Chapter 2

Prophylactic and Therapeutic Vaccines against Human Papillomavirus Infections

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Additional information is available at the end of the chapter

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Abstract

Human papillomaviruses (HPVs) are a large family of double-strand DNA viruses comprising more than 180 types. Infection with HPV is associated with benign and malignant proliferation of skin and mucosae. Low-risk HPVs produce warts, whereas high-risk viruses induce tumors. Because there are no anti-viral drugs for HPV infection, there is a lot of interest in vaccines that can prevent the infection and also in vaccines that can be used to treat established infections and HPV-related tumors. Two prophylactic vaccines have been approved for preventing HPV infection. They seem to be effective when very young people are vaccinated. Unfortunately, many older people are still at risk of infection, mainly in countries where vaccination coverage is not efficient and for those people, novel therapeutic vaccines are being developed. This chapter describes the properties of HPV vaccines used today and the current status of several therapeutic vaccines been developed to treat HPV-induced lesions.

Keywords: human papillomavirus, T cell, cytotoxicity, immunoglobulin, antibody, vaccinia virus

1. Introduction

About 40 years ago, human papillomavirus (HPV) infections were initially reported. This viral infection caused benign warts, which in most cases regressed spontaneously [1]. Since then, several types of HPV have been identified. Some of them have been associated with cervical carcinoma [2]. This form of cancer is very frequent around the world [3] and mostly among women [4].

HPV have selective tropism for cutaneous or mucosal epithelia [5]. More than 200 genotypes of HPV have been identified and classified into high-risk and low-risk groups according to

their degree of oncogenic capacity [6]. The two most common low-risk HPV are HPV 6 and HPV 11. They cause most genital warts and recurrent respiratory papillomas [7]. The HVP types, HPV 16 and HPV 18, are responsible for about 60% of all cancer cases [4, 8]. High-risk HPV are involved in other types of cancer, including tumors of anus, vagina, vulva, and penis [9]. In addition, many tongue, trough, and tonsil tumors are also caused by HPV [10–12].

Most sexually active women will be infected by at least one high-risk HPV during their lifetime [13]. Most of these infections will remain asymptomatic and are eliminated by the immune system [14]. However, for a fraction of infected women whose immune system fails to clear the infection, the virus can persist for a long time causing lesions that may further progress into cervical intraepithelial neoplasia (CIN) and even cervical cancer [15, 16]. Early detection of HPV-induced lesions is relevant for preventing the development of cancer. Confirming the presence of HPV DNA in the lesion is the most effective way to diagnose HPV infection [17, 18]. Unfortunately, this type of testing is expensive and difficult to implement in poor parts of the world [19]. Therefore, regular screening of cytological (Pap smear) or colposcopic abnormalities continues to be an effective preventive strategy for cervical cancer [20]. Still, this is not easy to accomplish in many parts of the world, and HPV-induced cancer continues to be a significant global health burden [3, 21].

The fact that most HPV infections are cleared spontaneously shows that the immune system can effectively eliminate virus-infected cells. This provides an opportunity for controlling HPV-induced cancers through immunization and other novel therapies. Vaccines have been successfully used as a preventative measure against many viral infectious diseases, including smallpox, polio, measles, yellow fever, and hepatitis B [22]. Similarly, a couple of prophylactic vaccines have been developed to prevent HPV infections. These vaccines direct the immune system toward the major capsid protein L1 of the HPV particle [23, 24]. Prophylactic vaccines have been effective in preventing vaccinated, healthy patients from acquiring HPV infections. They have also been effective in preventing reinfection by the same HPV type. However, these prophylactic vaccines have not shown any therapeutic effects on established HPV infections or HPV-induced lesions [25, 26]. Despite these advances in prevention of HPV infections, there is still a need for treatments of already existing HPV infections and their associated malignancies. Novel therapeutic approaches take advantage of our knowledge on how the immune system eliminates virus-infected cells through cytotoxic T cells [27]. Based on this, therapeutic vaccines and intralesion immunotherapeutic strategies are been developed. The idea behind them is to activate specific cytotoxic cells toward HPV-infected cells [28, 29]. In this chapter, we describe the current status of the prophylactic vaccines, and discuss the several therapeutic vaccines that are under development for treatment of HPV-induced lesions.

2. Papillomavirus

Papillomavirus belong to the Papovaviridae family of DNA viruses. The genome of these viruses is about 8000 base pairs and comprises eight defined genes (**Figure 1**). Six early genes code for proteins involved in virus replication and two late genes code for proteins that form

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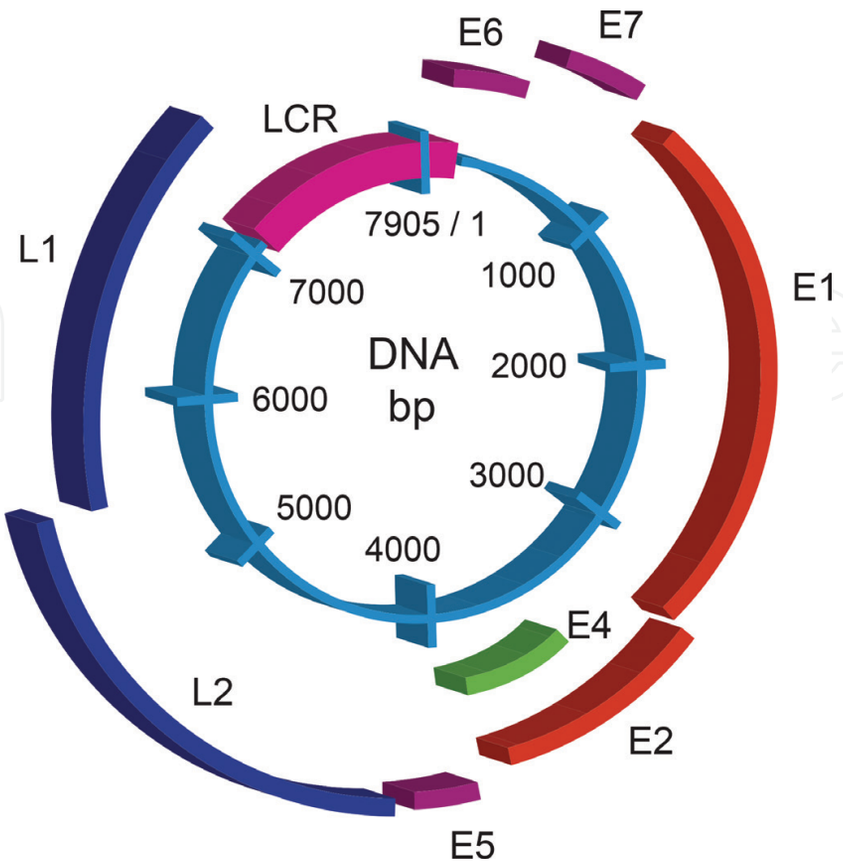

Figure 1. Human papilloma virus genome. The genomic organization of the human papilloma virus 16 is shown. The double strand DNA is about 8000 base pairs. The sequence LCR (long consensus repeat) comprises the promoter and enhancer elements. The early genes E1, E2, E6, and E7 code for proteins involved in viral replication and transcription. The E4 and E5 genes code for proteins involved in immune evasion and virus release. The late genes L1 and L2 code for the virus structural proteins. The E6 and E7 proteins alter the cell replication process and in consequence can function as oncogenes.

the capsid of virus. HPV gene expression is coordinated with the differentiation process of the epithelium. During infection, thousands of new virions are formed and released from the cells without causing cell death [30, 31].

2.1. Low-risk HPV

Infection with HPV is very common and is associated with benign and malignant proliferation of skin and squamous mucosae. Viruses that produce asymptomatic infections or that induce benign growth are classified as low-risk. HPV 6 and HPV 11 are the most common low-risk HPV. Other types are HPV 42, 43, 44, and 45. They produce genital warts and recurrent respiratory papillomas [7]. The standard therapy for low-risk HPV infections is usually the physical removal of the lesion. For this, cryotherapy, application of trichloroacetic acid, laser treatment, or surgical removal is most common.

2.2. High-risk HPV

HPV infections that do not clear spontaneously, usually persist for a long time, and eventually they induce tissue transformation leading to cancer. The viruses associated with tumor

formation are classified as high-risk HPV. In this group, we find the HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58. This group is very important because only about 15 high-risk HPV are responsible for around 95% of all cervical carcinomas [8]. Among these viruses, types HPV 16 and HPV 18 account for about 50 and 14% of all cases of cervical cancer, respectively [4]. High-risk HPV are also involved in other types of cancer, including tumors of the anus, the vagina, vulva, and penis. For these types of tumors, HPV 16 is the most common virus [9]. Also, tumors in tongue, trough [11], and tonsil are also caused by HPV [10]. Similar to most neoplasias, tumor development is a progressive disease. In the case of high-risk HPV infections, malignant lesions display various degrees of histological abnormalities. For the cervix, these lesions are classified as cervical intraepithelial neoplasia (CIN) 1, mild; CIN 2, moderate; and CIN 3, severe. All of these lesions can progress to invasive cancer.

2.3. Life cycle of HPV infection

The human papillomavirus (HPV) infect the epithelium of the cervix, and their replication is closely linked to the differentiation of the epithelium [30, 31] (**Figure 2**). The life cycle of the virus begins when it infects a keratinocyte in the basal layer of the epithelium. The virus usually gets access to the basal membrane through a micro trauma of the epithelium. Once inside the cell, the virus DNA is maintained in the proliferating cells at a low-copy number. During this time, the E1 and E2 genes are expressed and their proteins regulate viral DNA replication and expression of the other early viral genes. E1 is a viral enzyme with ATPase and helicase activity [32, 33]. E2 is a DNA-binding protein involved in activation or repression of different HPV promoters [34, 35]. As the infected cell migrates toward the superficial layers of the squamous epithelium, the viral genome gets integrated into the cellular

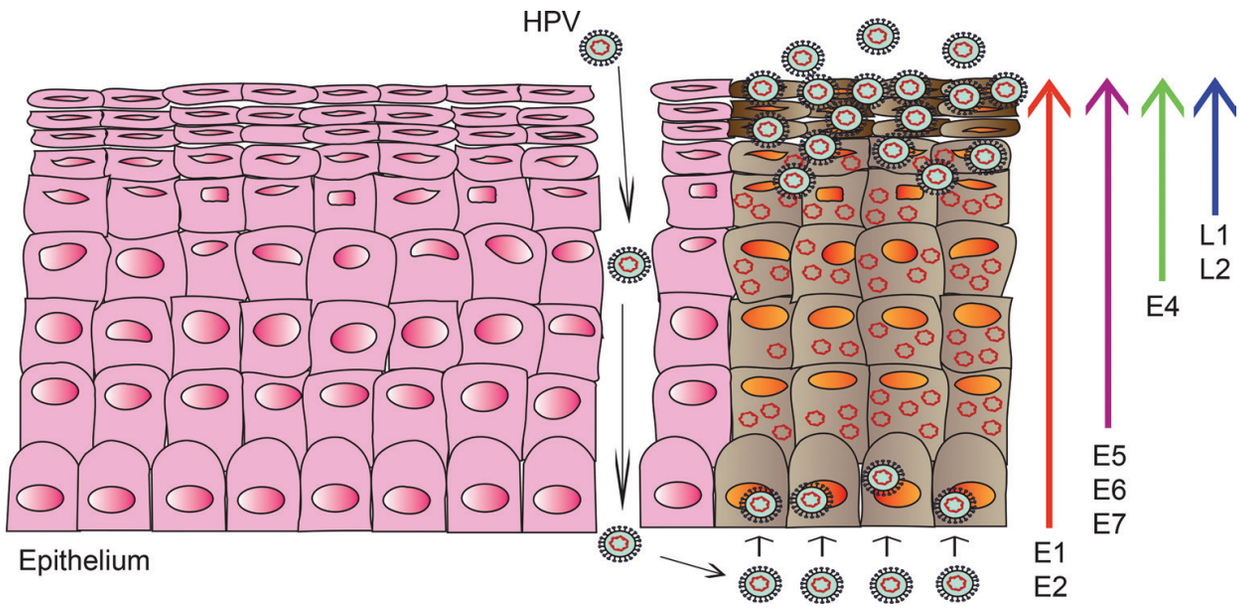

Figure 2. Papilloma virus life cycle. An HPV (human papilloma virus) can reach the base of an epithelium through small cuts and then infect cells. The virus DNA (circles) replicates in the proliferating cells first at a low-copy number, and later when cells differentiate at a high-copy number. In the cells at the top of the epithelium, new virions are formed and released without causing cell death. Expression of the virus proteins is shown at the right.

genome. This integration disrupts or inactivates the E2 gene, leading to a derepression of the E6 and E7 genes. The E6 and E7 gene products modify the cell cycle to maintain the infected keratinocyte in a state, which is advantageous for viral DNA amplification. The E6 protein can associate with and inactivate the p53 tumor suppressor protein. E6 ubiquitinates p53, thus labeling it for proteosomal degradation. The E7 protein binds to the retinoblastoma tumor suppressor gene product pRB, and in this way it competes for binding of pRB to the transcription factor E2F. The result is the release of E2F, which can bind and activate its DNA targets to promote cell cycle progression. With these effects, E6 and E7 are truly oncoproteins and are also responsible for cell transformation [36]. Expression of these oncogenes appears to be a critical step in the maintenance of the transformed stage and progression to invasive carcinoma. The classification of HPV as low- or high-risk types seems to be determined by the relative affinities of E6 and E7 to p53 and pRB, respectively [37]. The E4 gene is an open-reading frame (ORF) within the E2 ORF. This gene product is generated by spliced mRNA and is located centrally within the E2 gene. The E4 is involved in the amplification success and virus synthesis, suggesting a role in virus release and/or transmission [38]. The E5 gene is the least studied so far. Its function is not well characterized. However, HPV infection and transformation take place in complex regulatory patterns of gene expression, in which E5 gene is involved. E5 proteins are thought to act by modulating the activity of cellular proteins [39].

As the infected keratinocytes differentiate and move to the suprabasal and granular epithelial layers, the late genes L1 and L2 are expressed. The proteins L1 and L2 are the major and minor capsid proteins, respectively, and encapsidate the newly synthesized viral DNA (**Figure 2**). L1 can assemble spontaneously into a 72-pentamer icosahedral structure that closely resembles new virions [40]. These pentamers together with the L2 protein form the complete viral capsid [41]. This new capsid gains stability by disulfide bonds between L1 and L2 proteins, and provides resistance to environmental insults when the virus is shed from the epithelium [42], completing the HPV lifecycle (**Figure 2**).

3. Therapy for HPV infections

HPV infection of the anogenital area produces two types of lesions: warts (condyloma acuminata) and squamous intraepithelial lesions. These intraepithelial lesions can progress to neoplasia when a high-risk HPV is involved. Treatment for cervical intraepithelial neoplasia (CIN) usually contemplates elimination of the damaged HPV-infected tissue, leaving the healthy tissue of the cervix intact [43]. Ablative therapies commonly used include cryotherapy, excision procedures (conization), and electrosurgery [44, 45].

4. Immune response to HPV

Protection against viral infections is provided by both arms of the immune system. First, HPV infects cells in a damaged epithelium. The initial inflammation response attracts immune cells

to the tissue, mainly neutrophils, followed by macrophages and later lymphocytes. These innate immune cells can detect nonspecific viral molecules, such as double-stranded viral DNA. In response, cells produce inflammatory cytokines, such as interleukin (IL)-1β, IL-6, IL-8, IL-12, and α-, β-, and γ-interferon (IFN), which in turn activate natural killer (NK) cells [46]. Later, when the new viral proteins are produced, these proteins can be taken up by antigen-presenting cells (APCs), such as Langerhans cells or dendritic cells (DCs) [47]. These APCs process the proteins into small peptides and present them together with major histocompatibility complex (MHC) molecules on the cell membrane, to lymphocytes (T cells) for initiation of an adaptive immune response (**Figure 3**). Activated CD4+ helper T cells can differentiate into Th1, Th2, or Treg/Th3 phenotypes depending on the cytokines they produce. CD4+ helper T cells then, on one hand help activating B cells for the production of specific anti-virus antibodies. On the other hand, they help CD8+ T cells to differentiate into cytotoxic T lymphocytes (CTL) which secrete the proteolytic enzymes, granzyme, and perforin [48]. CTLs are the most efficient cells for destroying HPV-infected cells (**Figure 4**).

An adaptive immune response against the virus is important and for the most cases effective for controlling HPV infections [27]. This is supported by the fact that most HPV-related lesions are cleared spontaneously by immune-competent individuals [14, 30]. Also, in HPV-related regressing, but not in persistent lesions, infiltration of cytotoxic T cells has been detected [49]. Moreover, in immunosuppressed individuals, such as organ transplant recipients [50] or

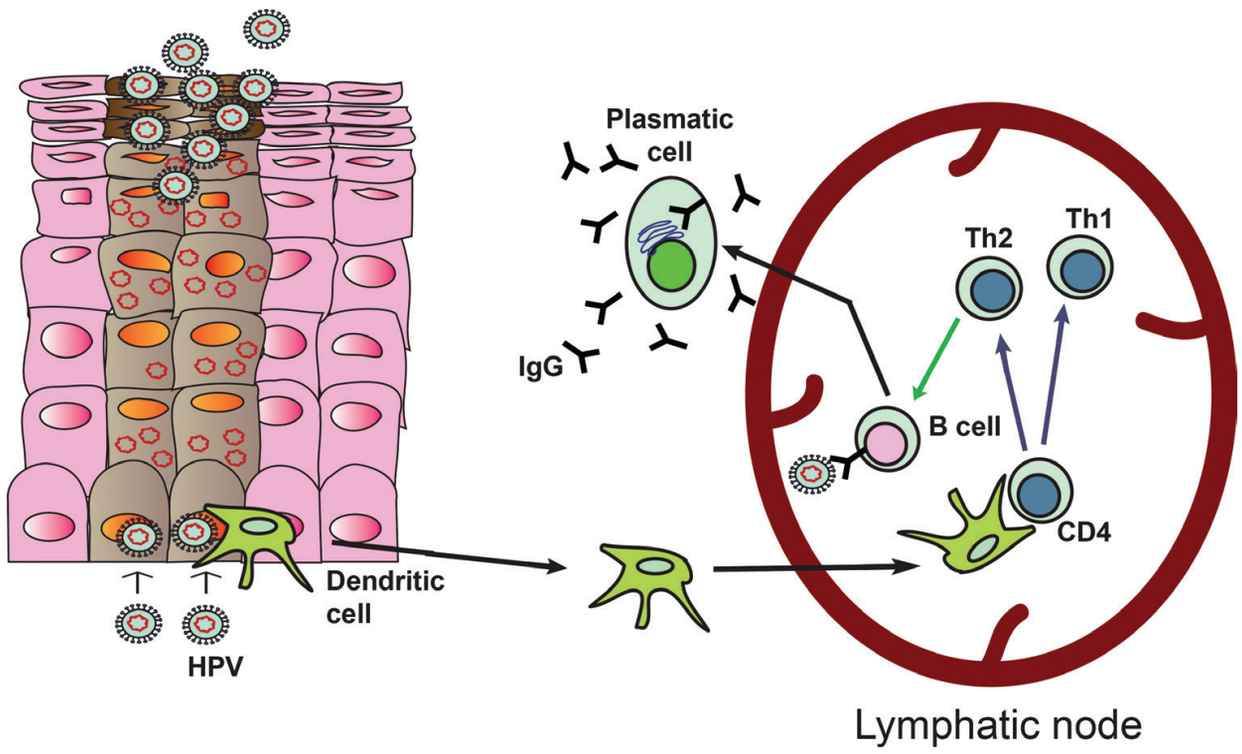

Figure 3. Humoral immune response to HPV. Dendritic cells (DC) capture HPV antigens from infected cells and migrate to lymph nodes, where they present the processed antigen to CD4+ T cells. These T cells then differentiate into T helper cells, either Th1 or Th2, depending on the type of cytokines they produce. B cells recognize native viral antigens and with help from Th2 cells, differentiate into antibody (IgG)-secreting plasma cells.

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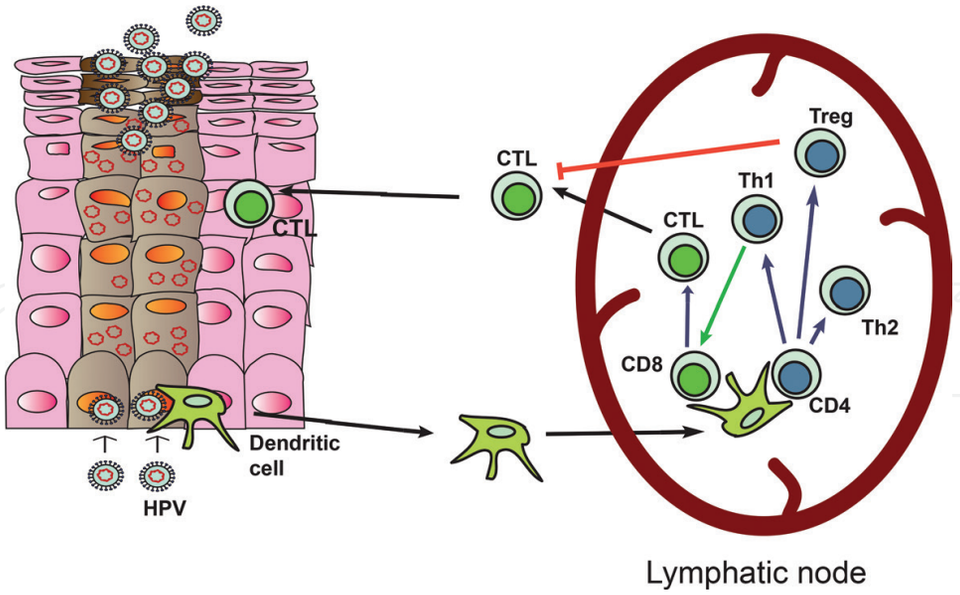

Figure 4. Cellular immune response to HPV. Dendritic cells (DC) take HPV antigens and migrate to lymph nodes. There, DC present processed viral antigens to CD8+ T cells in the context of MHC class I molecules and to CD4+ T cells in the context of MHC class II molecules. CD4+ T cells differentiate into T helper (Th1 or Th2) cells. With the help from Th1 cells, CD8+ T cells differentiate into cytotoxic T cells (CTL). These CTL migrate back to kill virus-infected epithelial cells. CD4+ T cells can also differentiate into regulatory T cells (Treg), which inhibit the cytotoxic activity of CTL.

human immunodeficiency virus (HIV)-infected people present a higher incidence of HPV-related lesions [51].

4.1. Humoral response

An efficient humoral immune response is detected in most patients with HPV infections. These patients have antibodies that recognize viral proteins, such as L1, E2, and E4 the first stage of infection. Later, when the virus DNA gets integrated into the cell genome, antibodies specific for the E6 and E7 proteins can be found in some lesions. Unfortunately, this antibody response is weak and variable and it does not seem to protect from future re-infections [52]. Thus, humoral responses are not efficient at eliminating established HPV lesions.

4.2. Cellular response

A cellular immune response is more important for eliminating HPV-related lesions. Activated CD8+ cytotoxic T cells, can efficiently destroy virus-infected cells, and in doing so they also prevent the onset of cancer lesions (**Figure 4**). The central role of T cells for controlling HPV infections is supported by many clinical observations where the elimination of lesions correlates with T cell functions. For example, patients who successfully eliminated previous HPV 16 infections present memory T cell responses to viral proteins [53], and in patients with spontaneous regression of grade 3 vulvar intraepithelial neoplasia strong CD4+ and CD8+ T cell responses are found [49]. In contrast, patients with cervical intraepithelial neoplasia or cervical cancer present deficient T cell responses [54].

4.3. Mechanisms of HPV to evade the immune system

HPV can be detected and eliminated by an efficient immune system. However, HPV also possess strategies to reduce the actions of the immune system. The best way to trick the immune system is to avoid detection. HPV infects tissues where immune surveillance is limited. In the epithelium of the cervix, the number of DCs greatly declines toward the external layers. Also, the virus replication is coupled with the differentiation state of the infected keratinocyte. Expression of viral proteins increases progressively with differentiation and upward migration of keratinocytes. Thus, the most immunogenic viral proteins are expressed last, in cells that are found in areas of poor immune surveillance (**Figures 3** and **4**). In addition, new virions are released through the normal rupture of surface epithelium. This action prevents inflammation and reduces the virus uptake by DCs. Therefore, HPV replication is a local phenomenon with minimal activation of the immune system.

In addition, HPV has other strategies that interfere with the immune response [27]. The E6 and E7 proteins block IFN production by the infected cell. E6 blocks the transcription factor IFR-3, which activates β-IFN gene expression. With less interferon produced, many interferon-responsive genes are downregulated [55]. Similarly, E7 also inhibits the expression of α-IFN-responsive genes [56, 57]. Also these two oncoproteins can reduce expression of Toll-like receptor (TLR) 9 [58], and some cytokines, such as IL-8 and IL-18 [59, 60]. TLR 9 is expressed in endosomal vesicles where it binds to unmethylated CpG sequences in viral DNA. TLR 9 then signals for production of anti-virus proteins, such as type-I interferon [58]. IL-8 is a potent chemoattractant for neutrophils and T lymphocytes [59], whereas IL-18 induces γ-IFN production by leukocytes [60]. Thus, E6 and E7 proteins can block several innate immune responses. In addition, the viral proteins E5, E6, and E7 can inhibit the expression of MHC class I molecules, reducing recognition of the HPV-infected cell by NK cells and by specific CTLs [61].

5. Prophylactic vaccines

Due to the strong correlation between the presence of HPV infection and tumors, it was thought that by preventing HPV infections, the HPV-induced cancers would disappear. Also as mentioned above, because HPV capsid proteins are recognized by antibodies from infected patients, it is clear that antibodies can bind virus particles. Thus, HPV vaccines that would induce the production of antibodies and could prevent infection were developed in the last decade. The pharmaceutical companies, Merck in the USA and GlaxoSmithKline (GSK) in Europe, created the two prophylactic vaccines approved and used today. Both vaccines, take advantage of the fact that capsid L1 proteins spontaneously assemble in virus-like particles (VLP) without viral DNA. These VLP, produced by overexpressing HPV L1 protein in yeast or insect cells, provide a source of the immunogenic L1 proteins in a non-infective form.

5.1. Cervarix® and Gardasil®

Cervarix® (GSK) is a bivalent vaccine against VLP of HPV types 16 and 18, produced in insect cells [62], whereas Gardasil® (Merck) is a quadrivalent vaccine made with VLP of HPV types

6, 11, 16, and 18, produced in yeast [63]. Both prophylactic vaccines are designed for HPV naïve individuals, since as mentioned above antibodies do not have a protective effect on already infected individuals. These vaccines generate a good antibody response that prevents new infections with high efficacy [64–66] from the HPV types included in the vaccines. Due to a small cross-reactivity [64], these vaccines also show some prophylactic effect on other HPV subtypes not included in the vaccine [67, 68]. However, for the most part these vaccines are effective only for those HPV types included. A new version of these vaccines including nine different types of HPV (6, 11, 16, 18, 31, 33, 45, 52, and 58) has also been developed. Since Gardasil 9® seems to be cost effective compared to the previous vaccines [69], it has also been licensed for clinical use [70].

These vaccines promise to reduce, in the future (30 years from now) the incidence of infection from the HPV types included in them [71]. However, this promise could only be possible if more than 50% of uninfected people get vaccinated. Unfortunately, this kind of coverage for boys and girls before the onset sexual activity, will be difficult and expensive in many parts of the world [72]. Thus, incidence of HPV-related diseases can increase despite HPV vaccination [73] due to many unvaccinated people, who will remain at a high risk and in need for treatment. In addition, distribution of HPV types among cervical malignancies changes all over the world [74–76]. Although, the high-risk HPV 16 and 18 are associated with most cervical cancers in Occident, this is not the case in Asia, where less than 60% of cervical cancer are related to these HPV types [28]. Therefore, the current prophylactic vaccines cannot cover all oncogenic types of HPV in different populations, and their general use in other parts of the world is questionable [68].

5.2. Limitations of current vaccines

As already mentioned, the current prophylactic vaccines against HPV have a limited coverage to only those types included in the vaccine. Since the antibody immune response to L1 proteins is highly specific, no general coverage can be achieved. In addition, the current prophylactic vaccines do not elicit cell-mediated immunity. This means that although these vaccines can protect from most HPV infections (70–80%), the rest of HPV types remain a serious threat for HPV-induced diseases even after vaccination [62, 77]. Despite government efforts to subsidize vaccination programs in order to achieve full coverage, it remains that even vaccinated females must continue cervical cancer screening [78].

These vaccines are directed against the L1 protein of only certain types of HPV. In order to increase coverage vaccines against all HPV types would need to be produced. This will increase the cost of production on multivalent vaccines. The use of adjuvants to augment the immunogenicity of the capsid proteins makes the vaccine thermolabile and also adds to the cost of the vaccine. The problem is that the population that is in need of these vaccines is exactly the one with fewer economical resources. Recently, a two-dose immunization protocol has been tried instead of the recommended three doses schedule. This seems to provide similar protection and thus it is a promising cost reducing strategy [79, 80].

The vaccines are designed for HPV naïve individuals. This requires that very young people get vaccinated before becoming sexually active. The benefit for immunizing older women

seems very limited, since no therapeutic effects have been detected for these vaccines [81]. The reason for this is that antibodies induced by these vaccines are directed against the L1 proteins and once the infection is established, these proteins are not expressed. Contrary to this, a therapeutic vaccine would need to be directed against proteins that are expressed throughout the lifecycle of the virus [82] (see next section).

Although, a good humoral immune response is obtained and antibodies are capable of blocking infection, the prevention of cancer by these vaccines is still presumptive. In all clinical trials, the end point has been prevention of only CIN 2/3 lesions. Also, because HPV infections may take a long time to develop cancer, the anti-cancer effect of these vaccines will be known in the future, when vaccinated people become adults and are exposed to the virus [83].

5.3. New prophylactic vaccines

Both current prophylactic vaccines are based on L1 VLP and are therefore very HPV type specific, thermolabile, and costly. The quest for newer vaccines continues with the aim of making them more affordable, more thermostable, and with more coverage toward larger number of HPV types. With these goals in mind, newer prophylactic vaccines are in development. Two kinds are worth mentioning, a L2 protein-based vaccine and a capsomere vaccine.

The capsid L1 protein is highly specific for each type of HPV. In contrast, the L2 protein contains a region that is highly conserved among most high-risk HPV types. This fragment between amino acids 20 and 38 is capable of inducing antibodies that are neutralizing for many HPVs [84]. Unfortunately, the L2 protein is not very immunogenic, and several approaches are being used trying to increase its immunogenicity. These include producing a recombinant protein in bacteria, an expression system in *Lactobacillus casei* for possible oral immunization, and production of L2 VLP derived from bacteriophage PP7 [82, 85].

A VLP formed with L1 protein requires 360 copies of the protein. Thus, a VLP is complex, more expensive to produce, and thermolabile. In contrast, a capsomere is much simpler, thermostable, and cheaper to produce. A capsomere is the basic component of the virus capsid. It has only five L1 copies of the protein, presents similar immunogenicity than an L1 VLP, and can be produced in bacteria [70]. A phase II clinical trial for a HPV 16 L1 capsomere vaccine is currently being conducted (NCT 01355823) [82, 86].

6. Therapeutic vaccines

Preventive vaccines are directed to the external proteins of the virus. By inducing a strong humoral immune response, the antibodies formed can bind to the virus capsid and block the interaction of the virus with endothelial cells. These antibodies can then neutralize the virus and prevent infection. However, this mechanism is not effective when the virus has already entered the cell. Antibodies induced by prophylactic vaccines cannot treat existing viral infections or established HPV-related diseases. Therefore, as discussed above, a high prevalence and mortality of cervical cancer still remains a serious health problem in the world, especially

in developing countries [3, 76]. In order to treat an established disease, the elements of the virus present during replication should be the target of the therapy involved. Also, since there are not anti-viral drugs, an effective treatment should be able to stimulate the immune system for elimination of virus-infected cells. An ideal therapeutic vaccine must activate both CD4+ (helper) and CD8+ (cytotoxic) T cells for elimination of the virus [37]. Cytotoxic cells need to recognize a viral antigen expressed in the infected cells. In the case of HPV, the capsid proteins L1 and L2 are expressed in terminally differentiated keratinocytes on the external part of the epithelium; a segment of the tissue where antibodies and cells cannot easily reach (**Figure 2**). In contrast, HPV early proteins, such as E1, E2, E6, and E7, are expressed in multiple stages of the virus infection (**Figure 2**). Consequently, these proteins are all good therapeutic targets.

E2 is a DNA-binding protein involved in activation or repression of different HPV promoters [35], and it also has a relevant role in controlling migration of viral DNA to daughter cells during mitosis of infected cells [87]. Due to these functions, E2 is expressed in all stages of the infection (**Figure 2**). Thus, it is an excellent target for stimulating the immune system for elimination of infected cells at multiple replication stages. In earlier studies, dogs, immunized against papilloma E1 and E2 proteins, did not show papilloma growth after viral challenge, or even presented complete regression of papilloma [88, 89]. These encouraging findings led to devise new vaccines that could activate cellular immune responses to the E2 protein. Clinical trials with these new vaccines have provided very encouraging results (see next section) [90, 91]. As indicated before, the E6 and E7 proteins are important for cancer. Therefore, they are also studied as probable antigens of therapeutic vaccines.

Different types of therapeutic vaccines have been designed and some have also been tested in clinical trials. These novel therapeutic vaccines can be grouped into five categories: peptide-based, protein-based, DNA vaccination, viral vectors, and dendritic cell-based immunization [44, 82]and are described in the following sections.

6.1. Peptide-based vaccines

Instead of using a whole protein, fragments of it can be prepared for immunization. Peptides are cost-effective and safe, but they are also usually poorly immunogenic. Thus, in general, peptides need to be mixed with adjuvants to improve their immunogenicity, deciding what peptides are useful is not easy, however. Recognizing what parts of an HPV antigen are immunogenic is almost impossible to predict and small peptides normally only present linear epitopes. Conformational epitomes that may be needed for an efficient immune response are usually not included. Thus, current preparations contain mixtures of peptides. In addition, because MHC molecules (HLA in humans) are polymorphic, it is possible that some peptides cannot be presented in some patients. An approach used to avoid this, has been the use of restricted HLA-binding peptides. Identification of these peptides is an even more complicated task, making the peptide approach unreliable and more expensive. Also, another complication with this strategy is that exogenously added peptides may load onto MHC class I molecules on cells other than antigen-presenting cells. In this case, the peptide-based vaccine may induce tolerance instead of stimulation [92]. In consequence, the best approach seems to be the use of long overlapping peptides, which appear to be processed and presented correctly by dendritic cells [93].

Despite these complications, some peptide-based vaccines have been tried. In phases I–II clinical trial, one vaccine made of two HPV E7 peptides and one T cell helper peptide, stimulated proliferation of T cells, but it did not induce cytotoxicity against E7 peptides [94]. In a different study, a mixture of long peptides from oncoproteins E6 and E7 in incomplete Freund's adjuvant, was administered to 20 patients with HPV 16-positive, high-grade vulvar intraepithelial neoplasia. In five patients, a T cell response was detected, together with complete regression [95].

Another vaccine (HPV 16-SLP) made of a mixture of long peptides from E6 and E7 proteins, has also been tested. In a group of patients with resected HPV 16-positive cervical cancer, this vaccine induced some HPV 16-specific T cell immune responses including γ-IFN-producing CD4+ T cells. Unfortunately, proliferation of T cells with a regulatory phenotype (Treg) was also detected, suggesting that the response against HPV was not completely effective [96]. In another group of women with high-grade cervical squamous intraepithelial lesions, this vaccine did not induce infiltration of HPV 16-specific T cells into the lesions or HPV clearance [97]. In a third group of patients with HPV 16-positive advanced or recurrent gynecological carcinoma, this vaccine was given with the adjuvant Montanide ISA-51. In this case, a T cell response was detected, but unfortunately no tumor regression or prevention of progressive disease were found [98].

6.2. Protein-based vaccines

Immunization with complete HPV proteins seems a more efficient approach. HPV recombinant proteins can be produced in large quantities and they would provide all possible epitopes of the protein, after processing by APC. However, complete protein still present low immunogenicity and they need to be mixed with adjuvants, or fused to other proteins with more immunogenicity. Some HPV protein vaccines consist of E6 and E7 proteins fused to immunogenic proteins as described next.

A chimeric protein made from the carboxyl-terminally part of HPV 16 L1 protein fused to the amino-terminal part of the HPV 16 E7 protein was produced. This recombinant fusion protein self-assembles into virus-like particles and it has been named L1VLPE7. In a small group of patients with HPV-induced CIN 2/3 lesions, these chimeric VLPs induced antibodies with high titers against HPV L1 and with low titers against HPV E7. Thus, the antibody response again was better toward the capsid protein than the early-gene protein. Consequently, no histological improvement in lesions was observed [99]. Another similar recombinant HPV 16 L1(ΔN26)-E7(ΔC38) protein also assembles into chimeric VLPs. These chimeric VLPs induced neutralizing antibodies and triggered some cell-mediated immune responses in a murine model of cervical cancer [100].

Another fusion protein (SGN-00101) consisting of a heat shock protein (Hsp) from *Mycobacterium bovis* and HPV 16 E7 protein, was administered to patients with CIN 3. Regression to CIN 1was seen in some patients, but it was not clear whether this result was caused by the vaccine or it was just natural regression [101]. Later, the same preparation was administered in several doses during 3 weeks. With this procedure, one-third of patients presented regression that correlated with immune response [102].

Yet, another fusion protein (HPV16 E6/E7) formed by HPV E6 and E7 was produced and tried mixed with the adjuvant ISCOMATRIX. In a group of patients with CIN, this preparation induced a cellular immune response. Unfortunately, the elimination of lesions detected in few patients did not correlate to this immune response [103].

Another recombinant fusion protein made of E6, E7, and L2 proteins (TA-CIN—tissue antigen-cervical intraepithelial neoplasia) has been given to a small group of patients with anogenital intraepithelial neoplasia (AGIN). Unfortunately, there was not a correlation between induction of systemic immunity and clinical outcome [104]. In another group of patients with vulvar intraepithelial neoplasia, a topical application of the immunomodulator, Imiquimod was given for 8 weeks before three doses of TA-CIN at 4-week interval were administered. With this protocol, an important local infiltration of CD8+ and CD4+ T cells in lesions of responding patients was detected, suggesting that the inflammatory state induced by Imiquimod enhances the immune response. Unfortunately, the therapeutic effect was only detected in few patients [105].

6.3. DNA-based vaccines

Another approach for immunization is the use of plasmid DNA coding for the protein of interest. It is known that plasmid DNA, when injected into the skin or muscle can induce immune responses to encoded antigens. The mechanism is poorly understood, and the response is rather inefficient. Yet, new physical methods for delivering DNA seem to induce better immune responses [106]. Some DNA preparations for HPV early proteins have been tried.

A DNA plasmid that encodes for HPV consensus E6/E7 fusion gene (pConE6E7) has been tested in mice and rhesus monkeys. Immunization induced a potent cellular immune response against both E6 and E7 proteins [107], and it was able to delay the growth of established HPV-tumors [108]. Another plasmid encoding E7-specific CTL epitopes from HPV 16 and 18 and embedded in biodegradable micro particles (ZYC101a) was tested in a group of patients histologically confirmed CIN 2/3 neoplasia. About 43% of patients presented regression, compared to 27% of patients receiving placebo, but the difference was not statistically significant, and no correlation between cytotoxic activity and clinical outcome was detected [109]. Another DNA preparation (Amolimogene) contains an encapsulated plasmid encoding some proteins of HPV. In a small group of patients with HPV-associated high-grade CIN, no correlation between cellular immunity and clinical response was reported [110]. Another DNA preparation (Sig-E7(detox)-HSP70) encoding a fusion protein between HPV E7 protein and heat shock protein 70 was tried in a small group of patients with HPV-induced CIN 2/3. Weak HPV E7-specific T cell responses were detected, but not correlation was found between this immune response and clinical outcome [111]. Although DNA vaccines are good tools to enhance the immune system, their approval from regulatory agencies seems unlikely. Regulatory agencies require that novel vaccines fulfill the followings requirements: laboratory demonstration of proof of concept, design end establishment of the manufacturing process, adequate quality and non-clinical safety, clinical trial approval, safety and efficacy, and a marketing authorization application. The use of naked DNA in humans remains a major

safety concern, and DNA vaccines, so far have not shown good activation of a specific cellular immune response.

6.4. Recombinant virus

Another approach that has shown better results for treatment of HPV-induced lesions is the use of recombinant viruses. A virus can deliver gene products directly into cells and because an active viral infection takes place, the immune system responds better activating the cellular effector functions. The highly attenuated poxvirus strain modified vaccinia virus Ankara (MVA) has become the vector of choice for novel HPV therapeutic vaccines [112]. This MVA virus is a non-replicating derivative from the virus of the smallpox vaccine. This exceptionally successful vaccine was given to millions of people without any complications. Thus the use of MVA in humans is completely safe. Other advantages of MVA are that it is genetically stable, very immunogenic, and easy to manufacture [113, 114]. The MVA immunogenic potential for cytotoxic responses is due in part to uptake of dying vaccinia virus-infected cells by antigen-presenting cells and cross-presentation of antigens to CD8+ T cells [115]. Several MVA vectors against various diseases are now being evaluated in phase I/II clinical trials [116]. The MVA vectors designed for treatment of HPV are described in the following section.

TA-HPV is a vaccinia virus encoding modified versions of the E6 and E7 genes from HPV 16 and HPV 18. Patients with high-grade vulval intraepithelial neoplasia were immunized intramuscularly with TA-HPV. Some of these (42%) patients presented partial reduction of lesions, but no increase in cytotoxic activity against selected HPV E6 or E7 peptides [117]. In another small group of patients, a partial reduction in lesion diameter, and an infiltration of T cells were observed [118].

Another modified MVA virus contains E6 and E7 proteins together with the human IL-2 gene. This vaccine (TG4001) was given subcutaneously in three weekly doses to 21 patients with CIN 2/3. About half of patients had some clinical responses 6 months later. However, no immune response was reported [119].

Another MVA recombinant virus (MVA E2), containing the bovine papilloma virus (BPV) E2 protein [120], has been has been assessed in several clinical trials. In a group of patients with HPV-induced CIN 1 to CIN 3 lesions, that received MVA E2 injected directly into the uterus once every week for 6 weeks, 94% (34) of patients had complete elimination of precancerous lesions. In addition, an important reduction (90%) in viral DNA load was observed in half of the patients. The others have completely eliminated the virus [90]. Next, in a phase II clinical trial for high-grade lesions, about half (56%) of patients presented complete regression, and in another third (32%) of patients, the lesions were reduced by 90–60% [91]. Importantly, specific cytotoxic activity against cancer cells correlated with clinical outcome [91]. More recently, in a phase III clinical trial, 1176 female patients with anogenital intraepithelial lesions were treated with MVA E2. Most of the patients (89%) showed complete elimination of lesions, and generated a specific cytotoxic response against HPV-transformed cells [121]. These clinical results indicate that MVA E2 is one of the most promising vaccines for therapy of HPV-induced malignancies.

MVA-E1 is a new MVA-based vaccine against HPV. This vaccine consists of the MVA vector encoding the E1 sequence of HPV 16. In a mouse model, immunization with MVA-E1 resulted in sustained HPV E1-specific cellular cytotoxic response [122].

6.5. Dendritic cell-based vaccines

As discussed earlier, dendritic cells (DCs) are major antigen-presenting cells that can efficiently activate cellular immune responses. Based on this, another approach to develop a therapeutic vaccine is the use of dendritic cells pulsed with HPV antigens. The idea is to generate DCs *in vitro* from monocytes taken from the same patient. Then, these DCs are presented with recombinant HPV proteins. The cells should process and present antigens on their membrane. Finally, the pulsed DCs are administered back to the patient to stimulate the immune system. The procedure is complex, time consuming, very costly, and has to be performed individually for each patient.

Earlier studies showed that autologous DCs loaded with HPV E7 protein could induce *in vitro* a specific T cell responses [123], and T cell proliferative responses *in vivo*, [124]. Another study found E7-specific γ-IFN secreting CD8+ T cells in patients treated with autologous DCs pulsed with HPV E7 [125], or with HPV E7 protein and keyhole limpet hemocyanin (KLH) [126]. These reports indicated that DC-based immunization improves T cell responses, but they did not evaluate the therapeutic potential. Recently, it was reported that DCs can be pulsed more efficiently if HPV antigens are directed toward the co-stimulatory molecule CD40 [127]. With a prototype vaccine (anti-CD40-HPV16.E6/7) consisting of a recombinant fusion protein of anti-human CD40 and HPV16 E6/7 protein), DCs could efficiently activate *in vitro* HPV E6/7-specific CD8+ T cells, from the blood of HPV16+ head-and-neck cancer patients [127].

These results show that this approach may be useful in the future if important questions still remaining on the nature and function of dendritic cells can be resolved. For example, are there any unique cell surface receptors that would allow for specific selection of DCs? What are the special DC subsets that can enhance the efficacy of vaccines? What are the DC activators that allow differentiation and/or maturation of a particular type of DC with the ability to promote effector T cells against tumors? Today, DCs are generated *in vitro* from peripheral blood monocytes. This procedure generates cells that vary greatly in their functional capacity, thus making their use in the clinical setting very uncertain.

7. Conclusion

Human papillomavirus (HPV) infections remain an important public health issue because they are associated to cervical carcinoma, the second most common cancer among women [4]. Two preventive vaccines have been approved and promise to achieve in the future, a reduction in HPV-related cancer incidence. However, these vaccines are not the complete solution. Since complete vaccination coverage is difficult and costly in many parts of the world [72], and since these vaccines are highly HPV type specific, only the high-risk HPV types responsible

for about 60% of all cervical cancers (HPV 16 and 18) are included in these preventive vaccines, a large population will remain at a high risk of HPV infections. Also, these preventive vaccines do not have any therapeutic potential. Therefore, many people remain in need of efficient treatment for HPV-related diseases.

Novel therapeutic vaccines for treatment of HPV-infected tissues are now being tested for their potential to activate an immune cellular response. Different types of therapeutic vaccines are considered. Studies, so far have shown variable results for most of them. However, the therapeutic vaccines using recombinant virus have demonstrated to be very effective in clinical settings. Thus, recombinant vaccinia therapies are today the most promising candidates for a successful treatment of HPV-induced cancers.

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