of smoking as a determinant of HPV acquisition or persistence have been inconsistent. However, smoking has consistently been associated with cervical cancer. The issue of whether this could be attributable to residual confounding by HPV (i.e the inability to separate the influence of the two factors) has been repeatedly discussed, as smoking habits correlate with sexual-risk-taking behaviour in several populations. A dose–response effect is seen, and randomised intervention studies of smoking cessation among women with low-grade CIN have shown a beneficial effect (Ref. 45). At present, the bulk of evidence indicates that smoking might indeed be a risk factor for cervical cancer. If so, it probably acts at the CIN progression stage in cervical carcinogenesis.

Oral contraceptives (OCPs) have been studied as a risk factor for cervical cancer. A large series of studies over a number of years have shown inconsistent associations with cervical cancer. OCP use is of course intimately associated with sexual habits, which might lead to confounding. However, recent systematic reviews of the literature have suggested that OCP use with a duration of more than five years is a risk factor for cervical cancer (Ref. 46).

Multiparity is consistently detected as a risk factor in populations where a substantial percentage of the women have multiple children (Ref. 47). The mechanism underlying this is unclear, with hormonal effects and/or repeated trauma to the cervix being the most commonly proposed explanations.

A series of early, cross-sectional, case–control studies found a strong association between infection with herpes simplex virus (HSV) and cervical cancer (Ref. 48). The case for HSV as a risk factor became weaker when several prospective cohort studies failed to find an association (Ref. 49). In recent years, history seems to have repeated itself: a large, cross-sectional,

case–control study found an association between HSV and cervical cancer (Ref. 50), but recent prospective studies have found no association (Ref. 51). Prospective studies are less prone to selection biases and, perhaps more importantly in this case, less prone to reverse causality issues. HSV is a common virus that persists in a latent form in the body and might be reactivated by serious disease such as cancer. It is therefore plausible that an association seen selectively in studies based on samples taken after cancer diagnosis might be due to the cancer causing virus reactivation rather than the virus itself being a risk factor for the cancer.

Infection with *Chlamydia trachomatis* has repeatedly been found to be associated with cervical neoplasia and invasive cancer in cross-sectional case–control studies. This association has commonly been attributed to confounding by HPV (Refs 52, 53). During recent years, an association with *Chlamydia* has also been a consistent finding of several biobank-based prospective studies with invasive cervical cancer as the endpoint (Refs 54, 55, 56). In a large, population-based, cohort study of HPV-positive women that examined most of the proposed risk factors for HPV persistence, a history of *Chlamydia* infection was the only significant risk factor for HPV persistence (Ref. 57). In the studies of cervical cancer it is noteworthy that *C. trachomatis* consistently associated with squamous cell carcinoma of the cervix but not with cervical adenocarcinoma or other anogenital cancers such as vulvar or vaginal cancer, even though these also have HPV and sexual behaviour as a risk factor (Ref. 58). More work is needed to clarify the true role of *Chlamydia* infection in cervical cancer.

Immunogenetic factors: an increased risk for cervical cancer?

Finally, immunogenetic factors have been implicated in cervical carcinogenesis, with class II HLA haplotypes being the best-studied factor. Although there has been a substantial heterogeneity between studies (Refs 59, 60), DQw3 and DR15/DQ6 are the haplotypes that have shown the most-consistent associations. DR15/DQ6 particularly increases the risk for cervical cancer among subjects positive for HPV 16, suggesting a specific interaction with HPV 16 epitopes rather than a more general immunoregulatory phenomenon. The association with DR15/DQ6 has in particular been found in

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a series of studies from different groups in various Scandinavian countries (Refs 55, 61). It is not entirely clear if the association is more pronounced in Scandinavia or if it has been more readily detected owing to the Scandinavian tradition of minimising selection biases by performing population-based studies. Similar tendencies have also been reported in other populations, notably from New Mexico and the UK (Refs 62, 63). DR15/DQ6 has been associated with HPV 16 infections becoming persistent rather than clearing, with CIN and with cervical cancer in longitudinal studies (Ref. 64).

Immune response to HPV infection and immunisation

The immune response to HPV infection is complex and incompletely understood. Studies in animal model systems performed over 40 years ago showed that there was a protective effect against papillomavirus infection that is mediated by immune serum reactions against the viral particle (Ref. 65). Neutralising antibodies for HPV can be generated by immunising animals with intact, infectious virus particles (Refs 66, 67). This approach cannot be utilised in humans because of the oncogenes contained in the HPV sequence. For other pathogens, generation of effectively neutralising antibodies appears to be essential in vaccine-induced protection (Ref. 68). Protection against infection from the pathogen relates to the amount of neutralising antibody present at the site of infection. This protection persists as long as sufficient levels of neutralising antibodies are present.

Natural HPV infection of the genital tract gives rise to a modest but measurable serum antibody response in most individuals (Ref. 69). A series of methodological issues make it difficult to study unambiguously whether immunity against type-specific re-infection occurs, but significant (although not complete) protection against re-infection has been found to be associated with the presence of HPV antibodies (Ref. 70). A cell-mediated immune (CMI) response also occurs, as mentioned below.

Eliciting humoral responses

A crucial discovery in the development of vaccines to prevent HPV infection was that the L1 protein could be expressed in eukaryotic cells and could self-assemble into so-called virus-like particles (VLPs; depicted in Fig. 2)

(Refs 71, 72, 73, 74, 75). HPV L1 VLPs contain the same conformationally dependent neutralising epitopes as are present on infectious viruses (Ref. 76). The L2 protein can also be expressed with L1 protein in yeast or *Spodoptera frugiperda* (Sf9) ovarian cells (natural hosts for baculovirus), giving rise to 'L1 + L2' VLPs (Ref. 77). However, there is as yet no evidence that virus particles, L1 VLPs or L1 + L2 VLPs would react differently with immune sera, whether from natural infection or from experimental infection in animals. VLPs can be purified and used to immunise animals and humans by intramuscular injection. The VLPs contain no nucleic acids and thus cannot transmit infection or induce expression of HPV proteins. In addition, plasmids or recombinant viruses (nonHPV) designed to express L1 and other HPV proteins can be administered by intramuscular injection.

The immunogenicity of HPV particles involves presentation to the immune system of conformational neutralising epitopes displayed on viral capsids. Although the HPV types are defined at the DNA sequence level, there is a very good correlation with serotypes – that is, individual HPV types have antigenically distinct
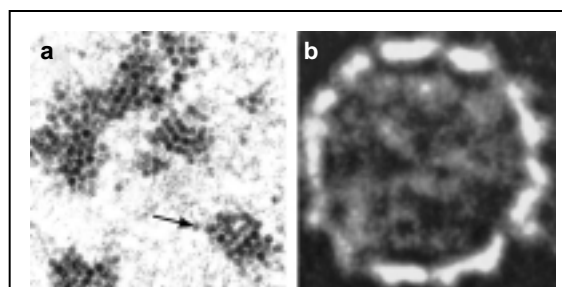
Transmission electron micrographs of human papillomavirus particles

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Figure 2. Transmission electron micrographs of human papillomavirus particles. (a) Human papillomavirus 83 (HPV 83) virus particles; the arrow indicates a cluster of ~25 virus particles. Original magnification: 90 000×. For further information on preparation of virions for electron microscopy, see Ref. 121. (b) A single HPV 11 virus-like particle (VLP). The VLP was photographed at an original magnification of 100 000×, and the image was further enlarged for this illustration. For further details, see Ref. 122.

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epitopes and there is limited or no cross-neutralisation. For example, Christensen et al. examined a series of neutralising monoclonal antibodies (mAbs) that targeted conformational epitopes of infectious HPV 11 virions and HPV 11 VLPs (Ref. 78). Three of the four HPV-11-neutralising mAbs did not bind to L1 VLPs of HPV 6, and the fourth bound very weakly. Also, a polyclonal anti-HPV-6 L1 VLP serum was only partially protective against infectious HPV 11 virions in the athymic mouse xenograft system, even when used in the assay at 100-fold higher concentration than a polyclonal anti-HPV-11 L1 VLP serum.

Vaccination of animals and humans with VLPs gives rise to neutralising antibodies. In a study by Rose et al., rabbits were immunised with VLPs of HPV 11 produced in Sf9 cells (expressed by a recombinant baculovirus) and purified by caesium chloride density gradient centrifugation (Ref. 79). Serum was then tested for neutralising antibodies in the athymic mouse model of HPV 11 infection. Pretreatment of infectious HPV 11 virions with the immune serum of VLP-treated animals caused a marked reduction of xenograft growth and viral gene expression, similar to the effects obtained using whole-virion postimmune serum, and consistent with immune neutralisation. To assess the serodiagnostic capabilities of VLPs, an enzyme-linked immunosorbent assay (ELISA) was developed and used to analyse sera from human subjects for anti-HPV-11 antibodies. VLPs were shown to substitute faithfully for native virions in this ELISA assay. In a related study by Christensen et al., baculovirus-expressed L1 VLPs of HPV 11 were highly immunogenic, and polyclonal rabbit antiserum to the VLPs was shown to neutralise infectious HPV 11 in the athymic mouse xenograft system (Ref. 80).

In another study, HPV 16 neutralisation was assayed using infection of an immortalised human keratinocyte cell line and detection of an HPV-16-specific spliced mRNA by reverse transcriptase (RT)-PCR (Ref. 81). Infection was blocked by preincubation of the virus with antiserum generated against L1 VLPs of HPV 16. To examine potential cross-neutralising activity among the different genital HPV types, rabbit antisera to L1 VLPs corresponding to HPV types 6, 11, 18, 31, 33, 35, 39 and 45 were assayed for the ability to block the HPV 16 infection of cultured cells. Antiserum raised against L1 VLPs of HPV 33 was the only heterologous antiserum that inhibited

HPV 16 infection to any degree. Thus, anti-HPV-16 VLP serum contained neutralising antibodies, but neutralisation was mainly type-specific.

African green monkeys immunised with L1 VLPs of HPV 11 produced high-titre immunoglobulin G (IgG) antibodies that effectively neutralised infectious HPV 11 in the athymic mouse xenograft system (Ref. 82). In addition, antibodies to HPV 11 VLPs were detected in cervical secretions of the immunised monkeys. However, the actual titre was less than 1% of the serum titre.

Young women who received intramuscular vaccination with L1 VLPs of HPV 11 (see below for details of the Phase I trial) developed serum antibodies that effectively neutralised infectious HPV 11 virus in the athymic mouse xenograft system (Ref. 83). A dose response in antibody titres and for HPV 11 neutralisation was evident. The degree of neutralisation correlated with radioimmunoassay titres of serum antibody, suggesting radioimmunoassay titres could serve as a surrogate marker of neutralisation.

As mentioned above, neutralisation seems to be fairly type-specific. Within individual HPV types, such as HPV 16, there are sequence variants, and there has been concern that vaccination with VLPs might not afford protection against infection with these variants. It has been shown that the immune response in human serum after natural infection is type-specific, but cannot distinguish between different variants of virus within the same type (Ref. 84). Certain neutralising mAbs (e.g. mAb V5 against HPV 16) are very effective for inhibiting the reactivity of immune sera from humans against VLPs. The mAbs that are able to block the natural antibody response will react with particles from all different variants within a virus type, but not with particles from other virus types. Also, serum from human volunteers vaccinated with VLPs will react with different virus variants. In a study of volunteers vaccinated with L1 VLPs of HPV 16 from variant 114K, effective cross-neutralisation (on the same level as homologous neutralisation) of variants from each of the five major phylogenetic branches of HPV 16 was found (Ref. 85). It was concluded that, from a vaccine perspective, HPV 16 variants belonged to a single serotype, and that vaccination with L1 VLPs of HPV 16 114K generates antibodies that should confer a similar degree of protection against all known phylogenetic branches of HPV 16.

HPV is presumably transmitted by contact with desquamated keratinocytes from an infected individual. This contact might be indirect, as in the case of cutaneous wart viruses, or sexual, in the case of cervical infection. These desquamated keratinocytes contain virus particles that escape the cell and infect the new host. As there is no viraemic phase, it is likely that the neutralising antibody would need to be present not only in serum but also in the immediate environment, such as the cervix or external genital epithelium, in order to prevent infection. How then do these antibodies arrive at the area of infection? Serum IgG antibodies probably reach the cervical epithelium and secretions by transudation (Ref. 86). It is commonly believed that exposure of the basal epithelial cell to infectious HPV results from microtrauma, and the same process could damage small blood vessels, leading to antibody in the cervical environment. However, the cervical antibodies detected in the vaccinated monkeys described above were in blood-free secretions, suggesting that no microtrauma was needed for antibodies to be present.

Eliciting CMI responses

As indicated above, a CMI response (i.e. involving CD4$^+$ and CD8$^+$ T cells) clearly occurs in natural HPV infection in addition to an antibody response. This subject was extensively reviewed recently by Man (Ref. 87). Also, Pinto et al. have recently described the CMI response to vaccination with L1 VLPs of HPV 16 (Ref. 88). They showed that vaccination of young women induced L1-specific T-cell responses detectable by proliferation of CD4$^+$ and CD8$^+$ T cells and in vitro production of T helper 1 (Th1) and Th2 cytokines.

Vaccine studies in animals

There is good evidence from animal studies that prophylactic vaccines can prevent papillomavirus infection and, in some cases, related disease (Refs 89, 90, 91, 92, 93). Table 1 summarises studies performed to date that have generated data on protective immunity, and includes both animal and human studies.

Two animal models have been used to examine papillomavirus-related disease: the cottontail rabbit papillomavirus (CRPV) and the canine oral papillomavirus (COPV) models. Immunisation has been performed either directly with VLPs or with plasmids expressing the L1 or other HPV proteins.

Immunisation with VLPs

VLPs produced from the L1 protein of several papillomaviruses have induced protection from infection after live challenge in animal models. Lin et al. used the CRPV model to show that protection from virus challenge occurred in animals immunised with full-length, nondenatured L1 protein (Ref. 90). In another study, CRPV VLPs consisting of the capsid proteins L1 or L1 + L2 of CRPV were produced in yeast, and used to immunise rabbits (Ref. 89). Three immunisations with VLPs formulated on aluminium adjuvant at 1–100 µg per dose efficiently protected rabbits from challenge with CRPV. Sera of immunised rabbits were shown to contain high-titred serum antibodies to CRPV L1 VLPs and to neutralise CRPV in vitro.

In another study, rabbits were immunised with recombinant baculovirus-produced VLPs of CRPV to determine whether these antigens could induce long-term protection against experimental challenge with CRPV (Ref. 93). Infectious CRPV and HPV 11 L1 VLPs were used as positive and negative control immunogens, respectively. Three groups of immunised animals were challenged with infectious CRPV after immunisations. Antibody titres in serum reached 1:10 000 immediately after the final booster immunisation and then decayed to 1:100 at 12 months in unchallenged rabbits. Serum neutralisation titres followed similar kinetics. Papillomas grew on control-immunised rabbits, but after CRPV L1 VLP immunisations the rabbits were protected against virus challenge.

Another study tested the ability of vaccination with CRPV VLPs to protect domestic rabbits against papillomas induced by infectious CRPV. A recombinant baculovirus system that expressed the CRPV L1 major capsid protein or L1 + L2 was used in insect cells as the source of VLPs (Ref. 92). Groups of rabbits were immunised with native or denatured VLPs from CRPV or bovine papillomavirus type 1 using Freund's adjuvant. Alum was used as the adjuvant for an additional group immunised with CRPV L1 + L2 VLPs. Animals were then challenged with infectious CRPV particles. No protection was seen in rabbits immunised with native or denatured bovine papillomavirus L1 + L2 or with denatured CRPV L1 + L2. Progression to carcinoma developed in 20 out of 30 challenged rabbits. Animals inoculated with native CRPV VLPs composed of L1 alone or L1 + L2 developed fewer lesions,

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Table 1. Summary of preventative human papillomavirus vaccine studies published to date that generated direct or indirect data on protective immunity

Species	Antigen	Efficacy	Comments	Refs
Rabbits	Nondenatured CRPV L1 protein produced in bacteria	Protection from CRPV challenge	Not a VLP vaccine, but showed the need for conformationally correct L1 protein	90
Rabbits	CRPV VLPs: L1 or L1 + L2 produced in yeast	Protection from CRPV challenge	High-titred, neutralising serum antibodies in animals	89
Rabbits	CRPV VLPs: L1 or L1 + L2 produced in Sf9 cells	Protection from CRPV challenge	No protection using denatured VLP preparation	92
Rabbits	CRPV L1 VLPs produced in Sf9 cells	Protection from CRPV challenge	High-titred, neutralising serum antibodies in animals	83
Rabbits	CRPV-L1-encoding DNA plasmid	Protection from CRPV challenge	Elicitation of conformationally specific neutralising antibodies	97
Rabbits	CRPV-L1-encoding DNA plasmid	Protection from CRPV challenge	Elicitation of neutralising antibodies	98
Rabbits	rVSV expressing L1 of CRPV	Protection from CRPV challenge	Both intranasal and intramuscular immunisation proved effective	99
Dogs	COPV L1 VLPs produced in Sf9 cells	Protection from COPV challenge	Serum Ig (passively transferred) conferred protection	91
Dogs	GST–COPV L1 fusion protein pentamers	Protection from COPV challenge	GST–COPV L1 protein retained its native conformation	96
Monkeys	L1 VLPs of HPV 11 produced in yeast	In vitro neutralisation of HPV 11 (serum)	Antibodies also detected in cervical secretions	82
Humans (Phase I trial)	L1 VLPs of HPV 11 produced in Sf9 cells	In vitro neutralisation of HPV 11 (serum)	Vaccine well tolerated	100
Humans (Phase I trial)	L1 VLPs of HPV 16 produced in Sf9 cells	In vitro neutralisation of HPV 11 (serum) and induction of L1-specific T-cell responses	Vaccine well tolerated	88, 101
Humans (Phase I trial)	L1 VLPs of HPV 11 produced in yeast	In vitro neutralisation of HPV 11 (serum); dose response in antibody titre	Vaccine well tolerated	83
Humans (Phase II trial)	L1 VLPs of HPV 16 produced in yeast	Reduction of infection and HPV-16-related CIN	Vaccine well tolerated	102

Abbreviations: CIN, cervical intraepithelial neoplasia; COPV, canine oral papillomavirus; CRPV, cottontail rabbit papillomavirus; HPV, human papillomavirus; GST, glutathione *S*-transferase; L1 and L2, late structural proteins; rVSV, recombinant vesicular stomatitis virus; VLP, virus-like particle.

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and none developed cancer within one year of infection. Rabbits vaccinated with native CRPV VLPs developed high-titre antibodies in an ELISA based on native VLPs, and passive transfer of serum or IgG from rabbits immunised with CRPV VLPs protected against CRPV challenge.

The L1 protein of COPV was expressed in Sf9 insect cells and VLPs were purified and used to immunise beagles. These animals developed serum antibodies against the L1 VLPs and were protected from experimental challenge with infectious COPV (Ref. 91). Serum immunoglobulin passively transferred from immunised to naive beagles conferred protection against infectious COPV.

Although these studies indicate that VLPs are immunogenic and can afford protective immunity in animals, VLPs are difficult and expensive to produce. 'Capsomeres' (a subunit of the protein coat of a virus particle) of L1 protein might represent an economical alternative to VLPs as an HPV vaccine. The L1 major capsid protein can be expressed and purified from bacteria, then trypsinised to generate recombinant capsomeres that retain HPV-genotype-restricted capsid antigenicity (Ref. 94). Capsomeres were used to generate high-titre polyclonal immune sera that demonstrated HPV-genotype-restricted reactivity by ELISA. The capsomere antisera were then tested in an in vitro infectivity assay and found to neutralise HPV 11 virions. In this assay, HPV 11 capsomere polyclonal antisera exhibited neutralisation titres comparable with those obtained with a virion-neutralising antiserum raised previously against intact HPV 11 VLPs (Ref. 95). These results indicated that highly immunogenic, genotype-restricted HPV capsid-neutralising antigenic domains are contained entirely within capsomeres, suggesting that L1 capsomeres might be viable vaccine candidates for the prevention of HPV disease.

To evaluate whether VLPs are necessary for effective vaccination, the L1 protein was expressed as a glutathione *S*-transferase (GST) fusion protein in *Escherichia coli* and its immunogenic activity was assayed in the COPV model (Ref. 96). The GST–COPV L1 fusion protein formed pentamers, but these capsomere-like structures did not assemble into VLPs. Despite the lack of VLP formation, the GST–COPV L1 protein retained its native conformation as determined by reactivity with conformation-specific anti-COPV antibodies. Most importantly, the GST–COPV L1 pentamers

completely protected dogs from high-dose viral infection of their oral mucosa.

DNA vaccines and other preparations
In the CRPV model, immunisation with an L1-encoding plasmid provided immunity against papilloma formation upon challenge with infectious CRPV (Ref. 97). Immunisation elicited conformationally specific neutralising antibodies to CRPV L1 protein.

Donnelly et al. immunised cottontail rabbits with a DNA plasmid expressing the L1 major capsid protein (Ref. 98). Rabbits responded by producing neutralising antibodies. In addition, rabbits were protected from challenge with CRPV. The authors suggested that immunisation with L1-expressing plasmids could be used in humans to protect against HPV, and could simplify the production of multivalent vaccines by combining plasmids encoding the L1 proteins of many HPV types.

Immunisations with live recombinant vesicular stomatitis viruses (rVSVs) expressing L1 of CRPV were tested for efficacy of protection following CRPV challenge (Ref. 99). An rVSV expressing L1 of CRPV (VSV–L1) was characterised for the protective ability afforded by intranasal, intradermal or intramuscular vaccination in rabbits subsequently challenged with CRPV. Specific humoral immunity to the L1 protein was consistently seen after a single VSV–L1 vaccination when administered through an intradermal or intramuscular route or after a boost via the intranasal route. Rabbits were completely protected from CRPV-inducted papillomas after vaccination and a boost given intranasally or intramuscularly.

Vaccine trials in humans
Several vaccine trials are under way in humans, although there have been only a few published results to date. Two large pharmaceutical companies (Merck; and GlaxoSmithKline in collaboration with the National Cancer Institute) are currently conducting vaccine trials in the USA and elsewhere. A blind, randomised, placebo-controlled, dose-escalation Phase I study of a vaccine of L1 VLPs of HPV 11 has been conducted (Ref. 100). The VLPs in the vaccine preparation were formed by self-assembly of HPV L1 capsid protein produced by baculovirus vectors in Sf9 cells. VLPs (3–100 µg) were administered intramuscularly and were

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formulated in aluminium hydroxide. Serum from vaccinated women was assayed for HPV 11 neutralisation by an in vitro RT-PCR-based assay that utilised infection of immortal human skin keratinocytes (HaCaT) cells. The vaccine was well tolerated and induced high levels of neutralising antibodies. Marked increases in lymphoproliferation to HPV 11 L1 antigens were noted after the second vaccination. In addition, lymphoproliferation was induced after vaccination in peripheral blood mononuclear cells (PBMCs) stimulated with heterologous L1 VLP antigens of HPV types 6 and 16. Statistically significant increases in HPV-antigen-specific interferon γ and interleukin 5 production were measured from PBMC culture supernatants.

In another Phase I vaccine trial, baculovirus-expressed L1 VLPs of HPV 16 were used (Ref. 101). This was a double-blind, placebo-controlled, dose-escalation trial in which volunteers were given intramuscular injections with placebo or with 10 µg or 50 µg doses of the vaccine (without adjuvant or with alum or MF59 as adjuvant) at 0, 1 and 4 months. All vaccine recipients were monitored for clinical signs and symptoms for seven days after each inoculation. Immune responses were measured by an ELISA based on L1 VLPs of HPV 16 and by an HPV 16 pseudovirion neutralisation assay. The vaccine was well tolerated. All subjects receiving vaccine seroconverted, and titres were approximately 40-fold higher than those observed in natural infection. Neutralising and ELISA antibody titres were highly correlated, confirming that ELISA titres are valid proxies for neutralising antibodies.

Recently, an interim analysis of a Phase II trial of a vaccine of L1 VLPs of HPV 16 was reported (Ref. 102). In this study, 2392 women (of age 16–23 years) were randomised to receive placebo or three doses of 40 µg of the vaccine produced in yeast cells in a 0, 2 and 6 month regimen. Genital samples for HPV DNA were obtained at enrolment and every six months. Biopsy tissue was evaluated for CIN and analysed by PCR for HPV 16 DNA. The mean duration of follow-up was 17 months. The incidence of persistent HPV 16 infection was 3.8/100 subject years at risk in the placebo group and 0.0/100 subject years at risk in the vaccine group (100% efficacy; 95% confidence interval, 90–100%; $P < 0.0001$). All nine cases of HPV-16-related CIN occurred among placebo recipients. It was concluded that administration of the HPV 16 vaccine reduced the

incidence of HPV 16 infections and HPV-16-related CIN. It should be noted that the follow-up of the study subjects continues, and it is likely that additional data on the long-term immunogenicity and protective effects of the vaccine will be forthcoming.

Unresolved questions about HPV vaccine trials

Endpoints
It is a challenge to find appropriate endpoints for trials of HPV vaccines. Evidence of incident HPV infection with a vaccine HPV type is an endpoint that would seem to be an obvious choice for a vaccine trial. However, a very high percentage of sexually active women, especially those with more than four or five lifetime sexual partners, are at least transiently infected with one or more genital HPV types. Since only a small proportion of infected subjects develop clinical disease, estimates of efficacy against infection cannot be assumed to also be estimates of protection against clinical disease. Therefore, HPV-induced clinical disease might be the most appropriate endpoint to determine vaccine efficacy. For example, genital warts or high-grade cervical dysplastic lesions of the cervix probably occur in no more than 5% of infected individuals within a 2–3 year period after infection. In addition, the clinical disease must be shown to be attributable to the specific HPV present in the vaccine before conclusions can be drawn about success or failure of protection. For example, cervical dysplasia can be caused by numerous HPV types. Therefore, cervical dysplasia in a vaccine recipient might either be due to infection with a vaccine HPV type, indicating lack of protection, or due to infection by a nonvaccine type. This is a complex issue and mandates the enrolment of large numbers of women in prospective trials of vaccine efficacy.

Inclusion of different HPV types
As indicated above, most evidence suggests that, in the case of L1 VLPs (that is, VLPs made exclusively of L1 protein with no L2 protein component), the antibody response elicited by immunisation is quite specific for the individual HPV type. This appears to be true even for closely related HPV types. At least 15 different 'high risk' types have been identified as causative agents of cervical cancer. With these considerations, an important question is: how many different HPV

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types can be included, given that each type requires a certain amount of antigen to be included in the preparation?

Hughes et al. attempted to determine the theoretical impact of a prophylactic HPV vaccine using mathematical models, with one model focusing on HPV infection and another on cervical cancer (Ref. 103). The authors determined that characteristics of sexual behaviour and characteristics of the vaccine affect the steady-state prevalence of HPV that would be expected if a vaccine program were begun. They determined that vaccinating women alone could reduce the prevalence of infection with the specific HPV type in the vaccine by 30%, and that vaccinating both males and females could reduce the prevalence by 44%. The analysis supported the concern that a 'fill-in' effect is possible; that is, if a vaccine gave protection against some but not all oncogenic HPV types, then disease could eventually be caused by HPV types not included in the vaccine. They concluded that a multivalent vaccine containing the majority of disease-causing HPV types would reduce the need for colposcopy (examination of the cervix for abnormal cells) and treatment, but that screening programs would remain necessary unless the vaccine was very effective and was given to a large percentage of the population. By contrast to less-common infections (such as HSV-2) that are restricted to defined core groups, an HPV vaccine would need to be given to a large percentage of the population since HPV infection is extremely common.

To reduce cervical cancer by 90%, eight to ten HPV types probably need to be included, assuming little or no cross-protection by vaccination. However, it cannot be assumed that each HPV type represented in a multivalent product would be 100% effective. Also, numerous other types that perhaps do not often cause invasive cancer are causative agents of cervical dysplasia, and therefore cytological screening programs (and possibly HPV typing) will continue to be necessary.

Vaccination against nononcogenic HPV
Since the biology and means of prevention by VLP vaccination is presumably the same for the benign HPV types as for the oncogenic ones, it is perfectly feasible to vaccinate against these viruses as well. HPV types 6 and 11 jointly cause more than 90% of genital condylomata acuminata lesions, a common clinical condition of significant impact

to sexual health. Low-grade dysplastic lesions of the cervix might also be caused by these and other nononcogenic types. An additional reason for vaccinating against HPV types 6 and 11 is the fact that these infections can cause serious disease in rare circumstances. HPV 11 is the major cause of recurrent respiratory papillomatosis, a severe disease that may be fatal. So-called giant condylomas or Buschke-Löwenstein tumours are usually caused by HPV 6. These tumours are regarded as having a low potential for malignancy, but may also be fatal.

In addition to the oncogenic HPV types 16 and 18, the Merck HPV vaccine undergoing testing includes HPV types 6 and 11 (low-risk types). To date, no efficacy data is available because the studies are not complete.

Inclusion of HPV proteins in addition to L1
As discussed above, most preclinical and clinical evaluations to date have used L1 VLPs. Are there other HPV proteins that should be included in a prophylactic vaccine? Addition of other proteins to the L1 VLPs requires increased technological challenges and costs. However, the L2 minor capsid protein is worthy of consideration for two reasons. First, this protein is present in the capsid structure of the natural virus. Second, the antibodies elicited to nondenatured L2 protein appear to have a degree of crossreactivity to heterologous HPV types, and therefore might be able to protect the immunised individual against these other types (Ref. 104). Other groups have studied the addition of early genes (E7 in particular) to determine if a CMI response could be elicited along with the antibody response to the L1 VLP component (Refs 105, 106, 107, 108, 109, 110).

Duration and consistency of the antibody response to VLPs
The duration of protection against HPV infection has not been evaluated. Encouraging data presented on the antibody response to HPV L1 VLP vaccines suggest only a slight decline in the high serum antibody titres over an evaluation period of six to eight months. The recombinant vaccines that have been produced to prevent hepatitis B infection might provide some insight into the possible duration of protection against infection. For the hepatitis B vaccines, neutralising antibodies are induced in high titres that slowly decrease over a time span of several

years. Protection from hepatitis B infection appears to be long lasting (at least five to ten years, and perhaps much longer).

As indicated above, it is likely that HPV infection of the cervix is prevented by the presence of neutralising antibodies at the site of infection. Do antibody levels to HPV VLPs remain constant and protective in women throughout the menstrual cycle? A study performed in mice showed that intramuscular administration of HPV 16 VLPs induced an IgG response in genital secretions, whereas nasal immunisation induced neutralising levels of both IgG and IgA (Ref. 111). The IgG response induced by intramuscular administration varied during the oestrous cycle of the mice, dropping to a non-neutralising level following dioestrus. By contrast, neutralising IgG and IgA antibody levels were found during the entire oestrous cycle when HPV 16 VLPs were administered by the intranasal route. These data suggested that mucosal administration might be more effective than parenteral immunisation at inducing continuous protection of the female genital tract from HPV infection.

A recent study in women indicated that antibody levels to HPV VLPs fluctuate during the menstrual cycle (Ref. 112). The levels of antibodies in the cervical secretions were measured in women who had been immunised with HPV 16 VLPs, and the influence of the menstrual cycle and OCP use on these levels was determined. The cervical titres of antibody to VLPs were relatively constant throughout the menstrual cycle in immunised women using OCPs. By contrast, antibodies to VLPs varied during the menstrual cycle in women who were ovulating, being highest in the proliferative phase, and dropping ninefold during ovulation. In addition, whereas serum and cervical antibody titres to VLPs correlated well in OCP users, there was no correlation in women who ovulated. The authors concluded that the decrease in cervical anti-VLP titres during the ovulatory phase of the menstrual cycle could suggest that parenterally administered VLP vaccines might be less effective during the peri-ovulatory phase. More studies are needed to determine fully the protective abilities of parenterally administered HPV VLPs in ovulating women.

Optimal timing for vaccination
Assuming the vaccines for HPV are eventually shown to be safe and effective, what would be

the optimal age for vaccination? Epidemiological studies indicate that many women become infected within several months of initiation of sexual activity. Thus, vaccination at an age of 8–10 years might be required to minimise the risk of infection before the vaccination takes place, perhaps adding a booster in adolescence or early adulthood if the duration of efficacy should turn out not to be life-long.

An alternative vaccination strategy that has advantages from the population perspective is to target the age groups that are most active in transmitting the infection in society – that is, adolescents and young adults. This strategy is likely to be important in particular in the first efforts to curb the infection in the early years after the vaccines have become available.

Immunisation of males
Genital-tract HPV is sexually transmitted. Therefore, immunisation of men might help prevent infection in women. Currently there are few data available regarding the immune response to HPV VLPs in men, although studies are being initiated. It is expected that immunisation of men with VLPs will elicit a serum immune response similar to that in women. Whether men will be protected against infection or clinical disease resulting from infection (genital warts, for example) has not been tested. A major obstacle in testing the efficacy of HPV vaccines in men has been the lack of safe, inexpensive and sensitive testing methods for HPV.

Costs, distribution and storage issues
The vast majority of deaths from invasive cervical cancer occur in women residing in developing countries, where basic services such as clean water, electricity and rudimentary medical care are not available to most people. Unfortunately, vaccines such as polio and tetanus that are essential for general health and are cheap, effective, easy to transport and easy to store are administered to a minority of children in some of these countries.

Will a vaccine directed against HPV be cheap enough to administer to large numbers of women in these countries, and thus have the potential to reduce the incidence of cervical cancer on a worldwide level? Although no cost information is available at this time for an HPV VLP vaccine, it is not likely to be cheap because of certain difficulties in vaccine production. The

recombinant hepatitis B vaccine produced by Merck, for example, is produced in a somewhat similar manner to the HPV VLP vaccine. According to the United Nations Children's Fund (UNICEF), the discounted cost of the recombinant hepatitis B vaccine is significantly more than the combined cost of vaccination against polio, measles, tetanus, diphtheria and pertussis. Certainly, women in wealthy nations will be able to afford the vaccine, but these same women enjoy the luxury of the availability of cervical cytological screening, which greatly reduces the likelihood that cervical cancer will develop. In addition, if precancerous cervical lesions occur in women in wealthy countries, treatment is readily available. There is a risk, therefore, that the women who need the vaccine the most will not receive it unless the cost is subsidised by governments or other organisations.

Antagonism between types

Although it is clear that multiple HPV types can be detected in the same individual, it is not known if different HPV types can infect the same cell, or interfere with infection or pathogenesis by each other. The possibility that different HPV types interfere with each other led to a seroepidemiological case–control study of 218 women with primary untreated cervical cancer and 219 healthy age-matched control women (Ref. 113). Possible interactions in cervical carcinogenesis between infections with several common HPV types (6, 11, 16, 18 and 33) were studied. As previously shown, HPV 16 seropositivity was associated with cervical cancer risk (Ref. 113), but HPV 16 was not associated with cervical cancer risk among women seropositive for HPV 6. The relative excess risk due to interaction between HPV 6 and HPV 16 was –2.35 (95% confidence interval, –0.04 to –4.65), indicating significant antagonism. The results suggest that infection with HPV 6 might interfere with HPV-16-associated cervical carcinogenesis. Further studies are needed to clarify this potentially important issue as new vaccine formulations are considered that contain numerous HPV types.

Future directions

There are many innovative developments in HPV vaccinology that are currently under way, although none of these new concepts has yet been tested in humans. Although only a few of these new concepts are mentioned in this section, many more deserve recognition and consideration for future trials in humans.

As indicated above, vaccination by injection appears to induce protective antibodies in the environment of the uterine cervix. However, vaccines formulated for intramuscular injection are generally more costly, more difficult to administer, and less acceptable to recipients than are mucosally administered vaccines. Can the level of protection be improved by introducing VLPs directly into the vagina? Several studies indicate that this is a possible route for induction of local immunity (Refs 114, 115).

Oral delivery represents another attractive alternative to parenteral injection for large-scale human vaccination. Rose et al. have studied this possible route for administration of HPV VLPs. HPV 11 VLPs given orally to mice induced a modest systemic neutralising antibody response (Ref. 116). This group also examined whether VLPs of other genotypes were immunogenic when administered orally and whether mucosal adjuvants could be used to enhance VLP oral immunogenicity (Ref. 117). HPV 16 and HPV 18 VLPs were found to be immunogenic when administered orally, and oral coadministration of these antigens with *E. coli* heat-labile enterotoxin (LT) mutant R192G (LT R192G) or CpG DNA improved anti-VLP humoral responses in peripheral blood and in genital mucosal secretions.

Transgenic plants expressing recombinant vaccine immunogens were also tested by this group as an inexpensive alternative to vaccination by injection. In one study, a plant-codon-optimised version of the HPV L1 major capsid protein coding sequence was introduced into tobacco and potato (Ref. 118). The plant-expressed L1 self-assembled into VLPs with immunological properties comparable with those of native HPV virions. Ingestion of transgenic L1 potato was associated with activation of an anti-VLP immune response in mice that was qualitatively similar to that induced by VLP parenteral administration, and this response was enhanced significantly by subsequent oral boosting with purified insect-cell-derived VLPs.

In another study, the gene encoding HPV 16 L1 was expressed in transgenic tobacco and potato plants under the control of the cauliflower mosaic virus 35S promoter (Ref. 119). The L1 protein was detected using an L1 gene optimised for expression in human cells, following the

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introduction of the translational enhancer Omega derived from the tobacco mosaic virus. The plant-derived L1 protein displayed conformation-specific epitopes and assembled into VLPs. Plant-derived L1 was as immunogenic as L1 expressed in baculovirus-infected insect cells. Feeding of tubers from transgenic potatoes to mice induced an anti-L1 antibody response in three of 24 mice.

Summary

At the time of this writing, there is ample evidence to support the supposition that genital HPV infection can be prevented with vaccination using L1 VLPs. The most compelling evidence to date derives from the trial by Koutsky et al. in which persistent HPV 16 infection and HPV 16-related cervical dysplastic disease were effectively prevented by vaccination with HPV 16 L1 VLPs (Ref. 102). The efficacy data from other trials of vaccines designed to prevent HPV infection remain unpublished. However, it is likely that Phase II trials of vaccines conducted by Merck and by GlaxoSmithKline will eventually provide efficacy data. In addition, large Phase III trials of VLP vaccines are under way by these companies and by government agencies in the USA. Thus, we should know in the next few years whether the vaccines are indeed effective in reducing both HPV infection and the consequences of infection for the HPV types in the formulation.

Many questions remain regarding the development of preventative HPV vaccines. What is the public's attitude and perception about the need for and the acceptability of a vaccine to prevent a disease resulting from sexual activity? Will parents be willing to permit their children to receive another immunisation in addition to the many already required or encouraged? These issues have recently been discussed (Ref. 120).

Will the vaccines protect against additional HPV types not included in the VLP formulations? Will a combination prophylactic and therapeutic vaccine be the optimal choice, as many women might already be infected at the time of immunisation? When marketed, this vaccine is likely to be expensive, perhaps prohibitively so for women living in countries with the highest burden of disease. Will the vaccine truly reduce the burden of cervical cancer worldwide? Indeed, 90% of all invasive cervical cancer occurs in countries in which a large percentage of the population is not given vaccines that, in developed countries, are considered routine and necessary. These and other important questions must be addressed if the worldwide burden of cervical cancer is to be significantly reduced.

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Further reading, resources and contacts

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UNICEF Information Newsline highlights the recent publication 'The State of the World's Vaccines and Immunization':

http://www.unicef.org/newsline/vpressr.htm

Features associated with this article

Figures
Figure 1. Schematic drawing of the human papillomavirus 11 genome.
Figure 2. Transmission electron micrographs of human papillomavirus particles.

Table
Table 1. Summary of preventative human papillomavirus vaccine studies published to date that generated direct or indirect data on protective immunity.

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