Immune Cellular Response to HPV: Current Concepts

Maria Alice Guimarães Gonçalves and Eduardo Antonio Donadi

Division of Applied Immunology, Clinical Medicine Department, Faculty of Medicine of Ribeirão Preto, University of São Paulo, São Paulo, SP, Brazil

Although cellular immunity is essential for the elimination of human papillomavirus (HPV), the mechanisms involved are still poorly understood. We summarize the main mechanisms involved in cellular immune response to infections caused by HPV. Immunotherapies for HPV-related cancers require the disruption of T-cell response control mechanisms, associated with the stimulation of the Th1 cytokine response.
Key Words: Human papillomavirus (HPV), cervical intraepithelial neoplasia (CIN), uterine cervical cancer, cellular immune response, cytokines.

Viruses are obligatory intracellular microorganisms that infect cells through various molecules present on their surface, and through other receptors. Viral replication interferes with the protein synthesis of normal cells, leading to lesion and death of the infected cells (cytopathic effect); the infection is considered lythic because the infected cell undergoes lysis. Non-cytopathic viruses can cause latent infections, during which the viruses live intracellularly and produce proteins that may or may not alter cellular functions. The purpose of innate and adaptive immune responses to viruses is to block the infection and to eliminate the infected cells [1].

Immunology of infection caused by human papillomavirus (HPV) and cervical cancer

Over 80 types of human papillomavirus (HPV) capable of infecting human beings are currently known. In tumors, and in most cancerous lesions of the uterine canal, and less frequently in lesions of the vulva, anus, and penis, high-risk HPV DNA are found, while in lesions of lesser gravity, low-risk HPV DNA are more frequent, sometimes associated with high-risk DNA [2,3].

HPV is a double strand DNA virus that contains around 8,000 base pairs. Two key regions have been described: the early region, with 7-8 genes (E1-E8) and the late region, with 2 genes (L1-L2). The first contains the viral DNA replication function (E1, E8), transcription control (E2, E8), and cellular transformation (E5, E6, and E7). The L1 and L2 genes code for the capsid's primary and secondary proteins respectively. Viral DNA replication takes place in the basal cells of the epidermis, where it remains in multiple copies in a stable manner, guaranteeing persistence in the epithelium's proliferative cells [4].

HPV infection has a transitory pattern, whereby most individuals (70% to 90%) eliminate the virus 12 to 24 months after initial diagnosis [4]. The persistence of HPV in 10% to 30% of cases is more common in oncogenic cases and is strongly associated with the development of high-grade squamous intra-epithelial lesions (SIL) [5]. There is evidence that the host's humoral or cellular immune response is responsible for the progression of HPV infection [6], since immune vigilance affects susceptibility to HPV-related lesions and their regression [7].

Received on 20 September 2003; revised 15 December 2003.
Address for correspondence: Dr. Maria Alice G. Gonçalves. Clinical Immunology Division, Clinical Medicine Department, Faculty of Medicine of Ribeirão Preto, University of São Paulo. Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brazil. Phone: (55 16) 602-2566, Fax: (55 16) 633-6695. E-mail:epigin@uol.com.br

The Brazilian Journal of Infectious Diseases 2004;8(1):1-9

Although the immune response to HPV cervical infections is not fully understood, it is known that local and systemic immune vigilance can explain the latency of HPV genital infection. HPV 16 infection manifests itself in the vast majority of cases of grade I and II cervical intra-epithelial neoplasias (CIN); even when this virus is associated with other potentially oncogenic viral types, it regresses spontaneously in most cases [2]. On the other hand, the virus is found unassociated with histopathological alterations; latent HPV infections occur asymptomatically in about 30% to 40% of sexually active adult women and in newborns [8], which demonstrates the activity of functional defense mechanisms.

Bowenoid papulosis is a typical example of the importance of the immune reaction for genital HPV infections; despite the association with HPV 16 and histological alterations, spontaneous regression frequently occurs in young patients. Biological response modifiers and some cytokines have been isolated from cellular lineages from vulvar bowenoid papulosis. The regulation of cytokine liberation (especially TNF-α), and the response to its action among carcinogenic and non-carcinogenic (HPV 16+) cellular lineages, are indications of the role of local immunological vigilance in controlling tumoral progression [9]. In other words, the interaction of cytokines/chemokines liberated during humoral/cellular immune reaction is responsible for the regression, persistence, or progress of lesions associated with HPV.

A polarization of the immune response for T-helper (Th)Th2 was observed in women with HPV infections that evolve into high-grade lesions [6]. These disequilibria may have various origins: inherited (HLA induced); acquired (due to acquired immunodeficiency - HIV); or unleashed according to the oncogenicity of the HPV type encountered. Whether SILs and in situ carcinomas get out of immunological control seems to depend on alterations in the cellular antigen presentation system, in the HLA receptors, and in the cellular recognition of oligopeptides [10].

There are other forms of control in proliferative cells infected by HPV that protect them from malignant transformation: 1) inhibition of the viral oncoprotein function, and 2) transcriptional blockage of the HPV DNA (cellular interference factor – CIF). In the first case, the consistent interruption of the function of the CDKN2A gene in immortalized E6 cells indicates that INK4A interferes functionally with the transforming activity of E6. The second case involves a modification of the AP1 transcription factor, essential for the expression of the HPV gene and the induction of the endogenous synthesis of antiviral interferon-β. This sequence does not function in cancer cells, indicating that the TNF-α-mediated cascade is interrupted when there is malignant transformation [10].

Penetration of the virus in the genital tract

Recently, two molecules of the integrin family, α6β1 and α6β4, expressed onthe surface of basal cells, have been identified as HPV receptors [11]. Due to abrasion or microlesions in the mucocutaneous surface, HPV affects basal layers, where proliferative cells are more susceptible; the squamocolumnar junction is the most common region for HPV-related lesions. The virus then replicates itself, expressing its early proteins in the proliferative layer and later in more differentiated cells, where capsid protein synthesis takes place and viral particles are structured [12].

The epithelium is an interface between the virus and the outside environment on one hand, and the immune system on the other. The effect of the immune barrier is reinforced by other elements of natural local immunity, i.e., Doderlein bacilli, vaginal acidity, secretion of defensins (peptides with bacterial and viricidal activity) by epithelial cells, and drainage through the cervicovaginal mucus. This set of factors in the presence of a viral infection activates a specific and adaptive immune response [4,13].

Mucosa-associated lymphoid tissue (MALT)

As with all MALT compartments, the genital mucosa has both inductor sites and effector sites involved in the immune response. The female genital tract has few lymphoid follicles, which are active units

of the inductor site of the MALT immune response [14]. The access to microbial antigens at this level seems also to be limited, since this epithelium has no M (Microfold) cells, which specialize in the transportation of antigens and are an essential part of the main means of access of viral pathogens to the lymphoid follicles [4].

Therefore, the afferent inductor paths in the female genital tract mainly involve Langerhans cells, macrophages, and lymphoid formations in the chorion [13]. MALT consists of various populations of lymphoid cells, including multiple Langerhans cells. These cells, as well as the keratinocytes of the cervical epithelium, express the major histocompatibility complex (MHC) class II molecules, and they participate actively in the presentation of antigens [15]. Cervical HPV infection apparently causes a local immune dysfunction, with a diminution of antigen-presenting intraepithelial cells [16] and T-lymphocytes [17]. Therefore, HPV infection can interfere with local immune vigilance mechanisms, both in the induction phase (antigen presentation) and in the effector phase (generation of cytotoxic T-cells and antibody-producing B cells). The mechanism of this interference is unknown; however, recent data suggest that the immunomodulating action of cytokines is a determining factor [18].

Antigen-presenting cells (APC)

Langerhans cells

The multiple layers of the cervical mucosa epithelium contain Langerhans cells, which have HLA class I and II molecules and participate actively in antigen presentation [15].

Langerhans cells are primarily located in the cervical squamous epithelium layer and they react positively to HLA-DR antigens [19]. Their concentration in the cervical epithelium is higher than in the vagina, indicating a localized inductor of genital immunity [4]. Langerhans cells capture the antigen in the epithelium and send it to the lymphoid nodules, where CD_4 lymphocytes are activated; these then multiply and differentiate. The increase in the number of Langerhans cells occurs because of the tumor necrosis factor (TNF-α) produced by the keratinocytes [20]. A diminution of the synthesis of TNF-α occurs in HPV infections, associated with a decrease in the expression of the B7.1 costimulatory molecule [12], resulting in a decrease in the presentation capacity of Langerhans cells.

Various subpopulations of Langerhans cells are known, and there are controversies about how they are affected by HPV and CIN. It has been suggested that infection by HPVs 16 and 18 reduces the number of Langerhans cells [20]. On the other hand, the average density of $S100^+$ Langerhans cells is significantly greater in patients with uterine cervical carcinoma than in those with cervicitis [16]. However, some studies have shown a decrease and others an increase in the number of Langerhans cells, with alterations of their morphology in patients in various stages of CIN. This phenomenon may be due to different types of HPV infections, creating different cytopathic effects. While certain types of HPV infections lead to cellular proliferation, the progression to CIN and invasive cancer is mediated by other initiating factors, such as tobacco smoking and herpes virus infection. In the case of persistent HPV infections, with koilocytosis associated with CIN, the continuous depletion of Langerhans cells results in intermediary densities. If the infection becomes non-permissive, the virus's cytopathic effect is lost; no more koilocytes are observed and the increase in the number of Langerhans cells is related to the presence of CIN [16,21].

The expression of HLA-DR molecules by Langerhans cells increases in koilocytic lesions and in CIN I and II, and the expression of HLA-DQ is also significantly higher in all grades of CIN, though it is more evident in CIN I [21].

Keratinocytes

Keratinocytes have the potential to present viral peptides, since HPV multiplies within these cells without inducing their lysis. However, keratinocytes do not express HLA class II molecules constitutively, but only in an inductive form, especially under the influence of IFN-γ. On the other hand, they also do not express

B7.1 (CD_{80}) and B7.2 (CD_{86}) costimulant molecules, which are essential for the induction of a specific response of the destructive type; therefore, these cells are more susceptible to a state of specific or anergic tolerance [11]. In sum, keratinocytes are both the main source of epithelial cytokines and the main target of HPVs [18].

Macrophages

Populations of monocytes/macrophages have been found in the mucosa of the uterine tract and in the vagina [14]. In high-grade lesions, an increase in the number of macrophages has been observed in parallel with a decrease in Langerhans cells, the contrary of what occurs in non-infected tissues. The presence of macrophages can be an indicator of the lesion regression. Macrophage participation in a protective immune response is evident through their indirect role in the negative control of the transcription of the genes that code E6 and E7 oncoproteins, and through their antitumoral functions in HPV-transformed cells [22].

Role of MHC molecules in the response to HPV

At the beginning of an efficient immune response, viral proteins are presented by APCs and the cells infected by the virus are destroyed. Viral antigens are fragmented by the proteasomes into short peptidic sequences, exported to the endoplasmic reticule by the TAP1 and TAP2 transportation molecules, and are finally associated with the MHC class I molecules. After passing through the Golgi apparatus and undergoing glycosylation, the resultant complex is rapidly expressed in the cellular membrane and the peptide is presented to the CD_8 T-lymphocytes. The interaction between the antigen/molecule of the MHC class I and the TCR (T-lymphocyte receptor) activates the CTLs. The synthesis and the release of perforin and granzyme by the activated lymphocytes provokes lysis of the infected cells [13,23].

However, even when the E6 and E7 proteins are strongly expressed by the keratinocytes, the CTLs appear not to recognize them. This may occur as a result of a deficiency in the presentation of viral epitopes during the long latency period between the beginning of infection and the appearance of cancer, independent of the type of APC. Therefore, the activity of MHC molecules seems to be a determining factor in the induction of an adaptive immune response [13].

However, in pre-cancerous and cancerous lesions, a defect in the presentation of the antigen, due to a decrease in the expression of MHC class I molecules and TAP proteins, would be responsible for the inefficiency of cellular immunity [23]. This deficiency is directly related to a modification in the expression of the MHC genes. Since the integration of the high-risk HPV DNA in the cellular genome sometimes occurs near the *c-myc* gene, the over-expression of this gene could diminish the expression of the HLA-A and B genes [24]. The expression of the proteasome genes and the TAP proteins located in the MHC genes can also be reduced. A reduction in the expression of TAP proteins could in turn prevent the formation of, or destabilize, the viral peptide/MHC class I complex, jeopardizing the presentation of the viral peptides, and alterations of the viral protein degradation proteasomes can modify the immunogenicity of the viral peptides.

Innate immunity against viruses

Interferons (IFNs)

Interferons are molecules (IFN-α, IFN-β) that are synthesized by infected cells, which combat the replication of the viruses in the host cells. They act by stimulating the synthesis of a protein-kinase that blocks the initiation of translation of viral proteins and of a 2'-5' A synthetase that, by activating endoribonucleases, allows the degradation of the viral and cellular mRNA. They also activate the synthesis and expression of the MHC class I molecules, facilitating the presentation of viral epitopes and increasing the cytotoxic capacity of the T-lymphocytes and the NK cells. Another IFN molecule (IFN-γ), produced by the activated T and

NK cells, induces the expression of MHC class II molecules on the surface of the keratinocytes and the monocytes/macrophages, allowing them to act as APC and, subsequently they activate the antiviral immune response. It has been found that IFN-γ blocks the expression of the HPV 18 mRNA of the cells derived from cervical cancer [25].

Tumoral necrosis factors (TNF)

These cytokines, coded by the genes situated at the heart of MHC, are essentially synthesized by monocytes/macrophages (TNF-α) and by T-lymphocytes (TNF-β), but specifically by T helpers with a Th1 profile. They have a strong antiviral and antitumoral action. TNF-α diminishes the expression of the E6/E7 genes in the cells infected by HPV 16 or 18 that have not yet been transformed; however, during tumoral progression, HPV-infected cells become insensitive to TNF-α [9].

TGF-β

This growth factor is produced by the epithelial cells and has an important role in immunosuppression, inhibiting the activity of NK cells, the expression of MHC class II molecules, and the production of cytotoxic lymphocytes. It inhibits the proliferation of most cells, including those infected by HPV [26]; it may or may not inhibit the expression of the E6 and E7 protein mRNA, since throughout tumoral progression the cells become resistant to TGF-β [18].

Natural killer cells (NK)

A small number of NK cells have been observed in condyloma acuminatum, and in mild to moderate dysplasias, whereas in severe dysplasias an increase occurs [27]. As these lesions progress toward cancer, they become resistant to NK cells. However, they are sensitive to lymphocyte lysis activated by lymphokines. This lysis can be strengthened when the cervical cells are treated with leukoregulin. In contrast, treatment with IFN-γ can result in reduced sensitivity to lymphokine-activated lysis, which illustrates the influence that cytokines can exert on immunological control of cervical cancer [7].

In patients presenting stage I or II invasive cervical carcinoma, NK cell activity is reduced. In many cases of uterine cervical cancer, NK cells are detected, but they are dispersed and their frequency is not related to the cancer's degree and prognosis [27]. However, patients with tumors that are not very differentiated or disseminated have a lower activity of NK cells than those with well-differentiated or localized tumors [27]. The reduction in the activity of NK cells in cancer patients is not, however, an irreversible phenomenon, since this activity can be restored through immunotherapy. Cellular activation can be increased through lymphokine-activated NK cells, as well as by increasing IFN-α levels [27].

Adaptive immune response to virus infections

T-lymphocytes

Most T-lymphocytes in the cervical epithelium belong to the CD_8 suppressor/cytotoxic subgroup and, in smaller numbers, to the CD_4 helper/inductor subgroup, both of which are found exclusively in the basal layers of the squamous epithelium and can also occur in large numbers in the ectocervical transformation zone [13]. The endocervix contains T-cells, B cells, and intraepithelial macrophages, with some CD_4 and CD_8 cells (< 2%) expressing the CD_{25} molecule (IL-2 receptor), which is a well-known sign of cellular activation [15].

The activation of a cytotoxic or helper cell is a complex process in which an APC must provide at least two signals. Signal 1 is provided by a foreign peptide linked to the MHC class II molecule on the surface of the presenting cell, which activates the T-cell receptor complex (TCR). Signal 2 is provided by a chemical signal, by interleukin-1, or by the B7 molecule, the latter being linked to the APC's plasmatic membrane. This molecule is recognized by a proteic co-receptor called CD_{28}, present on the surface of the T-helper cell and a member of the IgG superfamily [12,28].

In healthy women, the stimulation of peripheral lymphocytes with antigenic fragments of E6, E7, and L1 of the HPV induces the proliferation of specific cells,

especially memory cells [29]. Specific CTL lymphocytes for antigenic regions of E6 and E7 of HPV 16 and 18 have been detected in peripheral blood of patients with high-grade lesions [30] and those with infiltrating HPV+ cervical tumors [31]. Some authors observed that a CD_4 lymphoproliferative response to the C-terminal portion of E2 [3] was associated with a higher probability of eliminating the HPV infection. A cellular infiltration, composed essentially of CD_4 and macrophages, is frequently found in condylomas under spontaneous regression [32], while in patients with acquired or iatrogenic cellular immunodeficiency a greater prevalence of HPV-related lesions is observed, as well as a greater progression towards preinvasive lesions [33].

Cellular immunity plays a determining role in the early phase of infection and it is the only efficient response against non-lythic HPVs; it is also involved in the immune response to neoplasia. Usually, close to 60% of T-lymphocytes in peripheral blood are CD_4^+ and 20% to 30% are CD_8^+ (2/1); however, in patients with CIN/cancer the proportion of CD_4/CD_8 is lower (1.4). Locally, in the lymphocytic infiltrate, the opposite occurs, since in precancerous lesions the number of CD_4 is greater than the number of CD_8, which shows that the predominance of CD_4 cells favors regression, while CD_8 lymphocytes can mean the persistence and progression of the viral infection [34].

Cytokines

Cytokines are products of various lymphoid and non-lymphoid cells and constitute an important network of soluble mediators that facilitate cell-to-cell interactions in the MALT system and in skin-associated lymphoid tissue (SALT) [35]. Based on the pattern of cytokine production, Th cells are classified into two different groups: Th1 and Th2. Th1 cells produce Il-2, IFN-γ, and lymphotoxin; they promote cellular immunity, and are needed for effective responses in cases of intracellular pathogens (such as viruses) and for tumor cells. Th2 cells produce IL-4, IL-5, IL-6, and IL-10, and they favor humoral immunity against extracellular pathogens and allergic responses [9]. The regulation of the immune response is also controlled by cytokines produced by non-T-cells, known as type 1 and 2 cytokines. For example, IL-2 is produced by APCs (monocytes and dendritic cells) and B cells, and is necessary for the efficient differentiation of Th1 cells [36]. Another classification of cytokines is based on the type of effect produced: cytokines (IL-1, TNF-α), for example, exert pleomorphic effects *in vivo* and are known as primary cytokines. They have the ability to induce the synthesis of other mediators, the so-called secondary cytokines or chemokines, which are in turn responsible for activating the lymphoid cells and for chemotaxis [37].

The stratified epithelium cells and keratinocytes are the main sources of cytokines [37]. Cytokines act through specific receptors, which can be expressed in various forms in the cells infected by HPV or they can be released into the intracellular space, and from there to microcirculation. These soluble forms of cytokine receptors can significantly affect the immunoregulatory activity of cytokines, not only intermediating immune and inflammatory reactions, but also regulating proliferation, cellular differentiation, and apoptosis [1].

Polarization to the Th2 pattern explains a deficient cellular immune response against HPV and neoplasias, and seems to facilitate tumoral progression [20], since the deviation toward Th2 stimulates humoral response and inhibits cellular response. In patients with pre-neoplasic lesions [38], unbalanced mytogenic responses were detected, as well as reduced expression of the TCR-ξ receptor [39], and altered production of cytokines after cellular activation [40]. It has been found that the Th1 pattern (IL-2, IFN-α, and TNF-α) is associated with the regression of warts [41], while low levels of IFN-α are related to cervical carcinoma with a worse prognosis [42]. In addition, the production of IL-2 in preinvasive lesions decreases as the gravity of the illness increases [43].

Immune evasion mechanisms in viruses

Viruses, as well as tumor cells, are also capable of developing mechanisms of escape from the immune response. Barnard & McMillan [44] showed that the E7 protein of HPV 16 inhibits the

induction of IFN-α,activated genes, but not the induction of IFN-α inducible genes, therefore interfering with the effective elimination of HPV. Shneider et al. [45] also observed a weak response to treatment with IFN-α in HPV 16 and 18 lesions in comparison with the response to HPV6 and 11-induced lesions.

Although some HPV patients present a significant Th1 response targeting E7 and E2 and effectively eliminating the virus, in other patients with CIN or carcinoma the T-cell responses to the HPV's E7 do not resolve the lesion. This fact may be a reflection of escape mechanisms at the effector level [46] or at a target level [47].

CTL is the most common effector cell and is responsible for recognizing the MHC class I complex and the viral peptides present. However, in CINs and in cervical carcinoma there is a significant decrease in the expression of MHC class I molecules. At the same time, it appears that metastatic cells present a greater number of MHC class I cells compared to the cells of the primary tumor, which suggests that there is a selection for tumoral cells that are resistant to the action of the CTLs. Recently, studies with cancerous cells showed that these alterations in the regulation of the expression of MHC I molecules result from multiple genetic mechanisms and occur in around 90% of tumors [48].

Keratinocytes infected by HPV can modify the immune response in several ways: through the secretion of various cytokines (Il-1α, IL-6, IL-8, TNF-α, TGF-β) [26] and soluble receptors for TNF-α [9], or through the production of Th2 cytokines (IL-4 and 10) in squamous carcinomas, configuring an evasion mechanism against the T-cell mediated immune response [49].

Immunotherapies and vaccines

The purpose of studying factors that regulate the immune response to HPV is to help develop immunotherapies/vaccines that combat HPV-associated viruses and tumors. When prophylaxis of the infection is desired, L1 particles alone are used, or various types of HPV virus-like particles (VLPs) of L1 and L2; however, if the focus is on a therapeutic effect, some early proteins, in particular E6 and E7, have been proposed as vaccinal antigens because they are directly involved in uncontrolled proliferation and cellular transformation [50].

Recently, clinical tests have shown promising results when an L1 VLP prophylactic vaccine is used to reduce the onset of new cases of HPV infection. Serum conversion was observed in 99.7% of the women vaccinated, and adverse effects and toxicity were negligible [51]. However, it is important that vaccines focus on the 4 or 5 most prevalent types of high-risk HPV in order to address 80% to 90% of cervical cancers [10]. Prophylactic vaccines must be used in young women at the beginning of their sexual life, especially in developing countries, because they are considered the highest-risk population. However, some practical questions still need to be resolved before large-scale therapeutic use of this type of vaccine [52].

Immunotherapeutic vaccines can be based on peptides, proteins, chimerical VLPs, DNA, viral and bacteriological vectors, dendritic cells, and modified tumoral cells. In contrast with attenuated virus vaccines, which stimulate immunity through aberrant replication, DNA-based vaccines are incapable of propagating, and their ability to produce immunity is limited. Some efforts have been made to solve this issue, such as designing an HPV16 vaccine linked to M. tuberculosis heat shock protein 70. This composition was tested in animals and a 30-fold increase was observed in CD_8^+ cells specific for oncoprotein E7. However, the use of oncoproteins in human beings is still controversial, due to their mutagenic potential [53].

References

1. Abbas A.K., Lichtman A.H., Pober J.S. Cellular and Molecular immunology. WB Saunders Company, Philadelphia, USA, **2000**.
2. Barrasso R., De Brux J., Croissant O., Orth G.. High prevalence of papillomavirus-associated penile intraepithelial neoplasia in sexual partners of women with cervical intraepithelial neoplasia. New England Journal of Medicine **1987**;317:916.

3. Bontkes H.J., de Gruijl T.D., Bijl A., et al. Human papillomavirus type 16 E2-specific T-helper lymphocyte responses in patients with cervical intraepithelial neoplasia. Journal of General Virology **1999**;80:2453-9.
4. Ho G.Y., Bierman R., Beardsley L., et al. Natural history of cervicovaginal papillomavirus infection in young women. New England Journal of Medicine **1998**;338: 423-8.
5. Koutsky L.A., Holmes K.K., Critchlow C.W., et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. New England Journal of Medicine **1992**;327: 1272.
6. Scott M., Stites D.P., Moscicki A.B. Th1 cytokine patterns in cervical human papillomavirus infection. Clinical and Diagnostic Laboratory Immunology **1999**;6:751-5.
7. Wu R., Coleman N., Higgins G. Lymphocyte-mediated cytotoxicity to HPV 16 infected cervical keratinocytes. In: Stanley, M.A. Immunology of Human Papillomaviruses. Plenum Press, New York **1994**:255-9.
8. Tidy J.A., Parry G.C., Ward P., et al. High rate of human papillomavirus type 16 infection in cytologically normal cervices. Lancet **1989**;1:434.
9. Malejczyk J., Malejczyk M., Breitburd F., et al. Progressive growth of human papillomavirus type 16 transformed keratinocytes is associated with an increased release of soluble tumour necrosis factor receptor. British Journal of Cancer **1996**;74:234-9.
10. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nature reviews **2002**;2:342-50.
11. Evander M., Frazer I.H., Payne E., et al. Identification of the α6 integrine as a candidate receptor for papillomavirus. Journal of Virology **1997**;71:5449-59.
12. Frazer I., Tindle R. Cell-mediated immunity to papillomaviruses. Papillomavirus Report **1992**;3:53.
13. Greslin I., Mougin C., Seilles E. Biologie des infections à papillomavirus. II. Réponse immunitaire. Ann Biol Clin **1998**;56: 267-76.
14. Parr M.B., Parr E.L. Mucosal immunity in the female and male reproductive tract. In: Ogra P.L., et al. Handbook of mucosal immunology. San Diego, Academy Press **1996**:677-89.
15. Roncalli M., Sideri M., Gie P., Servida E. Immunophenotypic analysis of the transformation zone of human cervix. Laboratory Investigations **1988**;58: 141-9.
16. McArdle J.P., Muller H.K. Quantitative assessment of Langerhans cells in human cervical intraepithelial neoplasia and wart virus infection. American Journal of Obstetrics and Gynecology **1986**;154:509-15.
17. Tay S.K., Jenkins D., Singer A. Natural killer cells in cervical intraepithelial neoplasia and human papillomavirus infection. British Journal of Obstetrics and Gynecology **1987**;94:901-6.
18. Majewski S., Malejczyk J., Jablonska S. The role of cytokines and other factors in HPV infection and HPV-associated tumors. Papillomavirus Report **1996**;7:143-54.
19. Belec L., Georges A.J., Steenman G., Martin P.M. Antibodies to human immunodeficiency virus in vaginal secretions of heterosexual women. Journal of Infectious Diseases **1989**;160:385-91.
20. Mota F., Rayment S., Chong S., et al. The antigen environment in normal and human papillomavirus (HPV)-related premalignant cervical epithelium. Clinical and Experimental Immunology **1999**;116:33-40.
21. Hughes R.G., Norval M., Howie S.E. Langerhans cells and subtypes of human papillomavirus in cervical intraepithelial neoplasia. Journal of Clinical Pathology **1988**;41:253-9.
22. Banks L., Moreau F., Vousden K., et al. Expression of the human papillomavirus E7 oncogene during cell transformation is sufficient to induce susceptibility to lysis by activated macrophages. J Immunol **1991**;146:2037-42.
23. Keating P.J., Cromme F.V., Duggan-Keen M., et al. Frequency of down-regulation of individual HLA-A and B alleles in cervical carcinomas in relation to TAP-1 expression. British Journal of Cancer **1995**;146:944-52.
24. Cromme F.V., Snijders P.J., van den Brule A.J., et al. MHC class I expression in HPV 16 positive cervical carcinomas is posttranscriptionally controlled and independent from c-myc overexpression. Oncogene **1993**;8:2969-75.
25. Woodworth C.D., Lichti U., Simpson S., et al. Leukoregulin and gama-interferon inhibit human papillomavirus type 16 gene transcription in human papillomavirus-immortalized human cervical cell. Cancer Research **1992**;52:456-63.
26. Woodworth C.G., Simpson S. Comparative lymphokine secretion by cultured normal cervical keratinocytes, papillomavirus-immortalized and carcinoma cell lines. American Journal of Pathology **1993**;142:1544-55.
27. Satam M.N., Suraiya J.N., Nadkarni J.J. Natural killer and antibody-dependent cellular cytotoxicity in cervical carcinoma patient. Cancer Immunology and Immunotherapy **1986**;23:56-9.
28. Fiorentino D.F., Bond M.W., Mosmann T.M. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. The Journal of Experimental Medicine **1989**;170:2081-95.
29. Luxton J.C., Rowe A.J., Cridland J.C., et al. Proliferative T-cell responses to the human papillomavirus type 16 E7 protein in women with cervical dysplasia and cervical carcinoma and in healthy individuals. The Journal of General Virology **1996**;77:1585-93.
30. Nimako M., Fiander A.N., Wilkinson G.W., et al. Human papillomavirus-specific cytotoxic T lymphocytes in patients with cervical intraepithelial neoplasia grade III. Cancer Research **1997**;57:4855-61.

31. Evans E.M., Man S., Evans A.S., Borysiewicz L.K. Infiltration of cervical cancer tissue with human papillomavirus-specific cytotoxic T-lymphocytes. Cancer Research **1997**;57:2943-50.
32. Stanley M., Coleman N., Chamberst M. The host response to lesions induced by human papillomavirus. Vaccine against virally induced cancers. Wiley, Chichester (Ciba Foundation Symposium **1994**;187:21-44.
33. Palefsky J.M. Human papillomavirus infection and anogenital neoplasia in human immunodeficiency virus-positive men and women. Monogr Natl Cancer Inst **1998**;23:15-20.
34. Edwards R.P., Kuykendall K., Crowley-Nowick P., et al. T lymphocytes infiltrating advanced grades of cervical neoplasia. Cancer **1995**;76:1411-5.
35. Streilein J.W. Sunlight and skin-associated lymphoid tissue (SALT): if UVB is the trigger and TNF-α is the mediator, what is the message? Journal of Investigative Dermatology **1993**;100:47S-52S.
36. Scott P. IL-12: Initiation cytokine for cell mediated immunity. Sciences **1993**;260:496-7.
37. Schroder J.-M. Cytokine networks in the skin. Journal of Investigative Dermatology **1995**;105: 20S-4S.
38. Levy S., Kopersztych S., Musatti C.C., et al. Cellular immunity in squamous cell carcinoma of the uterine cervix. American Journal of Obstetrics and Gynecology **1978**;130:160-4.
39. Koch F., Stanzl U., Jennewein P., et al. High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD_{40} molecules and downregulation by IL-4 and IL-10. The Journal of Experimental Medicine **1996**;184:741-6.
40. Clerici M., et al. Cytokine production patterns in cervical intraepithelial neoplasia: association with human papilloma-virus infection. The Journal of National Cancer Institute **1997**;89:245-50.
41. Grassegger A., Rollinger-Holzinger I., Zelger B.W., et al. Spontaneous or interferon-?-induced T-cell infiltration, HLA-DR and ICAM-1 expression in genitoanal warts are associated with TH1 or mixed TH1/TH2 cytokine mRNA expression profiles. Archives of Dermathological Research **1997**;289:243-50.
42. Tartour E., Gey A., Sastre-Garau X., et al. Prognostic value of intratumoral interferon gamma messenger RNA expression in invasive cervical carcinomas. The Journal of National Cancer Institute **1998**;90(4):287-94.
43. Tsukui T., Hildesheim A., Schiffman M.H., et al. Interleukin 2 production *in vitro* by peripheral lymphocytes in response to human papillomavirus-derived peptides: correlation with cervical pathology. Cancer Research **1996**;56:3967-74.
44. Barnard P., McMillan N.A. The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-alpha. Virology **1999**;259:305-13.
45. Schneider A., Papendick U., Gissmann L., De Villiers E.M. Interferon treatment of human genital papillomavirus infection: importance of viral type. International Journal of Cancer **1987**;40:610-4.
46. Rudolf M.P., Small L.A., Velders M.P., et al. @Vaccine delivery and immunosupression in cervical cancer. In: Stern P.L., Beverley P.C.L., Carroll M.W. (Eds). Cancer Vaccines and Immunotherapy. Cambridge University Press, **2000**:82-106.
47. Garrido F., et al. HLA and Cancer. In: Ochoa (Ed). Tumor escape mechanisms. Academic Publishers, **2000**.
48. Brady C.S., Bartholomew J.S., Burt D.J. C.S., et al. Multiple mechanisms underlie HLA dysregulation in cervical cancer. Tissue Antigens **2000**;55:401-11.
49. Kim J., Modlin R.L., Moy R.L., et al. IL-10 production in cutaneous basal and squamous cell carcinomas. A mechanism for evading the local T-cell immune response. Journal of Immunology **1995**;155:2240-7.
50. WHO, International Agency for Research on Cancer. The current status of development of prophylactic vaccines against human papillomavirus infection. Report of a technical meeting. Geneva, **1999**.
51. Koutsky L.A. A controlled trial of a human papillomavirus type 16 vaccine. New England J Med **2002**;347:1645-51.
52. Tewari K.S., DiSaia P.J. Primary prevention of uterine cancer: focus on vaccine history and current strategy. Obstet Gynecol Clin N Am **2002**;29:843-68.
53. Moniz M., Ling M., Hung C.-F., Wu T.-C. HPV DNA Vaccines. Front Biosciences **2003**;8:55-68.