Biomedical Reviews 2003; 14: 75-93. Founded and printed in Varna, Bulgaria
ISSN 1310-392X

HUMAN PAPILLOMAVIRUS INFECTION AND CERVICAL CANCER

Toshiyuki Sasagawa
School of Health Science, Faculty of Medicine, Kanazawa University, Kanazawa, Japan

More than 50% of young women who have had sex early in life acquire cervical human papillomavirus (HPV) infection. Most of the infections are with the high-risk HPV types, which are potential ethiologic factor for cervical cancer. Although ten or more HPV types are thought to be of high-risk, perhaps less than 30% of such infections persist and less than 1.5% develop cancer. Many previous studies clarify the oncogenic potentials of HPV16 or 18 genes by the experimental transformation of cells, introduced with target genes. Some molecular events induced with E6 and E7 genes of high-risk HPV types could explain the mechanisms of HPV for promoting cancer development. One important step for the malignant progression by HPV is the stable expression of HPV E6 and E7 oncoproteins in the host cells, and this may be caused by the integration of the HPV genome into the host genome. The E6 and E7 proteins induce uncontrollable cell growth and accumulate mutations in the host genome after many cell divisions by inhibiting the functions of Rb and p53, which tightly regulate cell division cycle. However, it is postulated that such molecular events may occur only in cells able to escape from the immune-surveillance system, since all viral proteins are potentially immunogenic and targeted by the immune cells. Thus, immune responses to HPV and immune-evasion mechanisms of HPV play the most important role for persistent HPV infection and the progression of cervical diseases. It is reported that increased helper T type 2 (Th2) responses (suppression of cell-mediated immunity) and reduction of Th1 responses (no stimulation of cell-mediated immunity) are more frequently observed in cervical intraepithelial neoplasia (CIN) lesions than in normal cervices, suggesting that immune suppression is involved in CIN development. Correlations between certain major histocompatibility complex (MHC) class I and class II alleles and susceptibility to cervical carcinoma or protection against HPV infection are reported, suggesting that the genetic background related to immune responses may contribute to different outcome in the women who are infected with HPV. HPV appears to evade the host immune responses by several mechanisms: (i) avoidance of viral antigen recognition, (ii) induction of HPV16 E7 tolerance, (iii) modulation of the signal transduction pathways of interferons and other cytokines such as interkeukin-18, and MHC antigens, (iv) inhibition of surrounding immune cell activity by secreted E6 and E7 proteins. Both immune-suppressive and oncogenic actions induced with HPV infection may allow HPV-infected cells to transform into cancer cells in vivo by conferring survival abilities against the growth inhibitory or apoptotic stimulation of cytokines and the attack of killer cells.
Biomed Rev 2003; 14: 75-93.

INTRODUCTION

Cervical cancer is one of the most common cancers worldwide, accounting for 6% of all malignancies in women (1). Globally, approximately 470,000 new cases are diagnosed, and 230,000 women die from invasive cervical cancer each year. Cervical cancer predominantly afflicts economically disadvantaged women, in both developing and industrialized nations. Papillomavirus DNA was first identified in cervical cancer tissues and cloned by zur Hausen and colleagues

Received 27 July 2003 and accepted 4 December 2003.
Correspondence and reprint requests to Dr Toshiyuki Sasagawa, School of Health Science, Faculty of Medicine, Kanazawa University, 5-11-80, Kodatsuno, Kanazawa, Ishikawa 920-0942, Japan. Tel./Fax: 81 76 2652575.
E-mail: tsasa@med.kanazawa-u.ac.jp

(2-4). Human papillomaviruses (HPVs) are small, double-stranded DNA viruses. The virus particle is composed of 72 capsomeres (Fig. 1). HPV infection is known to induce hyperproliferative lesions and cutaneous warts on the skin, and *condylomata acuminata* in the genital epithelia. Accumulated evidence indicates that HPV is the major causative agent of anogenital cancers, which include cervical, vulvar, penile and anal squamous cell carcinomas. HPV infections of the genital areas are thought to be sexually transmitted. The majority (85-90%) of all cervical carcinomas are of squamous type, and develop in women aged 40-60 years. The precursor lesions to squamous cell carcinoma are known as cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesions (SIL), and are generally diagnosed in younger women at 20-30 years of age (5). This demonstrates the significant time lag between HPV infection and the development of invasive cervical cancer, which suggests that additional molecular events are required for progression to malignancy. More than 40 HPV types are known to infect the mucosal epithelium of the uterine cervix, and some of these types are implicated in cervical cancer. Thus, these HPVs are referred to as "high-risk types" for causing cervical cancer. Although not all HPV infections induce cancer, it is important to note that a high incidence of cervical HPV infection is observed in young women, and HPV-infected women may develop cervical cancer without displaying any symptoms. In this review, the numerous data on the gene function, natural history, and immune responses to human papillomaviruses are summarized. Understanding these mechanisms may provide an important insight for the rational management of cervical HPV infection and the development of an effective vaccine.

HPV GENE FUNCTION AND LIFE CYCLE

The genomes of all HPV types contain approximately eight open reading frames (ORFs), which are transcribed as polycistronic messages from a single DNA strand (Fig. 2). The regulatory sequences that are required for viral replication and transcription are concentrated in a noncoding region, which is termed the upstream regulatory region (URR) or long control region (LCR). The gene products can be divided into early and late classes. The early proteins (E) in high-risk HPV types are expressed from the early promoter (P97 in HPV31) prior to productive replication, while the late proteins (L) are expressed from the late promoter (P742 in HPV31) during the synthesis of new virions. The early proteins E1 and E2 are necessary for viral DNA replication, and they bind as a complex to sequences around the origin of replication (6-8). It has been suggested that E2 has repressor activity for viral gene transcription and has the ability to con-

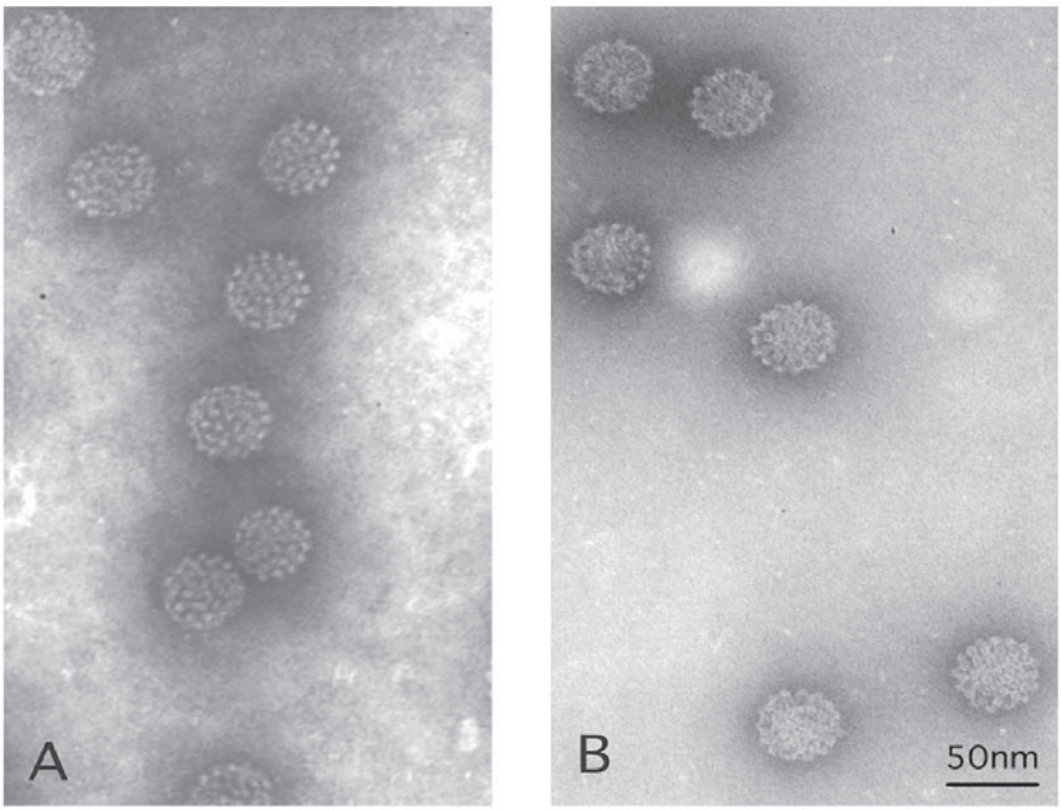

Figure 1. *Native HPV type 1 virions* ***(A)****, and HPV type 16 virus-like particles* ***(B)****.*

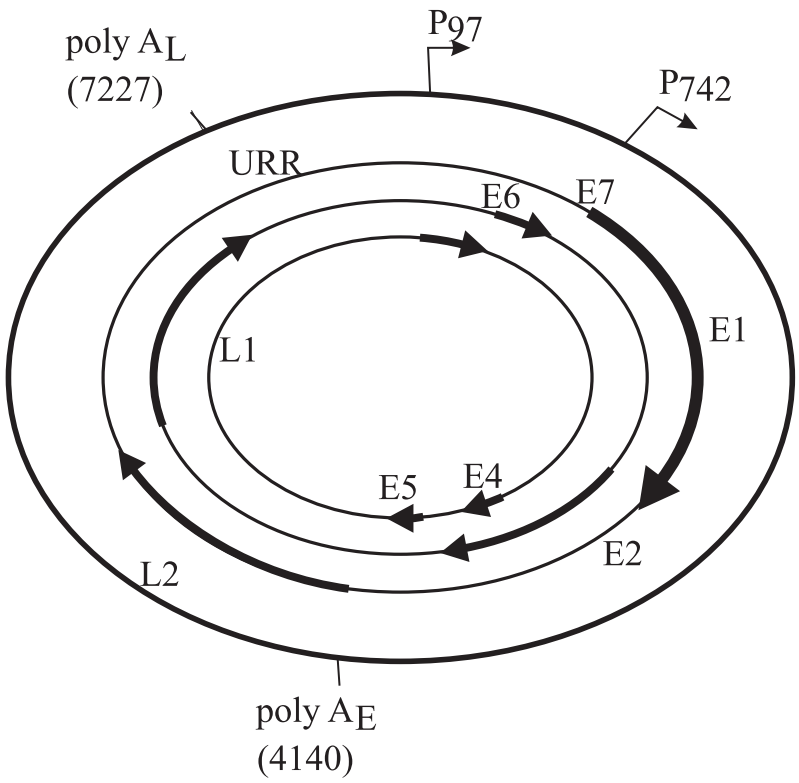

Figure 2. *The genome of the high-risk HPV31. The diagram indicates the ORFs of the early (E) and late (L) genes, the upstream regulatory region (URR), the two major promotors that drive viral expression (P97 and P742) and the two polyadenylation sites (AE4140 and AL7227).*

trol viral DNA replication (9,10). E4 is expressed as a fusion protein with amino acids from the N-terminus of E1 (E1, E4), and has been implicated in alterations to the cytoskeletal network (11). The function of the E5 gene is largely unknown, but it appears to encode a membrane protein with weak transforming activity for the infected cell (12). The *E6* and *E7* genes are thought to have oncogenic potential, and the late genes *L1* and *L2* encode the capsid proteins that are assembled to form the virus shell.

The productive life cycle of HPV is directly linked to epithelial cell differentiation. Papillomavirus infection is believed to occur in the epithelium at areas of microscopic trauma, where the basal cells are exposed to viral entry. Following entry into basal epithelial cells, the HPV genomes are established as episomes at approximately 50 copies *per* cell, and these episomes replicate in synchrony with the cellular DNA (13). The establishment and maintenance of HPV genomes are associated with the expression of the early HPV transcripts that encode the oncoproteins E6, E7 and E5, as well as the replication proteins E1 and E2. Following cell division, infected daughter cells leave the basal layer, migrate towards the suprabasal regions, and begin to differentiate. In contrast to uninfected keratinocytes, which exit the cell cycle as soon as they detach from the basement membrane, HPV-infected cells enter into the S phase after reaching the suprabasal layer. This entry into the S-phase results in amplification of the viral genomes to thousands of copies *per* cell, along with cell proliferation. Concurrent with viral DNA amplification, the E1 and E4 proteins and the capsid proteins are synthesized, which results in the assembly of infectious virions (14). Subsequently, the virions are released into the environment as the upper layer of the epithelium is shed. In low-grade cervical lesions, the HPV genomes are found exclusively as episomal DNA molecules, which suggests that these lesions are the result of reproductive viral infection. In contrast, in cervical carcinomas, high-risk HPV genomes are often found integrated into the cellular DNA of the host (15). HPV-DNA integration is considered to be an important event in the development of cervical cancer, because it ensures the stable expression of the E6 and E7 proteins (15,16).

The distinct functions of E6 and E7 were first reported by Yutsudo and coworkers, who showed that E6 had the ability to induce tumor formation in the nude mice, while E7 increased the saturation density of transformed mouse fibroblast cell lines (17). In addition, another study showed that both *E6* and *E7* genes are essential for the immortalization of human keratinocytes, which are the true target cells for HPV infection (18). Our group has demonstrated that the introduction of *E6* and *E7* genes induces tumor formation in various organs (19) and cervical premalignant lesions in the *in vivo*

mouse model systems (20,21). The E6 and E7 proteins of the high-risk HPV types modulate the activities of cellular proteins that regulate the cell cycle. The E7 protein interacts with the retinoblastoma gene product Rb (22), and E6 interacts with p53 (23), disrupting the cell cycle control. The E7-Rb interaction releases E2F, which is a transcriptional factor for genes that are involved in the induction of cell division, thereby promoting abnormal cell proliferation. E6 binding to p53 promotes the degradation of p53 (24), the major function of which is to stop cell division and induce cell senescence, during which DNA is damaged or the telomere is shortened. Our recent study has shown that DNA damage induces G2 arrest in most cell lines that lack the functional p53 protein, which suggests that G2 arrest be induced by a factor other than p53. When the G2 checkpoint is further abrogated by the administration of caffeine, which forces the cells into mitosis, cells that lack p53 exhibit a mitotic catastrophe (25). This suggests that HPV16 E6 expression and exogenous stimuli that induce DNA damage induce severe chromosomal alterations. Therefore, the induction of uncontrolled epithelial proliferation by E7, and the perturbation of DNA repair by E6 may cause genetic changes to accumulate in HPV-infected cells. These circumstances may confer a growth advantage on certain cells that acquire resistance to the anti-proliferative and anti-apoptotic stimuli mediated by immune modulators such as cytokines.

EPIDEMIOLOGICAL CLASSIFICATION OF HIGH-RISK (ONCOGENIC) HPV TYPES

To date, more than 100 HPV types have been identified, and about 40 types specifically infect the genital epithelium (26) (Fig. 3). Genital papillomavirus infection, which is passed between individuals through sexual contact, now represents one of the most common sexually transmitted diseases worldwide (27,28). Some HPV types, such as types 6 and 11, are referred to as low-risk types, since they induce only benign warts in the genital tract (*condylomata acuminata*) with a low risk of progression to malignancy. In contrast, the high-risk HPV types are associated with a high frequency of development of malignant lesion (5). More than 90% of cervical cancers contain HPV DNA of the high-risk type, with type HPV16 being the most prevalent, followed in order of decreasing prevalence by types 18, 31, 33, 39, 45, 51, 52, 58, 59 and 68 (29) (Fig. 4).

In a study of Japanese women using the PCR-based method established in our group for targeting the E6 and E7 genes, infection with multiple HPV types was identified in about 10-20% of the cervical cytological samples (28,30). With regard to infections with a given HPV type, the HPV types 16, 31, 51, 52, 58 and 67 were identified in squamous cell carcinoma

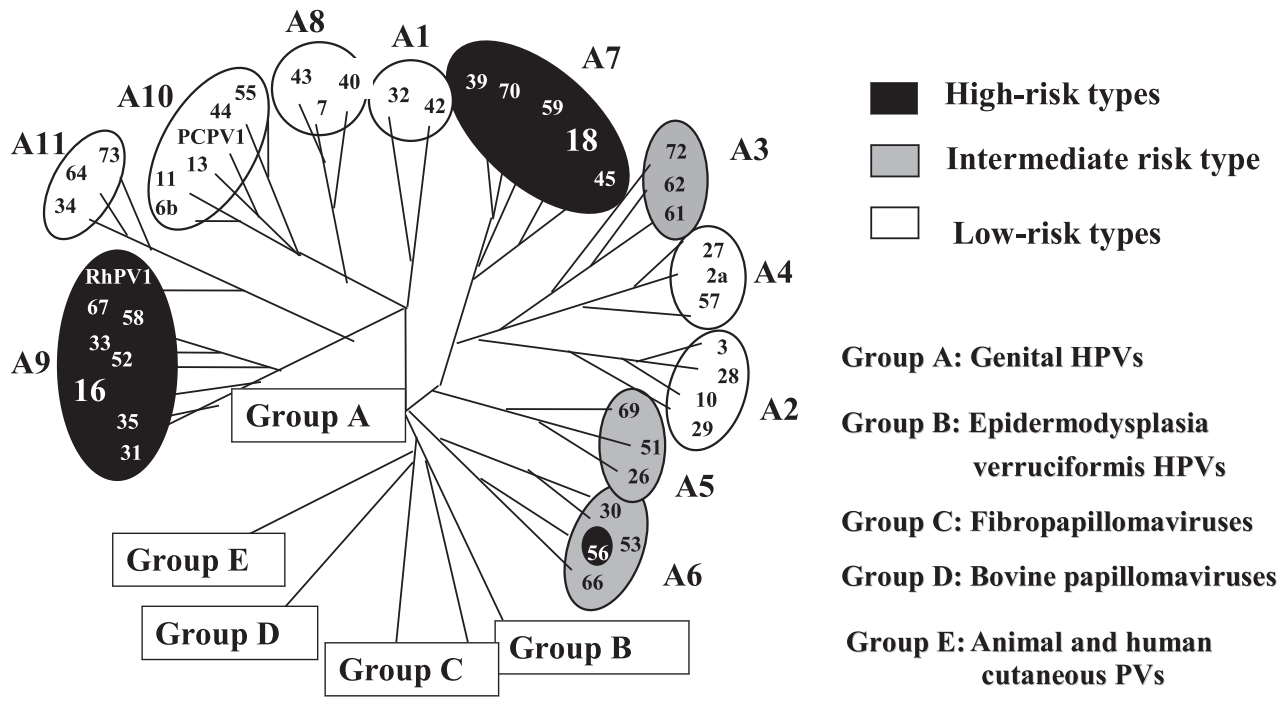

Fig. 3. *Phylogenetic classification of HPV types. HPV types are classified for L1 gene similarity by phylogenetic analysis.*

	Low-risk types	High-risk types
Multicenter Cervical Cancer Study Group	6, 11, 40, 42, 43, 44	16, 18, 31, 33, 35, 39, 45
	54, 61, 70, 72, 81	51, 52, 56, 58, 59, 68, 73
	CP6108	82, (26, 53, 66)
Our study in Japan	6, 11, 39*, 42, 44, 53*	16, 18, 31, 33, 35, 51, 52
	59*, 61, 62, 66*, 68*, 72	56, 58, 67
	(30, 54, 55, 61, 70, 73*)	

*: types show conflicted results between two studies.
(): possiblly low or high risk types

Figure 4. *Clinicopathological classification of mucosal HPV types.*

(SCC) of the cervix (comprising more than 95% of the cervical cancer cases). HPV18 was detected in both adenocarcinoma and adenosquamous cell carcinoma (5% of the cervical cancer cases), and HPV types 33, 35 and 56 were detected in high-grade SIL, which suggests that these types represent the high-risk HPV types for cervical malignancies in Japan (30) (Fig. 4). This study also showed that HPV52 and HPV58 were more prevalent in cervical cancer and high-grade SIL in Japan than in Europe and USA. Although HPV18 was identified in adenocarcinoma and adenosquamous cell carcinoma, other HPV18-related types, i.e. HPV types 39, 45, 59 and 68, were identified only in cases of SIL in our analysis. A recent worldwide study found that these types and others, including HPV types 73 and 82, and possibly HPV types 26, 53 and 66, were high-risk HPV types (31)(Fig. 4). The variability in the prevalent HPV type in cancers may be due to the different distributions of prevalent HPV types among different geographical areas. However, Matsukura and coworkers disagree with the categorization of HPV types according to PCR-based methods. In their analysis, using Southern blot hybridization under low-stringency condition, they identified many HPV types in cervical intraepithelial lesions of various grades (32). They have found that HPV18 DNA is rarely identified in high-grade CIN lesions or cervical SCC, whereas many of these specimens have been shown to be positive for HPV18 by PCR with the GP5/GP6 consensus primers (33). They speculate that the uneven amplification, due to sequence similarities between the HPV sequences and the consensus primers, may account for the disparate findings regarding HPV18 prevalence (33). Therefore, caution should be exercised in the interpretation of data on HPV types in clinical samples, particularly when PCR-based detection systems are used.

NATURAL HISTORY OF HPV INFECTION IN CASES OF CERVICAL CANCER

Various groups, including ours, have demonstrated a high prevalence of HPV infections in the uterine cervices of young women - more than 50% prevalence for any HPV types, and 30-40% for high-risk types in women in the age category of late teens to early 20s. This is associated with other risk factors in the Japanese study, including having multiple partners, younger age at first sexual intercourse, frequent sex and engaging in sex without condom usage (28). Although high-risk HPV infection is sexually transmitted, not all women with this infection are destined to develop cervical cancer. There is no evidence to associate a risky sexual behavior with cancer development. Epidemiological evidence for young women shows that about 30% of the infections with any HPV type persist and induce CIN1 lesions within three years (34). A recent cohort study that tested 227 cytologically normal

women, who were positive for HPV showed that 23% and 7% remained positive after one- and five-year follow-up periods, respectively (35). Persistent HPV infection is induced at higher frequency by high-risk HPV types than by low-risk HPV types (34-36). Molano *et al* (35) have demonstrated that HPV16 persists longer than other HPV types, including HPV18 and its related types, and that more than 20% of HPV16 infections persist longer than five years. The mean duration of oncogenic HPV infection is 13.5 months, while that for non-oncogenic types is 8.2 months. The age of the infected woman does not affect the mean duration of HPV infection (35). The clearance of HPV infection occurs chiefly in the first two years after HPV detection, and occurs rarely thereafter. Exact data on the percentage of infected women who go on to develop cancer are lacking. We postulate that 7-30% of HPV infections are persistent, and that the majority of persistent infections induce CIN lesions. The results of follow-up studies on CIN lesions, as analyzed by actuarial life-table methods (37-39), show that about half of the various forms of dysplasia (CIN1-2) progress to carcinoma *in situ* (CIS), and 43-66% of the CIS cases progress to invasive SCC. The transition time from dysplasia to CIS is estimated at 3-7 years, and the one from CIS to invasive cancer at 3-10 years. Taken together, 7% of women infected with HPV develop CIN1, 3.5% develop CIN3, and 1.5% develop cervical cancer, in the absence of any treatment (Fig. 5). However, the actual progression rates in industrialized countries may be lower, since the diagnostic procedures, such as repeated sampling and punch biopsy, which are applied to women with HPV-related lesions, may elicit HPV-specific immune responses, which induce spontaneous regression of the lesions.

PERSISTENT HPV INFECTION AND HOST IMMUNE RESPONSES

As shown in the previous section, many HPV infections are transient and regress spontaneously over a period of several months to two years, whereas a few cases persist and progress to invasive carcinoma. Therefore, the establishment of persistent high-risk HPV infection is a critical step towards malignant progression. It is well known that immunocompromised women, who have undergone renal transplantation (40), or who are infected with HIV (41) are at high risk of anogenital HPV infection and cervical cancer, which suggests that immunity is a determining factor for viral persistency. However, the mechanisms underlying persistent HPV infection in healthy women are unclear. It has been suggested that HPV has evolved certain strategies to evade the host immune responses. The human immune system consists

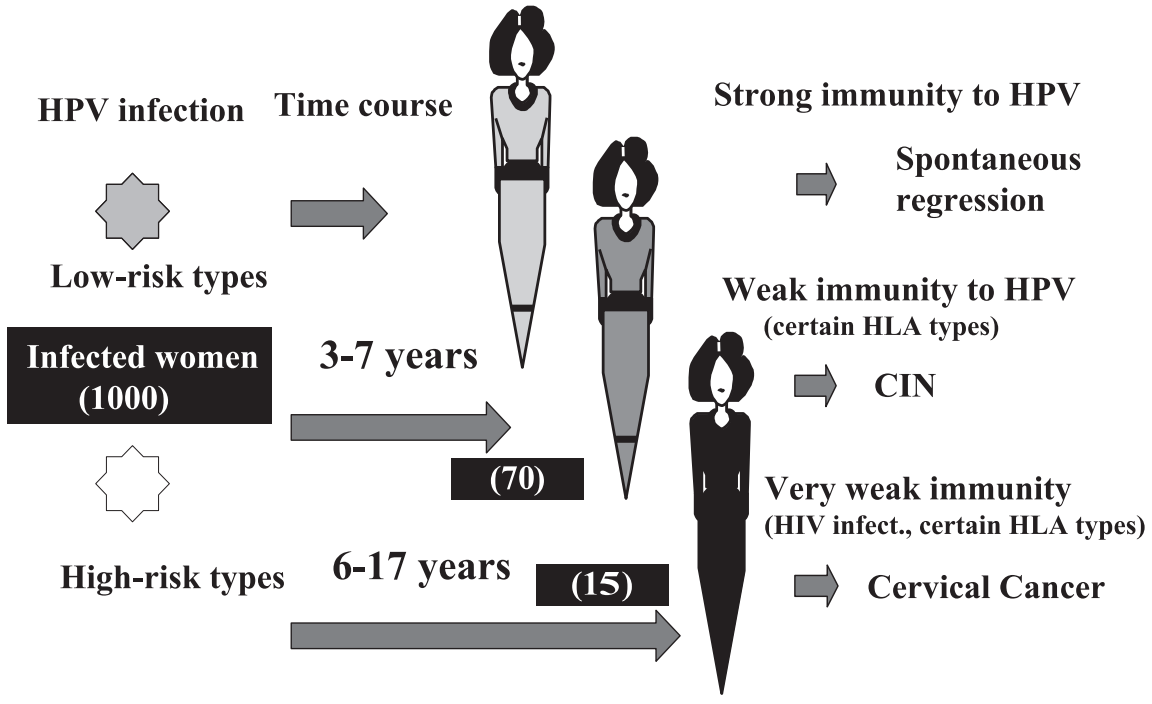

Figure 5. *Natural history of HPV infection.*

of two major subsystems: *innate* and *adaptive* immunity. In the following section, we review the literature on immune responses against HPV infection during cancer development, and explore the potential mechanisms employed by HPV and HPV-infected keratinocytes to evade the host immune responses.

Growth inhibition properties of cytokines, and the acquired resistance of HPV-infected cells to anti-proliferative factors; innate immunity

HPV and HPV-infected epithelial cells initially encounter the innate immune system, which involves major histocompatibility complex (MHC)-non-restricted, non-specific reactions. Immune cells, such as macrophages, neutrophils and dendritic cells engulf foreign materials, such as bacteria and viruses, while natural killer (NK) cells eliminate virus-infected and cancer cells. The virus-infected cells also encounter growth inhibitory stimulation by cytokines that are released from both immune cells and the infected cells themselves (a phenomenon known as "autocrine regulation"). Several studies have investigated the ability of cytokines, particularly transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, and the interferons (INF), to inhibit the proliferation of both normal and HPV-transformed keratinocytes, as well as to inhibit the expression of the *E6* and *E7* genes. TGF-β1 has been shown to be both a product of and a growth inhibitor of cells that are transformed with the nontumorigenic HPV types 16 and 18 (42). Woodworth *et al* concluded that TGF-β1 is an autocrine regulator of HPV gene expression in HPV-infected genital epithelial cells (43). In this system, not only the expression of a HPV gene, but also that of *c-myc* (a stimulator of proliferation), was suppressed. Keratinocytes also produce TNF-α, which may have antiproliferative effects on HPV-infected cell (44) (Fig. 6). This effect involves growth arrest in the G0-G1 phase of the cell cycle (45). TNFα was shown to repress HPV16 *E6* and *E7* expression at the transcriptional level in HPV16-immortalized human keratinocytes, a property it shared with interleukin (IL)-1α (46). In contrast, INF-γ and IL-6 do not exhibit such an effect. In general, all INFs suppress the proliferation of HPV-immortalized keratinocytes and down-regulate the expression of the HPV *E6* and *E7* genes (47-49), although these effects are likely to differ from cell to cells. The down-regulation of *E6* and *E7* gene expression by cytokines, such as TNF-α, IL-1α, INF-α and INF-β, probably occurs during the innate immune response, since these cytokines are released by phagocytic cells or NK cells. In addition, since epithelial cells are also capable of producing these cytokines (except INF-γ), some authors have suggested that these molecules play autocrine roles in keratinocyte growth regulation during HPV infection (Fig. 6). It seems likely that many cell types contribute to the growth inhibitory effect. Additional functions of these and other cytokines in HPV immunity are discussed in the sections on adaptive immunity.

Accumulated evidence suggests that malignant transformation involves a loss of responsiveness to the inhibitory effects of certain cytokines (42,50-52)(Fig. 6). For example, TGF-β1 has been shown to inhibit cell growth and HPV gene transcription in nontumorigenic HPV16-immortalized cell lines, but not in HPV16-positive cervical cancer cell lines, such as CasKi and SiHa (42). Another group reports that the cytopathic effect of INF-γ on HPV16-immortalized cells is not induced in HPV16-harboring SiHa cells (50). These results support the notion of escape from cytokine-mediated growth inhibition of HPV-infected cells during the stage of malignant progression. Malejczyk *et al* compared HPV16-transformed cell sublines with different levels of tumorigenicity in nude mice and showed correlations between increased tumorigenicity, resistance to TNF-mediated inhibition of proliferation *in vitro*, and significantly decreased expression of TNF receptors (51). These authors also show increased shedding of soluble type I TNF receptor in the more potently tumorigenic subline (52). Interestingly, the serum levels of soluble type I and type II receptors are significantly elevated in patients with HPV16- or HPV18-associated carcinomas or anogenital Bowen's carcinoma (53). Conversion of nontumorigenic HeLa-fibroblast hybrids to tumorigenic cells is accompanied by the development of resistance to TNF-mediated suppression of HPV18 gene transcription, as well as changes in the composition of the activator protein 1 (AP-1) complex, which plays a role in the expression of E6 and E7. Moreover, it has been proposed that the loss of TNF sensitivity may be causally related to alterations in the AP-1 transcription activator complex (54) (Fig. 6).

Inhibitory effect of HPV genes on the signal transduction mediated by interferons and antiviral proteins

One of the possible mechanisms of acquired resistance to cytokines may involve the functions of HPV E6 and E7, which inhibit the signal transduction associated with interferon-mediated antiviral responses (Fig. 7). In response to viral infection, several signal transduction pathways are activated (55), which ultimately lead to the activation of transcription factors that regulate immediate early genes, including the genes that encode type I interferons, such as INF-α and INF-β. Once secreted, these INFs interact with specific receptors on the surfaces of the surrounding cells, thereby inducing the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling pathway. This leads to the activation of transcription factors called INF-stimulated gene factor (ISGF)-3 and interferon regulatory factor (IRF)-7. Upon virus infection, IRF-3 and IRF-7 contribute to the expression and amplification of the INF response by inducing delayed-type I INF genes and several other genes that modulate antiviral effects, including RANTES, IL-15, interferon-stimulated gene

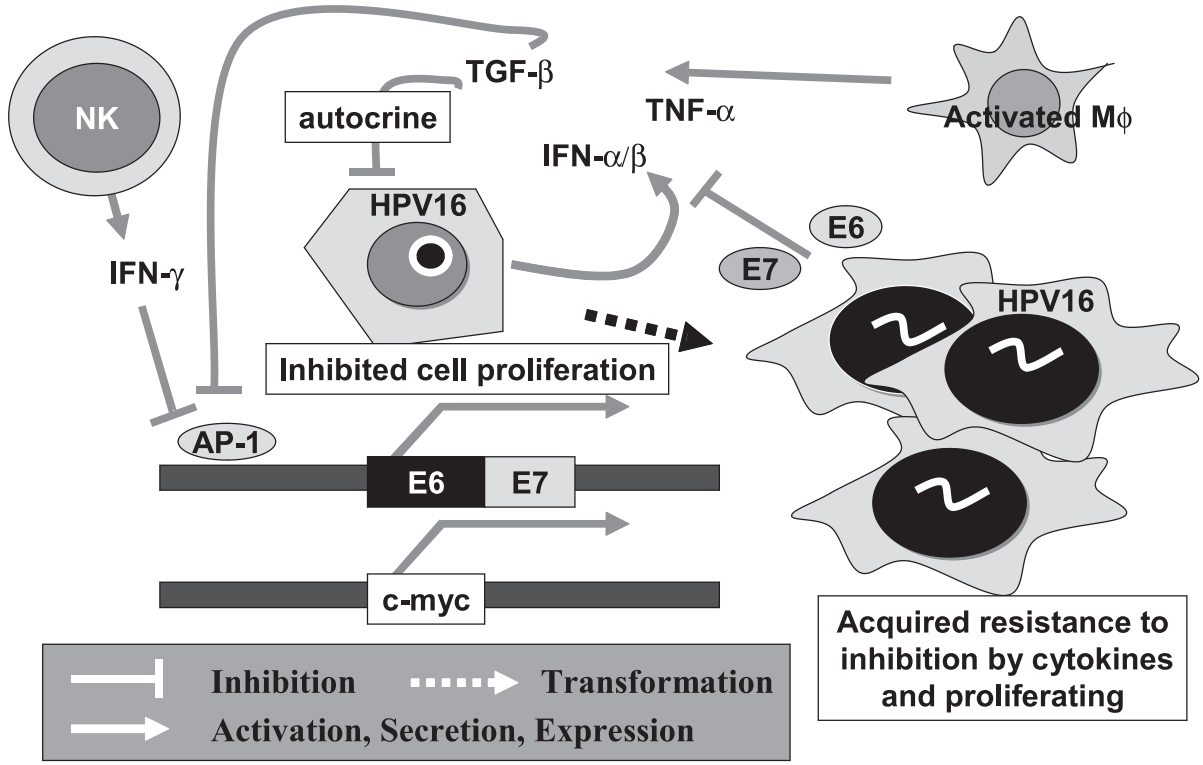

Figure 6. *Cytokine effect on the keratinocytes infected with HPV. Proliferation of keratinocytes infected with HPV is inhibited by cytokines released from immune cells: macrophages, NK cells, and keratinocytes themselves. The cytokines secreted from the keratinocytes sometimes play an autocrine role for this inhibition. However, HPV-transformed cells which have acquired resistance to this inhibitory effect are able to grow regardless of the presence of cytokines. AP-1, activator protein-1.*

(ISG), double-stranded RNA activator kinase (PKR), 2'-5'-OASs/RNase L, and others (55) (Fig. 7).

During HPV infection, E6 and E7 are associated with proteins that transduce signals inducing the expression of INFs and other antiviral signals, such as those mentioned above. It has been reported that HPV16 E6 binds to IRF-3, thereby inactivating the IRF-3 transactivating function (56), which plays a critical role in the regulation of INF-α and INF-β. In the delayed response stage, HPV18 E6 interacts with tyrosine kinase 2 (Tyk2), which plays an essential role in signal transduction *via* the INF receptor by binding INF-α or INF-β (57). HPV16 E7 also interacts with IRF-9 (58), which is one of the components of ISGF-3, resulting in the reduced expression of IRF-7. The reduction in the levels of IRF-7 decreases the expression of INF-α, INF-β and other antiviral proteins (55) (Fig. 7). The ability of E7 to interfere with IFN signaling is further demonstrated by its binding and inactivation the transcription factor IRF-1 (59). IRF-1 was originally identified as an INF-β promoter-binding transcription factor, and was characterized as a critical mediator of INF signaling induced by viral infection or INF treatment (60). IRF-1 overexpression inhibits cell growth, and the introduction of activated *c-Ha ras* oncogene alone is sufficient to transform embryonic fibroblasts from IRF-1 knockout mice (60). Cho *et al* recently demonstrated that HPV16 E6 downregulates IL-18, a stimulator of type 1 helper T-cell responses, by inducing INF-γ expression (61). Although macrophages are the major source of IL-18, this cytokine is also secreted by keratinocytes, suggesting that the down-regulation of IL-18 expression in HPV-infected keratinocytes contributes to the inhibition of cell-mediated immunity. In addition, the production of type 1 INFs enhances the expression of IL-12 and IL-18 receptors. Therefore HPV interference with both the INF pathway and IL-18 expression comprises a double-edged sword to block

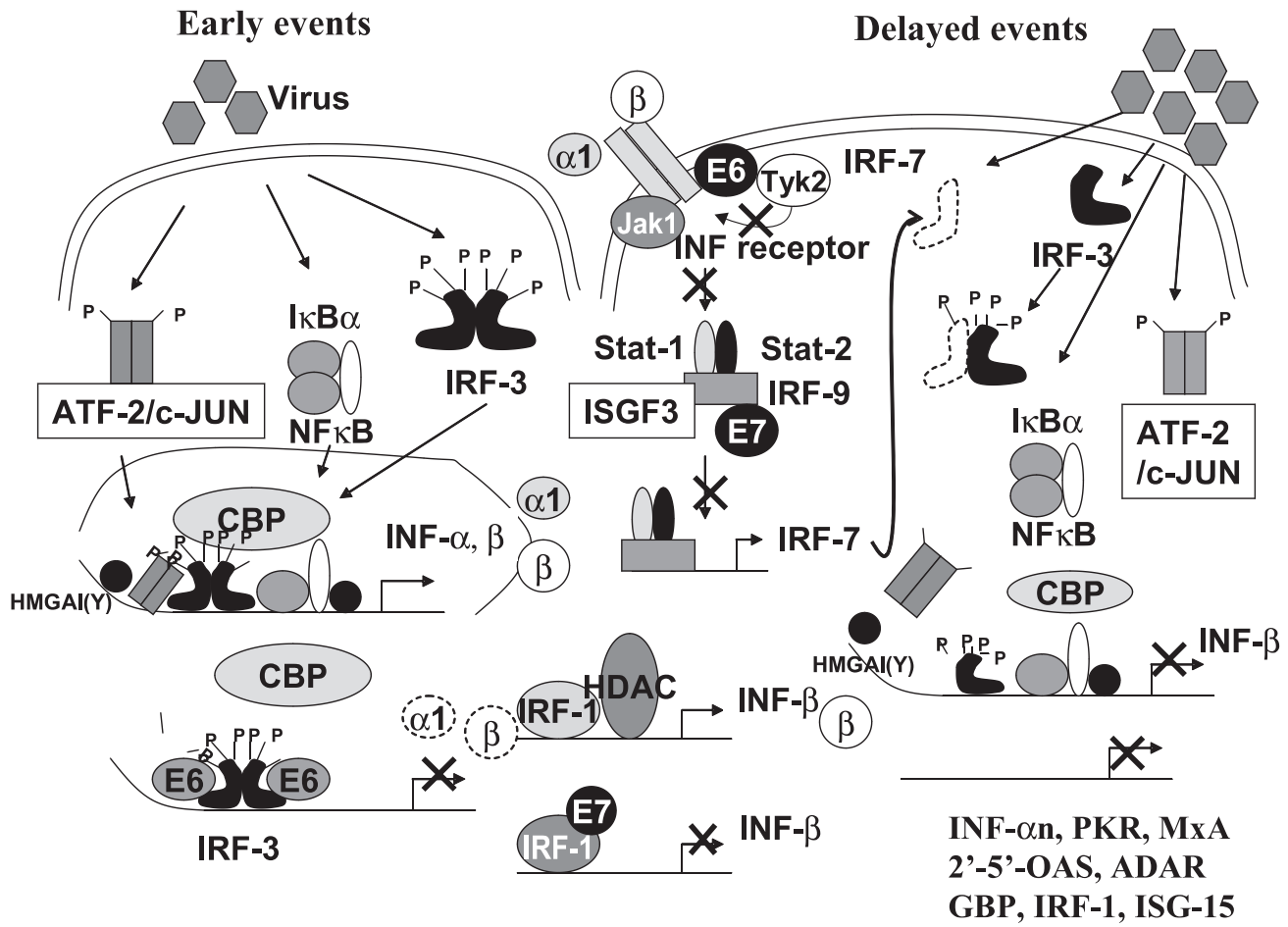

Figure 7. *Control of interferon signaling in HPV infection. Virus infection induces early and delayed events in the interferon signaling pathways. In early events, type I interferons (α1 and β) are expressed upon stimulation. They induce gene expression in neighboring cells via binding to cell surface receptors and transducer signals, and provoke anti-viral effect on the cells. Second viral attack induces stronger response by expressing many additional anti-viral genes such as PKR, MxA, 2'-5'-OAS, ADAR1/2, GBP, IRF-1, ISG-15, -54, -56 as well as INF-α and -β. HPV16 or 18 E6 and E7 proteins block these signaling pathways by protein to protein interactions. IkB, inhibitory factor of kB; IRF, interferon regulatory factor; ISGF, interferon stimulated gene factor; Jak, Janus kinase; NFkB, nuclear factor-kB; PKR, double stranded RNA activated kinase; STAT, signal transducer and activator of transcription; ISG, interferon-stimulated gene; 2'-5'-OAS, oligoadenylate synthase; MxA, Mx protein; GBP, guanylate-binding protein; ADAR, RNA-specific adenosine deaminase.*

an effective immune response. These immune evasion mechanisms, which are mediated by the HPV *E6* and *E7* genes, may explain the resistance of HPV-transformed keratinocytes to the anti-proliferative effects of cytokines.

Inhibition of antigen recognition. The recognition phase of adaptive immunity

HPV16 *E6* and *E7* gene expression is limited to the basal epithelial cell layers, and the expression levels of these genes are very low in pathological studies. The late genes *L1* and *L2*, which encode the viral capsid proteins, are expressed only in the superficial squamous epithelial layers. Viral antigen exposure to the antigen-presenting cells (APCs) in the skin, known as Langerhans cells, is the first and crucial step in the induction of immune responses against HPV. Limited or localized expression of viral proteins facilitates viral evasion of immune surveillance (62). Human trials of a HPV16 vaccine conducted by Koutsky and co-authors showed that intramus-

cular injection of HPV16 virus-like particles induced anti-HPV16 neutralizing antibodies that protected against HPV16 infection in 100% of the vaccinated subjects (63). This result suggests that HPV particles that are administered (albeit together with an adjuvant) into the stromal tissues effectively elicit anti-HPV neutralizing antibodies. In natural HPV infections, anti-HPV (capsid) antibodies are not induced in every HPV16-infected woman (64). IgG antibodies against high-risk HPV are induced in women with persistent HPV16 infection (64,65), but not during transient infections (65). Our recent study demonstrates that both IgA and IgG antibodies against high-risk HPV types 16, 18, 31 and 45 are secreted in the uterine cervix in approximately 70% of women with SIL (66). In this study, some of the women elicited IgG responses after treatment for SIL, which suggests that the surgical procedure induces inflammation that enhances the immune response. We postulate that HPV infection does not always induce inflammation, and consequently, no immune response is elicited, since many cervical HPV infections are clinically asymptomatic.

Several investigators have suggested that the density of Langerhans cells decreases during genital HPV infection, condylomas (67) and SILs (68). The decrease of Langerhans cells during HPV infection may, along with other local immune deficiencies, contribute to prolonged infection and possibly to malignancy. Other studies have suggested that the Langerhans cells in HPV lesions are functionally impaired, which may also contribute to the persistence of infection. One group has shown a defect in S-100 protein expression in Langerhans cells within SILs, although the marker of CD1 expression on the same cells was intact, which suggests disrupted maturation of the Langerhans cells (69). Once Langerhans cells have captured an antigen, the recognition phase continues with their migration from the infection site to the draining lymph nodes. Certain cytokines, particularly IL-1α and TNF-α (mainly produced by keratinocytes) and IL-1β (mainly produced by Langerhans cells), promote this migration (70). In contrast, IL-10, which is produced by keratinocytes and type 2 helper T cells, acts as an inhibitor of Langerhans cell migration (71). Other cytokines, e.g. the keratinocyte-produced granulocyte-macrophage colony-stimulating factor (GM-CSF), promote the initiation of maturation of Langerhans cells into mature dendritic cells (72). An association between defective production of these cytokines and persistent HPV infection has been postulated, based on the findings of reduced expression of IL-1α, IL-1β, TNF-α, GM-CSF, etc. by several HPV-immortalized cervical cell lines and several cervical cancer lines, as compared to normal cervical cells (73). Mota *et al* found that TNF-α expression by keratinocytes was absent in some SIL biopsy specimens, but was present in all of the biopsy specimens from normal cervical squamous epithelium (74). Conversely, IL-10 expression by keratinocytes was present in many SIL biopsy specimens, but absent in samples of normal epithelium. Our ongoing study has shown high-level secretion of IL-10 and relatively low-level secretion of TNF-α in low-grade cervical SIL. The circumstance of high-level IL-10 and low-level TNF-α at the early stage of HPV-infected lesion development may have the effect of inhibiting Langerhans cell activation and migration, resulting in impaired antigen recognition. Tindle *et al* have postulated that the disrupted maturation of Langerhans cells by HPV16 E7 may induce immune tolerance, rather than immune activation (75).

HPV antigen-specific helper T cell and cytotoxic T cell responses. The effector phase of adaptive immunity

Naive helper T cells are activated following the recognition of viral antigens that are exposed on class II MHC molecules on the surface of APCs, such as Langerhans cells. This activation converts naive T cells into mature helper T cells. Following activation, these cells proliferate rapidly upon secondary exposure to the same antigen (T-helper response). In three of five cross-sectional studies, T-helper responses to HPV16 E6 and E7 proteins and peptides were observed more frequently in subjects who were cytologically normal, than in subjects who had developed SIL, which suggests that these antigens are important in SIL prevention (76-78). In one study, responses to HPV16 E7 peptides were observed more frequently in SIL subjects, who were infected with HPV types 16, 31 and 33, than in controls without SIL (HPV status unknown) (79). In contrast, another study found no correlation between these responses and the presence of SIL (80).

The results from studies that have focused on helper T cell response against other HPV16 proteins are equally confusing. T-cell proliferative responses to HPV16 L1 were observed more frequently in subjects with SIL, than in healthy, age-matched controls (81,82). These results are concordant with the results of serological investigations showing that anti-high-risk HPV L1 IgG responses are induced more frequently in women with SIL and cancer than in control women (64,65). In cross-sectional studies, the E2 responses were associated with viral clearance, but not with the resolution of SIL (83), whereas the E5 responses were associated more frequently with low-grade SIL (LSIL) subjects than with HPV-positive normal and high-grade SIL (HSIL) subjects (84). A longitudinal study of E5 expression would be of great interest in determining whether the higher responses in subjects with LSIL are associated with disease resolution. One limitation is that the activities of helper T cells alone may not correlate directly with the clearance of the virus and virus-associated lesions, since they are not the effector cells that kill HPV-infected cells.

On the other hand, CD8-positive, MHC class-I restricted cytotoxic T-lymphcytes (CTLs) are known to be responsi-

ble for the recognition and killing of virus-infected host cells and virus-induced tumors (85). CTLs that recognize specific parts of the HPV antigen kill those keratinocytes that present the corresponding antigenic peptides on the cell surface. In humans, HPV16 E6- and/or E7-specific CTLs have been detected in women with cervical cancer and in women with SIL (86,87). Lack of E6 but not E7 correlates with persistent HPV16 infection, which suggests that the CTL response to E6 is important in the clearance of HPV16 infection (88). However, the CTL responses noted in this study were frequently transient. The sensitivity of the current assay for the detection of circulating T cells that are specific for localized infection may be limited. Recently, a novel technique for identifying antigen-specific T lymphocytes, which uses peptide-MHC class I tetramers, has been used to identify and isolate CD8-positive T lymphocytes with specificity for HPV. Positive tetramer responses to the HPV16 E7 (11-20) peptide were observed in patients with cervical cancer and CIS (89). However, the interpretation of positive responses in cancer patients is problematic, since these patients have defective immune responses.

MHC-restricted antigen presentation. The recognition and effector phases of adaptive immunity

Foreign antigens are presented by MHC class II molecules on the cell surface of APCs, after being processed inside the cell. INF-γ treatment of HPV16-, HPV18-, and HPV33-immortalized keratinocytes has been shown to enhance the transcription of MHC class II (90). Several studies have reported evidence of impaired MHC class II expression in high-grade cervical lesions (73,91,92), whereas other studies have provided rather confusing results, showing more frequent expression of Human Leukocyte Antigens (HLA)-DR in HSIL than in LSIL (74,93). The hypothesis is that HPV evades cellular immune responses and relies on the inhibition of MHC class II expression. This idea was suggested by a study in which two groups of patients with genital condyloma were compared: those who responded and those who did not respond to interferon treatment (92). The impaired up-regulation of this antigen in the nonresponders, as compared to the responders, was associated with the overexpression of the HPV *E7* gene, which suggests a possible causative link between high-level E7 expression and reduced inducibility of HLA-DR expression in nonresponders. A recent report showed that HPV E5 downregulates the expression of MHC class II (94). Sequential degradation of the invariant chain within the acidic endocytic compartments is a key process in the successful loading of antigenic peptides onto MHC class II molecules. The E5 protein may affect appropriate peptide loading onto MHC class II by inhibiting the acidification of the late endosomes in human keratinocytes.

Normal human keratinocytes constitutively express MHC class I molecules, and are susceptible to class I-mediated lysis by alloantigen-primed CTLs (95). Moreover, exogenous INF-γ enhances this susceptibility. A drastic reduction in MHC class I expression in cutaneous warts was reported, but with only a mild reduction in condyloma and laryngeal papillomas (96). At least 30% of cervical cancer cases exhibit a significant reduction in MHC class I expression (97). Torres *et al* found that the loss of MHC class I expression in cervical cancer biopsy specimens correlated with tumor invasiveness and more aggressive histology, as assessed by the Glanz histoprognostic index of malignancy (98). Furthermore, some reports have demonstrated the down-regulation of the transporter associated with antigen presentation (TAP1) and MHC class I proteins in laryngeal papilloma biopsies from patients with recurrent respiratory papillomatosis (RRP), which is a disease that is refractory to treatment (99). A similar result was observed in cervical cancer biopsy specimens (100). Ashrafi *et al* have demonstrated that the bovine papillomavirus E5 downregulates MHC class I expression (101), and they have confirmed that the human papillomavirus E5 also has this function. Therefore, HPV might be able to disrupt CTL recognition of HPV-infected cells by down-regulating the expression of TAP-1 and MHC class I. However, the down-regulation of MHC class I may result from altered cytokine levels or sensitivity to cytokines, such as INF-γ and TNF, rather than a direct effect of viral infection (102). Regardless of the reasons for the changes in MHC class I expression, the data suggest that the loss or down-regulation of MHC class I expression may contribute in a significant way to the defective recognition and removal of infected cells by CTLs. This may explain why HPV-specific CTL responses have been detected in some women who are developing cancer.

REGULATION OF T-CELL RESPONSES BY CHEMOKINES AND ADHESION MOLECULES

An important component of the effector phase of the cutaneous immune response is the recruitment and retention of activated lymphocytes at sites of inflammation, reflecting processes that involve both soluble chemokines and membrane-bound adhesion molecules. Cervical keratinocytes constitutively produce IL-8, the secretion of which is enhanced by activation with IL-1 or TNF-α. The T-cell product INF-γ has been shown to synergize with TNF-α in the enhancement of keratinocyte IL-8 production, which may provide a positive feedback loop whereby T cells migrate to sites of inflammation (103). Spear *et al* demonstrated significantly higher IL-8 levels in women with genital tract dysplasia or with cytological or histological evidence of HPV infection than in women without these signs of disease, whereas the levels of other chemokines, such as RANTES and MIP-1α, were not significantly different (104). In contrast, other au-

thors have reported significantly diminished production of IL-8 in HPV16- and HPV18-immortalized cell lines and in cervical carcinoma cell lines (73). Treatment with IL-1 or TNF-α failed to upregulate IL-8 production in these cancer cells.

The chemokine macrophage chemoattractant protein 1 (MCP-1) may also be important in HPV control. HeLa cells that were transformed with the *MCP-1* gene showed significant growth retardation, as well as marked macrophage infiltration when inoculated into nude mice, whereas HeLa cells that lacked the transformed *MCP-1* gene rapidly produced growing tumors in the absence of macrophage infiltration (105). Keratinocytes are capable of producing MCP-1, and the endogenous gene in HeLa cells is not rearranged structurally. However, the expression of MCP-1 in HeLa cells appears to be suppressed and is only marginally upregulated by TNF-α (106). This suggests another potential mechanism of escape from host defenses in HPV-associated malignancies.

Keratinocytes express intercellular adhesion molecule 1 (ICAM-1) and its ligand, leukocyte function-associated antigen 1 (LFA-1). The LFA-1 interaction with ICAM-1 appears to be involved in specific antigen recognition and is critical for CTL-mediated killing (103). The constitutive expression of ICAM-1 in keratinocytes is very low, but is markedly increased by stimulation with the cytokines TNF-α and INF-γ (106). In a study comparing regressing and nonregressing genital condylomas, significant induction of ICAM-1 and two other adhesion molecules, E-selectin and vascular cell adhesion molecule 1 (VCAM-1), was observed in the regressing lesions (92). The regressing lesions also contained significantly more T cells and macrophages than the nonregressing controls. Some investigators have presented evidence that malignant transformation of cervical keratinocytes is associated with impaired ICAM-1 inducibility (73), whereas others have reported the upregulation of ICAM-1, VCAM-1 and E-selectin in HSIL, as compared to the respective expression levels in LSIL or normal cervical epithelium (107). These data suggest a role for adhesion molecules in anti-neoplastic immune responses in HSIL, but do not support the hypothesis that the impaired expression of adhesion molecules is associated with the progression of HPV-induced lesions.

EXPRESSION OF TH1 AND TH2 CYTOKINES DURING HPV INFECTION

In recent years, studies of immunoregulation have focused on the functional dichotomy of cytokines, i.e. those that support cellular immune responses and those that support humoral responses, as well as the parallel dichotomy of cytokine-producing helper T cells. The phenotypic classification of activated helper T cells into INF-γ-, TNF-α-, and IL-2-producing T helper type 1 (Th1) cells, which stimulate cellular responses, and into IL-4-, IL-5-, IL-6-, IL-10-, and IL-13-producing T helper type 2 (Th2) cells, which stimulate humoral responses, has been reviewed previously (108). Accessory cell-derived cytokines, such as IL-12, which promote Th1 cell development, are also important regulators of T cell function. In addition, accessory cells and even keratinocytes contribute to the production of various Th1 and Th2 cytokines, such as TNF-α and IL-10. The imbalance between Th1 and Th2 cells induces severe infectious diseases in humans, as confirmed in several studies. The Th1-Th2 hypothesis of immune regulation may also be applicable to the natural history of HPV infection.

Some studies have suggested that HPV infection normally elicits a Th1 response. The immune responses to HPV vaccination in a mouse model were characterized by the secretion of the Th1 cytokines INF-γ and IL-2 (109). Tsukui *et al* demonstrated IL-2 production in response to HPV16 peptides, by the peripheral blood lymphocytes of women with SIL and cervical cancer and in cytologically normal women with a history of HPV16 infection (78). The contention that the Th1 response is important for clearance of HPV infection, or conversely, that the lack of such a response may be associated with persistent HPV infection or the development of HPV-related neoplasia, is supported by several studies. In a study of cytokine mRNA patterns in exfoliated cervical cells, seven HPV-positive subjects, who showed HPV clearance on examination four months later (110), displayed a Th1-like pattern of cytokine secretion (INF-γ-positive, IL-4-negative) prior to clearance, as compared to the highly variable patterns observed in HPV-negative women with a previous history of HPV infection. Another investigation, using cervical biopsy specimens, showed decreased levels of INF-γ expression in women with SIL or cervical cancer as compared to normal cervical tissues (111). The normal cervical tissues were obtained from HPV16- and HPV18-negative women, although it was not stated whether these women were positive for any other HPV type. Yet another recent study examined cervical biopsies by immunohistochemistry, and found that SIL, particularly HSIL, biopsies contained fewer Thl (IL-2-postive) cells and had an increased density of Th2 (IL-4-positive) cells as compared to normal cervical epithelium (112). In a report by Tsukui *et al* using peripheral blood lymphocytes (78), the production of IL-2 following *in vitro* stimulation with HPV16 peptides correlated with cervical disease grade - the highest IL-2 levels were detected in cytologically normal HPV16-positive women, intermediate levels of IL-2 were established in women with SIL, and the lowest levels of IL-2 were reported in women with cervical cancer. Clerici *et al* (113) examined the antigen- and mitogen-stimulated cytokine production levels in peripheral blood mononuclear cells from women with SIL, and reported a shift from Th1 to Th2 cytokines in women whose HPV infection extended beyond the cervix to the vagina. The authors speculated that augmented

IL-10 production decreases the immune recognition of HPV-associated tumors by downregulating the expression of MHC class I and/or class II molecules. As described above, both of these molecules have been reported to show diminished expression in persistent or progressing HPV-associated lesions, although the evidence for the loss of MHC class II expression is controversial at this time.

We examined the levels of cytokine secretion in the cervices of ten women who had HPV infection that was followed for a year. Most of the women (with only one exception) had persistent infection and showed high-level IL-10 secretion, regardless of the level of INF-γ. On the contrary, most of the women who have cleared the HPV infection had low levels of IL-10 and high levels of INF-γ secretion (data not shown), which suggests that high-level secretion of IL-10 may be the predominant factor in immune suppression against HPV. This hypothesis appears to be supported by the findings of our cross-sectional study, which showed that the Th2 cytokine IL-10 was secreted at higher levels in women with LSIL than in healthy women, whereas the secretion levels of INF-γ (Th1) and TNF-α remained unchanged (unpublished data). Surprisingly, TNF-α was overexpressed in women with malignant lesions, and immunohistochemical analysis revealed that malignant cells that infiltrated into the lesion contributed to this increased expression rather than monocytes. Although the reason for this finding is currently unknown, we postulate that it reflects a strong immune response to inhibit the malignant cell growth in vain. As has been noted, this process may not be effective against malignant cells that have already acquired resistance to the anti-proliferative effects of TNF-α.

IMMUNE EVASION DURING HPV INFECTION

The potential mechanisms by which HPV evades the host immune responses have been outlined in the preceding sections and are summarized in this section.

First, HPV may have evolved mechanisms that limit the extent to which viral antigens are exposed to immune recognition. HPV delays the expression of abundant viral proteins (the capsid proteins encoded by the late viral genes) until the terminal differentiation stage of the squamous epithelium, which is an anatomically superficial location in which dendritic cells, such as Langerhans cells have less access to these antigens. In contrast, in the basal epithelium, where the early genes (such as E6 and E7) are expressed, the level of protein expression is low and confined to a nuclear location, which potentially limits an effective immune response against those cells in which the virus is active. Some authors have proposed that the delayed expression of the capsid-encoding genes may be due to a pattern of codon usage that inhibits their expression in basal epithelial cells (114).

Second, viral proteins such as E5, E6 and E7 may modulate the immune responses. Either E6 or E7 can modulate the signal transduction pathway of interferon and other antiviral proteins. This apparently corroborates the finding that HPV-infected keratinocytes acquire the ability to resist the antiproliferative effects of many cytokines. E5-mediated acidification of endosomes affects antigen processing and presentation in keratinocytes (115). HPV16 E7 is tolerogenic in the mouse model system, if the inflammatory stimulus is absent at the time of E7 recognition (75).

Third, immune evasion may be achieved by inhibiting antigen recognition, mainly *via* the downregulation of MHC class I and class II antigens. HPV-specific CTLs have been detected in some women who develop cervical cancer. A possible explanation for this phenomenon is the downregulation of TAP-1 and/or MHC class I antigens on the surfaces of cancer cells, which results in the CTL attack being averted. The increased secretion of immunosuppressive cytokines, such as IL-10, the downregulation of IL-18 by HPV E6, and the non-responsiveness to TNF-α of HPV-transformed cells are all responsible for the downregulation of MHC expression. Correlations between certain MHC class I and II alleles and susceptibility to, or protection against, CIN lesions and cervical carcinoma probably reflect T-cell responses that are directed against the HPV oncoprotein (116). The fact that only some HPV-infected women are susceptible to cervical cancer indicates that immune-response-driven selective pressures have shaped the sequences of native oncoproteins so that the derived peptides do not bind to any of the MHC molecules in the repertoire of that individual and, thus, these peptides are not presented to T cells.

Alternative immunosuppressive mechanisms have been presented previously (75). HPV16 E7 shows extensive similarity to several human proteins. Molecular mimicry of self-antigens may underlie the limited immunogenicity of E7. The E6 and E7 proteins secreted by cervical carcinoma cell lines can reduce the production of immunostimulatory IFN-γ in NK cells. HPV E7 expressing cells are resistant to lysis by NK cells (117).

CONCLUSION

Many molecular events induced by oncogenic HPV types could explain the mechanisms of HPV infection for cancer development. However, such molecular events were not prosecuted in many women, if HPV-infected cells could not escape from the immune surveillance system. The possible mechanisms by which HPV promotes cancer development are summarized as follows:

- The stable expression of HPV E6 and E7 oncoproteins in the cells, and this may be established by the integration of HPV genome into host genome.
- E6 and E7 proteins induce uncontrollable cell growth and accumulate mutations in host genome by inhibit-

ing the functions of Rb and p53, which tightly regulate the cell division cycle.

The immune-evasion mechanisms of HPV can be summarized as follows:

- Avoidance of antigen recognition of APCs by limited expression of viral antigens such as E6, E7 and capsid proteins.
- Induction of HPV16 E7 tolerance if there is no augmented inflammatory stimulation at the stage of E7 recognition.
- HPV proteins suppress the immune stimulation from the host cells: HPV 16 or 18 E6 and E7 proteins block the signal transduction pathway of IFN and other antiviral proteins, E5 downregulates antigen processing and presentation of MHC antigens, and HPV16 E6 downregulates expression of IL-18 enhancing cell-mediated immune response.
- Secreted E6 and E7 suppress the immune responses of surrounding immune cells. E6 and E7 secreted from cervical carcinoma cell lines can reduce the production of IFN-γ in NK cells. E7-expressing cells are resistant to lysis by NK cells.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001; 94: 153-156.
2. Durst M, Gissmann L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc Natl Acad Sci USA* 1983; 80: 3812-3815.
3. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Sheurlen W, zur Hausen H. A new type of papillomavirus DNA, its prevalence in genital cancer biopsies and in cell lines derived from cervical cancer. *EMBO J* 1984; 3: 1151-1157.
4. zur Hausen H. Human papillomaviruses in the pathogenesis of anogenital cancer *Virology* 1991; 184: 9-13.
5. Van Oortmarssen GJ, Habbema JD. Duration of preclinical cervical cancer and reduction in incidence of invasive cancer following negative pap smears. *Int J Epidemiol* 1995; 24: 300-307.
6. Mohr IJ, Clark R, Sun S, Androphy EJ, MacPherson P, Botchan MR. Targeting the E1 replication protein to the papillomavirus origin of replication by complex formation with the E2 transactivator. *Science* 1990; 250: 1694-1699.
7. Ustav M, Stenlund A. Identification of the origin of replication of bovine papillomavirus and characterization of the viral origin recognition factor E1. *EMBO J* 1991; 10: 449-457.
8. Stubenrauch F, Hummel M, Iftner T, Laimins LA. The E8E2C protein, a negative regulator of viral transcription and replication, is required for extrachromosomal maintenance of human papillomavirus type 31 in keratinocytes. *J Virol* 2000; 74: 1178-1186.
9. Thierry F, Yaniv M. The BPV1-E2 trans-acting protein can be either an activator or a repressor of the HPV18 regulatory region. *EMBO J* 1987; 6: 3391-3397.
10. Stubenrauch F, Colbert AM, Laimins LA. Transactivation by the E2 protein of oncogenic human papillomavirus type 31 is not essential for early and late viral functions. *J Virol* 1998; 72: 8115-8123.
11. Doorbar J, Ely S, Sterling J, McLean C, Crawford L. Specific interaction between HPV-16 E1-E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. *Nature* 1991; 352: 824-827.
12. Leptak C, Ramon y Cajal S, Kulke R, Horwitz BH, Riese II DJ, Dotto GP, *et al*. Tumorigenic transformation of murine keratinocytes by the E5 genes of bovine papillomavirus type 1 and human papillomavirus type 16. *J Virol* 1991; 65: 7078-7083.
13. Munger K, Howley PM. Human papillomavirus immortalization and transformation functions. *Virus Res* 2002; 89: 213-228.
14. Doorbar J, Foo C, Coleman N, Medcalf L, Hartley O, Prospero T, *et al*. Characterization of events during the late stages of HPV16 infection in vivo using high-affinity synthetic F-abs to E4. *Virology* 1997; 238: 40-52.
15. Schwarz E, Freese UK, Gissmann L, Mayer W, Roggenbuck B, Stremlau A, *et al.* Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. *Nature* 1985; 314: 111-114.
16. Baker CC, Phelps WC, Lindgren V, Braun MJ, Gonda MA, Howley PM. Structural and transcriptional analysis of human papillomavirus type 16 sequences in cervical carcinoma cell lines. *J Virol* 1987; 61: 962-971.
17. Yutsudo M, Okamoto Y, Hakura A. Functional dissociation of transforming genes of human papillomavirus type 16. *Virology* 1988; 166: 594-597.
18. Hawley-Nelson P, Vousden KH, Hubbert NL, Lowy DR, Schiller JT. HPV16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. *EMBO J* 1989; 8: 3905-3910.
19. Kondoh G, Murata Y, Aozasa K, Yutsudo M, Hakura A. Very high incidence of germ cell tumorigenesis

(seminomagenesis) in human papillomavirus type 16 transgenic mice. *J Virol* 1991; 65: 3335-3339.
20. Sasagawa T, Inoue M, Inoue H, Yutsudo M, Tanizawa O, Hakura A. Induction of uterine cervical neoplasias in mice by human papillomavirus type 16 E6/E7 genes. *Cancer Res* 1992; 52: 4420-4426.
21. Sasagawa T, Kondoh G, Inoue M, Yutsudo M, Hakura A. Cervical/vaginal dysplasias of transgenic mice harbouring human papillomavirus type 16 E6-E7 genes. *J Gen Virol* 1994; 75: 3057-3065.
22. Munger K, Werness BA, Dyson N, Phelps WC, Harlow E, Howley PM. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. *EMBO J* 1989; 8: 4099-4105.
23. Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 1990; 248: 76-79.
24. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as an ubiquitin-protein ligase in the ubiquitination of p53. Cell 1993; 75: 495-505.
25. Minemoto Y, Uchida S, Ohtsubo M, Shimura M, Sasagawa T, Hirata M, *et al*. Loss of p53 induces M-phase retardation following G2 DNA damage checkpoint abrogation. *Arch Biochem Biophys* 2003; 412: 13-19.
26. Bernard, HU, Chan, SY, Manos, MM. Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphism, nucleotide sequence, and phylogenetic algorithms. *J Infect Dis* 1994; 170: 1077-1085.
27. Jacobson JL. The other epidemics. Sexually transmitted diseases. *Links* 1993; 9: 3-5.
28. Sasagawa T, Tani M, Yasuda H, Azar KK, Fukae T, Ukita T, *et al*. Sexual behavior and high risk human papillomavirus infection in Japanese women. *Sex Trans Infect* 2004, in press.
29. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, *et al*. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst* 1995; 87: 796-802
30. Sasagawa T, Basha W, Yamazaki H, Inoue M. High-risk and multiple human papillomavirus infections associated with cervical abnormalities in Japanese women. *Cancer Epidemiol Biomarkers Prev* 2001; 10: 45-52.
31. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, *et al*. International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003; 348: 518-527.
32. Matsukura T, Sugase M. Identification of genital human papillomaviruses in cervical biopsy specimens: segregation of specific virus types in specific clinicopathologic lesions. *Int J Cancer* 1995; 61: 13-22.
33. Matsukura T, Sugase M. Human papillomavirus genomes in squamous cell carcinoma of the uterine cervix. *Virology* 2004; in press.
34. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med* 1998; 338: 423-428.
35. Molano M, Van den Brule A, Plummer M, Weiderpass E, Posso H, Arslan A, *et al*. HPV Study Group. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. *Am J Epidemiol* 2003; 158: 486-494.
36. Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, Desy M, *et al*. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. *J Infect Dis* 1999; 180: 1415-1423.
37. Filder HK, Boyes DA, Worth AJ. Cervical cancer detection in British Columbia. *J Obstet Gynecol Brit Commonw* 1968; 75: 392.
38. Richart RM, Baron BA. A follow-up study of patients with cervical dysplasia. *Am J Obstet Gynecol* 1969; 105: 386.
39. Barron BA, Richart RM. A statistical model of the natural history of cervical carcinoma. II. Estimates of the transition time from dysplasia to carcinoma in situ. *J Natl Cancer Inst* 1970; 45: 1025.
40. Lefebvre G, Darbois Y, Bitker MO, Luciani J, Mouquet C. Human papilloma virus infection and renal transplantation. Two personally observed cases and a review of the literature. *J Gynecol Obstet Biol Reprod (Paris)* 1995; 24: 600-603.
41. Schrager LK, Friedland GH, Maude D, Schreiber K, Adachi A, Pizzuti DJ, *et al.* Cervical and vaginal squamous cell abnormalities in women infected with human immunodeficiency virus. *J Acquir Immune Defic Syndr* 1989; 2: 570-575.
42. Braun L, Durst M, Mikumo R, Gruppuso P. Differential response of nontumorigenic and tumorigenic human papillomavirus type 16-positive epithelial cells to transforming growth factor beta 1. *Cancer Res* 1990;

50: 7324-7332.
43. Woodworth CD, Notario V, DiPaolo JA. Transforming growth factors beta 1 and 2 transcriptionally regulate human papillomavirus (HPV) type 16 early gene expression in HPV-immortalized human genital epithelial cells. *J Virol* 1990; 64: 4767-4775.
44. Malejczyk J, Malejczyk M, Kock A, Urbanski A, Majewski S, Hunzelmann N, *et al*. Autocrine growth limitation of human papillomavirus type 16-harboring keratinocytes by constitutively released tumor necrosis factor-alpha. *J Immunol* 1992; 149: 2702-2708.
45. Vieira KB, Goldstein DJ, Villa LL. Tumor necrosis factor alpha interferes with the cell cycle of normal and papillomavirus-immortalized human keratinocytes. *Cancer Res* 1996; 56: 2452-2457.
46. Kyo S, Inoue M, Hayasaka N, Inoue T, Yutsudo M, Tanizawa O, *et al*. Regulation of early gene expression of human papillomavirus type 16 by inflammatory cytokines. *Virology* 1994; 200: 130-139.
47. Khan MA, Tolleson WH, Gangemi JD, Pirisi L. Inhibition of growth, transformation, and expression of human papillomavirus type 16 E7 in human keratinocytes by alpha interferons. *J Virol* 1993; 67: 3396-3403.
48. Nawa A, Nishiyama Y, Yamamoto N, Maeno K, Goto S, Tomoda Y. Selective suppression of human papilloma virus type 18 mRNA level in HeLa cells by interferon. *Biochem Biophys Res Commun* 1990; 170: 793-9
49. Perea SE, Lopez-Ocejo O, Garcia-Milian R, Arana MJ. Interferon-alpha elicits downregulation of human papillomavirus 18 mRNA in HeLa cells by selective repression of endogenous viral transcription. *J Interferon Cytokine Res* 1995; 15: 495-501.
50. De Marco F, Giannoni F, Marcante ML. Interferon-beta strong cytopathic effect on human papillomavirus type 16-immortalized HPK-IA cell line, unexpectedly not shared by interferon-alpha. *J Gen Virol* 1995; 76: 445-450.
51. Malejczyk J, Malejczyk M, Majewski S, Breitburd F, Luger TA, Jablonska S, *et al*. Increased tumorigenicity of human keratinocytes harboring human papillomavirus type 16 is associated with resistance to endogenous tumor necrosis factor-alpha-mediated growth limitation. *Int J Cancer* 1994; 56: 593-598.
52. Malejczyk J, Malejczyk M, Breitburd F, Majewski S, Schwarz A, Expert-Besancon N, *et al*. Progressive growth of human papillomavirus type 16-transformed keratinocytes is associated with an increased release of soluble tumour necrosis factor (TNF) receptor. *Br J Cancer* 1996; 74: 234-239.
53. Malejczyk M, Jozwiak J, Osiecka A, Roszkowski PI, Mazurkiewicz-Smoktunowicz W, Rogozinski TT, *et al*. Serum levels of soluble tumor-necrosis-factor receptors in patients with benign and malignant HPV-associated anogenital lesions. *Int J Cancer* 1997; 73: 16-9.
54. Soto U, Das BC, Lengert M, Finzer P, zur Hausen H, Rosl F. Conversion of HPV 18 positive non-tumorigenic HeLa-fibroblast hybrids to invasive growth involves loss of TNF-alpha mediated repression of viral transcription and modification of the AP-1 transcription complex. *Oncogene* 1999; 18: 3187-3198.
55. Grandvaux N, tenOever BR, Servant MJ, Hiscott J. The interferon antiviral response: from viral invasion to evasion. *Curr Opin Infect Dis* 2002; 15: 259-267.
56. Ronco LV, Karpova AY, Vidal M, Howley PM. Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. *Genes Dev* 1998; 12: 2061-2072.
57. Li S, Labrecque S, Gauzzi MC, Cuddihy AR, Wong AH, Pellegrini S, *et al*. The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak-STAT activation by interferon-alpha. *Oncogene* 1999; 18: 5727-5737.
58. Barnard P, McMillan NA.The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-alpha. *Virology* 1999;259: 305-313.
59. Park JS, Kim EJ, Kwon HJ, Hwang ES, Namkoong SE, Um SJ. Inactivation of interferon regulatory factor-1 tumor suppressor protein by HPV E7 oncoprotein. Implication for the E7-mediated immune evasion mechanism in cervical carcinogenesis. *J Biol Chem* 2000; 275: 6764-6769.
60. Taniguchi T, Tanaka N, Taki S. Regulation of the interferon system, immune response and oncogenesis by the transcription factor interferon regulatory factor-1. *Eur Cytokine Netw* 1998; 9: 43-48.
61. Cho YS, Kang JW, Cho M, Cho CW, Lee S, Choe YK, *et al*. Down modulation of IL-18 expression by human papillomavirus type 16 E6 oncogene via binding to IL-18. *FEBS Lett* 2001; 501: 139-145.
62. Fausch SC, Da Silva DM, Kast WM. Differential uptake and cross-presentation of human papillomavirus virus-like particles by dendritic cells and Langerhans cells. *Cancer Res* 2003; 63: 3478-3482.
63. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, *et al.* Proof of principle study investigators. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002; 347: 1645-1651.
64. de Gruijl TD, Bontkes HJ, Walboomers JM, Schiller

JT, Stukart MJ, Groot BS, *et al.* Immunoglobulin G responses against human papillomavirus type 16 virus-like particles in a prospective nonintervention cohort study of women with cervical intraepithelial neoplasia. *J Natl Cancer Inst* 1997; 89: 630-638

65. Sasagawa T, Yamazaki H, Dong YZ, Satake S, Tateno M, Inoue M. Immunoglobulin-A and -G responses against virus-like particles (VLP) of human papillomavirus type 16 in women with cervical cancer and cervical intra-epithelial lesions. *Int J Cancer* 1998; 75: 529-535.
66. Sasagawa T, Rose RC, Azar KK, Sakai A, Inoue M. Mucosal immunoglobulin-A and -G responses to oncogenic human papilloma virus capsids. *Int J Cancer* 2003; 104: 328-335.
67. Morris HH, Gatter KC, Sykes G, Casemore V, Mason DY. Langerhans' cells in human cervical epithelium: an immunohistological study. *Br J Obstet Gynaecol* 1983; 90: 412-420.
68. Tay SK, Jenkins D, Maddox P, Campion M, Singer A. Subpopulations of Langerhans' cells in cervical neoplasia. *Br J Obstet Gynaecol* 1987; 94: 10-15.
69. Connor JP, Ferrer K, Kane JP, Goldberg JM. Evaluation of Langerhans' cells in the cervical epithelium of women with cervical intraepithelial neoplasia. *Gynecol Oncol* 1999; 75: 130-135.
70. Wang B, Amerio P, Sauder DN. Role of cytokines in epidermal Langerhans cell migration. *J Leukoc Biol* 1999; 66: 33-39.
71. Lappin MB, Kimber I, Norval M. The role of dendritic cells in cutaneous immunity. *Arch Dermatol Res* 1996; 288: 109-21.
72. Wang B, Amerio P, Sauder DN. Role of cytokines in epidermal Langerhans cell migration. *J Leukoc Biol* 1999; 66: 33-9.
73. Woodworth CD, Simpson S. Comparative lymphokine secretion by cultured normal human cervical keratinocytes, papillomavirus-immortalized, and carcinoma cell lines. *Am J Pathol* 1993; 142: 1544-1555
74. Mota F, Rayment N, Chong S, Singer A, Chain B. The antigen-presenting environment in normal and human papillomavirus (HPV)-related premalignant cervical epithelium. *Clin Exp Immunol* 1999; 116: 33-40.
75. Tindle RW. Immune evasion in human papillomavirus-associated cervical cancer. *Nat Rev Cancer* 2002; 2: 59-65.
76. Luxton JC, Rowe AJ, Cridland JC, Coletart T, Wilson P, Shepherd PS. Proliferative T cell responses to the human papillomavirus type 16 E7 protein in women with cervical dysplasia and cervical carcinoma and in healthy individuals. *J Gen Virol* 1996; 77: 1585-1593.
77. Nakagawa M, Stites DP, Farhat S, Judd A, Moscicki AB, Canchola AJ, *et al*. T-cell proliferative response to human papillomavirus type 16 peptides: relationship to cervical intraepithelial neoplasia. *Clin Diagn Lab Immunol* 1996; 3: 205-210.
78. Tsukui T, Hildesheim A, Schiffman MH, Lucci J 3rd, Contois D, Lawler P, *et al.* Interleukin 2 production in vitro by peripheral lymphocytes in response to human papillomavirus-derived peptides: correlation with cervical pathology. *Cancer Res* 1996; 56: 3967-3974.
79. Kadish AS, Romney SL, Ledwidge R, Tindle R, Fernando GJ, Zee SY, *et al*. Cell-mediated immune responses to E7 peptides of human papillomavirus (HPV) type 16 are dependent on the HPV type infecting the cervix whereas serological reactivity is not type-specific. *J Gen Virol* 1994; 75: 2277-2284
80. Cubie HA, Norval M, Crawford L, Banks L, Crook T. Lymphoproliferative response to fusion proteins of human papillomaviruses in patients with cervical intraepithelial neoplasia. *Epidemiol Infect* 1989; 103: 625-632.
81. Luxton JC, Rose RC, Coletart T, Wilson P, Shepherd PS. Serological and T-helper cell responses to human papillomavirus type 16 L1 in women with cervical dysplasia or cervical carcinoma and in healthy controls. *J Gen Virol* 1997; 78: 917-923.
82. Shepherd PS, Rowe AJ, Cridland JC, Coletart T, Wilson P, Luxton JC. Proliferative T cell responses to human papillomavirus type 16 L1 peptides in patients with cervical dysplasia. *J Gen Virol* 1996; 77: 593-602.
83. Bontkes HJ, de Gruijl TD, Bijl A, Verheijen RH, Meijer CJ, Scheper RJ, *et al*. Human papillomavirus type 16 E2-specific T-helper lymphocyte responses in patients with cervical intraepithelial neoplasia. *J Gen Virol* 1999; 80: 2453-2459.
84. Gill DK, Bible JM, Biswas C, Kell B, Best JM, Punchard NA, *et al*. Proliferative T-cell responses to human papillomavirus type 16 E5 are decreased amongst women with high-grade neoplasia. *J Gen Virol* 1998; 79: 1971-1976.
85. Greenberg PD. Adoptive T cell therapy of tumors: mechanisms operative in the recognition and elimination of tumor cells. *Adv Immunol* 1991; 49: 281-355.
86. Alexander M, Salgaller ML, Celis E, Sette A, Barnes WA, Rosenberg SA, *et al*. Generation of tumor-specific cytolytic T lymphocytes from peripheral blood of cervical cancer patients by in vitro stimulation with a

synthetic human papillomavirus type 16 E7 epitope. *Am J Obstet Gynecol* 1996; 175: 1586-1593.

87. Evans EM, Man S, Evans AS, Borysiewicz LK. Infiltration of cervical cancer tissue with human papillomavirus-specific cytotoxic T-lymphocytes. *Cancer Res* 1997; 57: 2943-2950.
88. Nakagawa M, Stites DP, Patel S, Farhat S, Scott M, Hills NK, *et al*. Persistence of human papillomavirus type 16 infection is associated with lack of cytotoxic T lymphocyte response to the E6 antigens. *J Infect Dis* 2000; 182: 595-598.
89. Youde SJ, Dunbar PR, Evans EM, Fiander AN, Borysiewicz LK, Cerundolo V, *et al*. Use of fluorogenic histocompatibility leukocyte antigen-A*0201/HPV 16 E7 peptide complexes to isolate rare human cytotoxic T-lymphocyte-recognizing endogenous human papillomavirus antigens. *Cancer Res* 2000; 60: 365-371.
90. Woodworth CD, Lichti U, Simpson S, Evans CH, DiPaolo JA. Leukoregulin and gamma-interferon inhibit human papillomavirus type 16 gene transcription in human papillomavirus-immortalized human cervical cells. *Cancer Res* 1992; 52: 456-463.
91. Arany I, Tyring SK. Activation of local cell-mediated immunity in interferon-responsive patients with human papillomavirus-associated lesions. *J Interferon Cytokine Res* 1996; 16: 453-460.
92. Coleman N, Birley HD, Renton AM, Hanna NF, Ryait BK, Byrne M, *et al*. Immunological events in regressing genital warts. *Am J Clin Pathol* 1994; 102: 768-774.
93. Coleman N, Stanley MA. Analysis of HLA-DR expression on keratinocytes in cervical neoplasia. *Int J Cancer* 1994; 56: 314-319.
94. Zhang B, Li P, Wang E, Brahmi Z, Dunn KW, Blum JS, *et al*. The E5 protein of human papillomavirus type 16 perturbs MHC class II antigen maturation in human foreskin keratinocytes treated with interferon-gamma. *Virology* 2003; 310: 100-108.
95. Symington FW, Santos EB. Recognition of keratinocytes by cytotoxic T cells specific for conventional HLA class-I alloantigen. *J Invest Dermatol* 1990; 95: 224-228.
96. Viac J, Soler C, Chardonnet Y, Euvrard S, Schmitt D.Expression of immune associated surface antigens of keratinocytes in human papillomavirus-derived lesions. *Immunobiology* 1993; 188: 392-402.
97. Connor ME, Stern PL. Loss of MHC class-I expression in cervical carcinomas. *Int J Cancer* 1990; 46: 1029-1034.
98. Torres LM, Cabrera T, Concha A, Oliva MR, Ruiz-Cabello F, Garrido F. HLA class I expression and HPV-16 sequences in premalignant and malignant lesions of the cervix. *Tissue Antigens* 1993; 41: 65-71.
99. Vambutas A, Bonagura VR, Steinberg BM. Altered expression of TAP-1 and major histocompatibility complex class I in laryngeal papillomatosis: correlation of TAP-1 with disease. *Clin Diagn Lab Immunol* 2000; 7: 79-85.
100. Keating PJ, Cromme FV, Duggan-Keen M, Snijders PJ, Walboomers JM, Hunter RD, *et al*. Frequency of down-regulation of individual HLA-A and -B alleles in cervical carcinomas in relation to TAP-1 expression. *Br J Cancer* 1995; 72: 405-411.
101. Ashrafi GH, Marchetti B, Sibbet G, Tsirimonaki E, Andrew L, O'Brien PM, *et al*. Down regulation of surface MHC class I by bovine papillomavirus E5 protein. *Oncogene* 2002; 21: 428-459.
102. McFadden G, Kane K. How DNA viruses perturb functional MHC expression to alter immune recognition. *Adv Cancer Res* 1994; 63: 117-209.
103. Barker JN, Sarma V, Mitra RS, Dixit VM, Nickoloff BJ. Marked synergism between tumor necrosis factor-alpha and interferon-gamma in regulation of keratinocyte-derived adhesion molecules and chemotactic factors. *J Clin Invest* 1990; 85: 605-608.
104. Spear GT, Sha BE, Saarloos MN, Benson CA, Rydman R, Massad LS, *et al*. Chemokines are present in the genital tract of HIV-seropositive and HIV-seronegative women: correlation with other immune mediators. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; 18: 454-459.
105. Kleine K, Konig G, Kreuzer J, Komitowski D, Zur Hausen H, Rosl F. The effect of the JE (MCP-1) gene, which encodes monocyte chemoattractant protein-1, on the growth of HeLa cells and derived somatic-cell hybrids in nude mice. *Mol Carcinog* 1995; 14: 179-89.
106. Norris DA. Cytokine modulation of adhesion molecules in the regulation of immunologic cytotoxicity of epidermal targets. *J Invest Dermatol* 1990; 95: 111S-120S.
107. Coleman N, Stanley MA. Characterization and functional analysis of the expression of vascular adhesion molecules in human papillomavirus-related disease of the cervix. *Cancer* 1994; 74: 884-892.
108. Paul WE, Seder RA. Lymphocyte responses and cytokines. *Cell* 1994 28; 76: 241-251.
109. Dupuy C, Buzoni-Gatel D, Touze A, Le Cann P, Bout D, Coursaget P. Cell mediated immunity induced in mice by HPV 16 L1 virus-like particles. *Microb Pathog*

1997; 22: 219-25.
110. Scott M, Stites DP, Moscicki AB. Th1 cytokine patterns in cervical human papillomavirus infection. *Clin Diagn Lab Immunol* 1999; 6: 751-755.
111. Pao CC, Lin CY, Yao DS, Tseng CJ. Differential expression of cytokine genes in cervical cancer tissues. *Biochem Biophys Res Commun* 1995; 214: 1146-1151.
112. al-Saleh W, Giannini SL, Jacobs N, Moutschen M, Doyen J, Boniver J, *et al*. Correlation of T-helper secretory differentiation and types of antigen-presenting cells in squamous intraepithelial lesions of the uterine cervix. *J Pathol* 1998; 184: 283-290.
113. Clerici M, Merola M, Ferrario E, Trabattoni D, Villa ML, Stefanon B, *et al*. Cytokine production patterns in cervical intraepithelial neoplasia: association with human papillomavirus infection. *J Natl Cancer Inst* 1997; 89: 245-250.
114. Zhou J, Liu WJ, Peng SW, Sun XY, Frazer I. Papillomavirus capsid protein expression level depends on the match between codon usage and tRNA availability. *J Virol* 1999; 73: 4972-4982.
115. Straight SW, Herman B, McCance DJ. The E5 oncoprotein of human papillomavirus type 16 inhibits the acidification of endosomes in human keratinocytes. *J Virol* 1995; 69: 3185-3192.
116. Wang SS, Wheeler CM, Hildesheim A, Schiffman M, Herrero R, Bratti MC, *et al*. Human leukocyte antigen class I and II alleles and risk of cervical neoplasia: results from a population-based study in Costa Rica. *J Infect Dis* 2001; 184: 1310-1314.
117. Routes JM, Li H, Steinke J, Cook JL. Dissimilar immunogenicities of human papillomavirus E7 and adenovirus E1A proteins influence primary tumor development. *Virology* 2000; 277: 48-57.