INTRODUCTION

Cervical cancer remains a major health problem affecting thousands of women across the globe. Being a preventable disease by virtue of the availability of a screening method to detect and treat precancerous lesions, it remains a mystery as to why so much morbidity and mortality is prevalent in modern society. A classic example of control of this disease is the Nordic countries where the incidence and mortality of cervical cancer has been reduced by about 80% with an established cervical cancer screening program. Lack of political will, inadequate or non-existent facilities, failure of the health care provider to educate women and failure of women to avail themselves for screening have contributed to the devastating effects affecting women predominantly in the productive years of their lives. The Human Papillomavirus (HPV) is a necessary pre-requisite for the development of precancerous and cancerous lesions not only of the cervix, but also of the lower female genital tract. However, co-factors are necessary for the progression to invasive cancer. Two commercially available vaccines against the human papillomavirus have been developed and are being provided to women as part of a government initiative in many developed countries. In the long term, it remains to be seen if the HPV vaccines will become an armamentarium of women's rights world-wide to prevent HPV-related diseases.

1.0 EPIDEMIOLOGY OF CERVICAL CANCER

Cervical cancer is the most common malignancy amongst women of developing countries and the second commonest cancer amongst women worldwide. In South Africa, the incidence of cervical cancer has been reported to be approximately 30 per 100 000 women (Sitas et al, 1993). Although the worldwide incidence and mortality of cervical cancer has declined over the last four decades (32.6 per 100 000 to 8.3 per 100 000 in the United States in the late 1940s), cervical cancer continues to be the leading cause of cancer–related mortality in the developing world (Brinton, 1992). Annually, there are about 500 000 new cervical cancer cases with a mortality of just over 250 000 women, representing about 10% of all female cancers. This is mainly due to the fact that about 85% of women in the developing world present with late-stage disease (59.3% stage III versus 5.2% stage 1B) (Cronje, 2004; Moodley et al, 2001 & Lomalisa et al, 2000). This pattern is similar in other developing regions such as Southeast Asia, Africa, Central America and the Caribbean, where the incidence rate of cervical cancer approaches 30 cases per 100 000 women (Fowler & Sayegh, 2005) . Estimates from Globocan 2002 have reported that there were 6742 cases of cervical cancer in South Africa, with mortality figures of 3681 and an age-standardized rate of 37.5. In comparison with breast cancers (Ferlay et al, 2004), there were 6018 cases of breast cancer in South Africa with a mortality of 2790 and an age-standardized rate of 35. This is in contrast to the incidence rate of cervical cancer in the United States of about 7.2 cases per 100 000 women with an overall mortality rate of 2.9 cases per 100 000 women (Fowler & Sayegh, 2005). This

disproportionate burden of cervical cancer is mainly due to the lack of well-organized screening programs (Womack et al, 2000). In many developing countries where cervical cancer screening exists, women's knowledge of cervical cancer and Papanicolaou (Pap) smears is very limited. Data from the United States Surveillance, Epidemiology and End-Results (SEER) program has demonstrated that survival amongst Black women with cervical cancer is far inferior to their White counterparts. The five-year survival rate for White women was 71% compared with 60% for Black women.

Cervical cancer has well recognized precancerous stages, which, if detected early by screening, can be treated to prevent the development of full-blown cancer. Unlike the developed countries such as the Nordic countries following the introduction of national screening in the 1960s, where the mortality has been reduced by about 80%, lack of screening facilities or inadequate screening facilities and poor uptake account for screening failures in the developing world (Wellensiek et al, 2002). The data also shows that the largest decline was in Iceland (84% from 1965 to 1982), where a large percentage of the population was screened. The pathogenesis of cervical cancer and its precursors, is causally linked to the high-risk human papillomaviruses (HPV). Human papillomaviruses are reported to be present in more than 99.7% of cervical cancers (Bosch, 2002). The most common high-risk HPV viruses found in cervical cancers are types 16 and 18 (70%). Human papillomaviruses are a necessary but insufficient cause of cervical cancer. Co-factors are therefore necessary for the expression of the malignant phenotype. It is estimated that only 5% of women in the developing world receive screening compared with 40% to 50% of women in the developed world (Sherris et al, 2001).

A further problemmatic aspect of cervical cancer is co-infection with the human immunodeficiency virus (HIV) epidemic in so many parts of the developing world. Although the HIV / Acquired Immunodeficiency Virus Syndrome (AIDS) pandemic is global in distribution, its maximum impact has been in Sub-Saharan Africa, where two-thirds of people (21 million) are reported to be living with HIV/AIDS (Ateka, 2000). By some estimates, up to 40% of patients with AIDS will develop some type of malignancy (Smith, 1998). In 1993, the Centres for Disease Control (CDC) expanded the case definition and included invasive cervical cancer as an AIDS-defining illness (CDC, 1993). Women who are infected with HIV are more likely to be infected with multiple HPV-types as well as to develop cervical intraepithelial neoplasia. They are also at greater risk of persistent HPV infection and lower regression of cervical intraepithelial neoplastic lesions (Schuman et al, 2003). Immunosuppression with CD4 counts of less than 200 cells/μL is associated with a 10-fold risk of developing cervical intraepithelial neoplasia (Heard et al, 2000). The seroprevalence of human immunodeficiency virus infection amongst antenatal attendees in KwaZulu Natal, South Africa was reported as 38.7% for 2003 (Moodley, 2006). Hence, in KwaZulu Natal, South Africa, the combination of deficient cervical cancer screening and the HIV epidemic predisposes many young women to cervical cancer with its attendant morbidity and mortality.

1.1 RISK FACTORS AND AETIOLOGY OF CERVICAL CANCER AND ITS PRECURSORS

1.1.1 PARITY

Although parity has been regarded as a risk factor for cervical cancer, it is the number of live births that has been consistently found to be of importance, irrespective and independent of other factors related to sexual activity such as number of partners. Brinton et al (1989) noted that the risk increased to 5.1 for women with 14 or more pregnancies related to live births, suggesting that hormonal factors and cervical trauma are putative mechanisms. It has also been suggested that the pregnancy-associated hormonal influence may have an impact on the HPV genomic elements which are responsive to progesterone (Pater et al, 1994). According to the data from the International Agency for Research on Cancer (IARC), there is a clear link between parity and HPV-positivity in that high parity in HPV-positive women increases the risk of cervical cancer even further (Munoz et al, 2002).

1.1.2 THE NUMBER OF SEXUAL PARTNERS AND FREQUENCY OF SEXUAL INTERCOURSE

Many studies have reported a link between the number of sexual partners and the risk of cervical cancer (Slattery et al, 1989; La Vecchia et al, 1986). There is three times the risk associated with ten or more partners compared with one partner (Brinton et al, 1987; Peters et al, 1986). Although Boyd & Doll (1964) did not find any significant association between frequency of intercourse and risk of cervical cancer, Herrero et al (1990a) reported a significant link between the frequency of coitus, multiple exposures prior to age 20 and risk of cervical cancer.

1.1.3 SMOKING

Smoking is a well recognized risk factor not only for precancerous and invasive cervical caners, but also for dysplasia of the vulva. This link has been alluded to in many epidemiological studies. Although initially the effect of smoking was thought to be linked to associated factors such as sexual behaviour, several studies which controlled for extraneous factors have still established a link between smoking and cervical cancer (Brinton et al. 1986; Clarke et al, 1982). Atalah et al (2001) reported an odds ration of 2.8 for smokers. The risks are highest for long-term or high- intensity smokers. More recent studies have shown a positive link between smoking and cervical cancer (Kjellberg et al, 2000) Thomas et al (2001) demonstrated that the odds of ever being a smoker was higher

in women with cervical carcinoma in-situ, invasive cervical carcinoma and cervical intraepithelial neoplasia grade 3. This implies that not only is smoking important as a causative agent, but has an important role in the later stages of disease pathogenesis. Castellsague and Munoz (2003) in a review of risk factors for cervical cancer reported an odds ratio of 2 to 5 for those who ever smoked among HPV-positive women. They also noted most studies which reported risk estimates according to intensity, duration or pack-years demonstrated an increased risk of cervical cancer with increasing exposure to tobacco smoking. Shields et al (2004) demonstrated a two-fold increased risk of precancerous and invasive cervical cancers in current or ever-smokers versus non-smokers. Syrjanen 2008 reported data from a cohort study of 3187 women to determine the influence of risk factors for cervical cancer. In multivariate analysis smoking was found to be an independent risk factor for cervical cancer because of the increased acquisition of high-risk HPV infections. The possible mechanisms involved include high levels of nicotine and cotine found in cervical mucus (Schiffman et al, 1987; Prokopczk, 1997), the immunosuppressive effects of smoking and the enhanced effects of infectious agents such as HPV (Barton et al, 1988). These immunosuppressive effects include the reduction of Langerhans cells in the cervix. Melikian et al (1999) reported the presence of benzo[a]pyrene and its metabolites in cervical mucus to be two times higher amongst smokers versus non-smokers. Yang et al (1996) demonstrated that the malignant transformation of immortalized endocervical cells occurs in the presence of cigarette condensate. Giuliano et al (2002) reported that smokers retain HPV infections significantly longer and have a lower chance of clearing HPV infection than non-smokers.

1.1.4 ROLE OF THE MALE PARTNER

Support for male partner contribution is derived from geographic clusters of cervical and penile cancers and elevated rates of cervical cancers among wives of men with penile cancer (Franco et al 1988; Graham et al 1979). Kessler (1977) showed in a follow-up study that wives of men previously married to cervical cancer patients had elevated rates of cervical neoplasia compared to a control group. Direct evidence for a male factor derives from studies in which the sexual histories of husbands of cervical cancer patients have been compared with those of control husbands (Brinton et al 1989; Buckley et al 1981). In all these studies, husbands of cervical cancer patients were likely to have histories of genital conditions, including venereal warts, gonorrhea and herpes simplex. Poor hygiene of the male partner has been thought to contribute to the aetiology of cervical cancer especially in relation to the issue of circumcision. Although initial studies showed a protective effect of circumcision (Terris & Oalmann, 1960), most other studies have shown no significant difference between case and control husbands (Boyd & Doll 1964; Rotkin 1967). The issue, however, remains to be resolved as Kjaer et al (1991) showed that wives of circumcised men were at a significantly lower risk (0.3). The issue of male circumcision also remains unresolved due to problems in assessment, with most studies showing no effect on cervical cancer risk (Brinton et al 1989).

1.1.5 ROLE OF DIETARY FACTORS

The protective effects of dietary factors such as beta-carotene, Vitamins A,C and E have been consistently reported (Garcia-Closas et al, 2005; Potischman and Brinton,1996 & Shannon et al, 2002). However, Kjellberg et al (2000), found no link between diet and risk of invasive cervical cancer or precancerous cervical lesions. Other dietary factors which have been shown to reduce the risk of invasive cervical cancer include tocopherols, folates and lycophene (Palan et al, 1991; Goodman et al, 2001 & van Eenwyk et al, 1991). The biological basis for the protective role of these dietary factors is thought to be due to their antioxidant actions on intracellular free radicals. Beta-carotene is thought to be a metabolic precursor to retinoic acid which modulates epithelial cell growth and differentiation (Potischman and Brinton, 1996). However, it is difficult to determine the exact role of dietary factors due to confounding by other risk factors known to be associated with the development of cervical cancer.

1.1.6 ROLE OF SEXUALLY TRANSMITTED INFECTIONS OTHER THAN HPV

Since HPV is present in virtually all cervical cancers (99.7%), all other agents are thought to act as co-factors with HPV in the pathogenesis of cervical intraepithelial and invasive neoplasias. These agents include the Herpes simplex Type 2 virus, *Chlamydia trachomatis, Neiseria gonorrhoea, Treponema pallidum*, cytomegalovirus, Epstein-Barr virus and bacterial vaginosis. Although many studies have reported an association of

these infectious agents with cervical cancer, some studies have reported unadjusted risks, resulting in difficulties in interpreting relationships independent of those associated with socioeconomic status and other risk factors. Brinton (1987) reported consistent increases in risk associated with histories of condyloma acuminate after adjusting for the number of sexual partners. However, genital warts are usually linked to HPV types 6 and 11 rather than HPV types 16 and 18 which are associated with cervical cancer (Koutsky et al, 1988). There has been a fairly consistent relationship between invasive cervical cancer and *Chlamydia trachomatis* (Koskela et al, 2000; Anttila et al, 2001). Smith et al (2002) reported a relative risk of 2.1 for *Chlamydia trachomatis* and HPV infections. Zur Hausen et al (1982) proposed that the HSV type 2 virus may be a co-factor in the pathogenesis of cervical cancer and that HPV sequences may be required to maintain the transformed phenotype. McDougall et al (1986) demonstrated that HSV can transform cells in culture and HSV type 2 proteins and HPV integrated DNA are found in cervical cancers. The IARC case-controlled studies have demonstrated an increased risk of 2-fold and 3-fold for invasive cervical cancers in HPV-positive women. However, other studies did not detect HSV type 2 virus consistently in cervical tumours (Lehtinen et al, 2002; Vonka et al, 1984). Inconsistent associations have been observed for other infections such as *Treponema pallidum*, cytomegalovirus, Epstein-Barr virus and bacterial vaginosis.

1.1.7 ROLE OF THE HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION

In 2003, it was estimated that there were about 5 million new HIV infections and 3 million deaths from HIV-related illnesses. In the same year the joint United Nations Programme on HIV/AIDS concluded that there were about 40 million adults and children infected with HIV/AIDS. Majority of these infections were in persons living in sub-Saharan Africa, where majority of women do not have access to cervical cancer screening (Chirenje, 2004). The majority of these infections are in developing regions such as sub-Saharan Africa where cervical cancer is also most prevalent. Although the human immunodeficiency virus (HIV) / Acquired Immunodeficiency Virus Syndrome (AIDS) is a global phenomenon, the bulk of the disease occurs in developing regions of the world such as Sub-Saharan Africa (Ateka, 2000). By previous estimates, up to 40% of patients with AIDS will develop some type of malignancy (Smith et al, 1998). Women who are HIV-infected have a higher rate of developing human papillomavirus (HPV) infections, especially of the high-risk types. Such women are at greater risk of developing squamous intraepithelial neoplasia and invasive cervical cancers (Ellerbrock et al, 2000).

In areas ravaged by the HIV virus, the occurrence of female genital tract malignancies in association with the HIV virus has become a reality, creating new challenges for clinicians. The prevalence of HPV in HIV-infected and non-infected women has been shown to be 65% and 29%, respectively (Duerr et al, 2001). However, a more recent report by Levi et al (2002) documented the prevalence of HPV DNA in 98% of HIV-

infected women. Whilst HPV infection is usually transient in HIV non-infected women, HIV-infected women have higher rates of persistent infection and are less likely to clear high-risk HPVs than HIV non-infected women (20% versus 3%, respectively)(Sun et al, 1995). Jamieson et al (2002) reported increased prevalence of multiple HPV infections in HIV-infected compared with HIV non-infected women. HPV type 16 was the commonest high–risk type amongst HIV-infected women in the New York Cervical Disease Study (NYCDS) and Women's Interagency Study (WIHS) (Barkan et al, 1998) compared with HPV type 53 which was reported to be the commonest HPV type in the HIV Epidemiology Research Study (HERS) (Smith, 1998). Ahdieh et al (2001) reported higher HPV-positivity (75%) in HIV-infected women with CD4 counts of less than 200 cells/µL compared with women with CD4 counts >500 cells/µL (54%). Blossom et al (2007) conducted a cross-sectional study amongst Ugandan women to determine the prevalence of HIV infection, HPV infection and cervical abnormalities. Of the 106 women studied, the HPV and HIV prevalence was 46.2% and 34.9%, respectively. High-risk HPV genotypes 52, 58 and 16 were the commonest types detected. Only 18% of women had HPV types 16 and 18. There were 73% of HIV-infected women compared with 16% of HIV non-infected women with cervical cytological abnormalities ($p<0.0001$). Abnormal pap smears was significantly associated with HIV sero-positivity ($p<0.001$). Therefore, the majority of women had HPV types other than 16 and 18. HPV type 52 was also the commonest type reported by de Vuyst et al (2003) amongst Kenyan women. Lin et al (2006) also reported that types 16, 52 and 58 were the commonest types in South Taiwanese women. Women who are HIV-infected with severe immunosuppression are five times more likely than HIV non-infected women to have

lower genital tract neoplasia (Ferenczy et al, 2003). Ellerbrock et al (2000) demonstrated a 55% risk of developing cervical intraepithelial neoplasia in HIV-infected women over a two year follow-up period. Cervical dysplastic lesions are more likely to progress and recur after conventional treatment. Spitzer (1999) reported recurrent lesions in 87% of women at 36 months post treatment.

Some studies have supported the concept that there is an increased prevalence of invasive cervical cancer amongst HIV-infected women (Royansky et al, 1996; Franceschi et al, 2003 & Goedert, 2000), whereas other studies failed to establish this relationship (Newton et al, 2001 & Mbulaiteye et al, 2003). International HIV seroprevalence rates amongst women with cervical cancer vary from 1.6% in Hong Kong (Chan et al, 2004), 15% in Kenyan women (Gichangi et al, 2003) to 21% reported in Durban, South Africa (Moodley et al, 2001). In 1990, the prevalence for HIV infection amongst the antenatal population in the province of KwaZulu- Natal, South Africa, was 1.6% (Webb, 1997). In the same year the incidence of HIV infection in women with invasive cervical cancer in this province was 5%. In 1999 the prevalence of HIV in women attending antenatal clinics for this province was 32.5% (Ateka, 2000). Sitas (2000) reported that the relative risk of HIV with cervical cancer to be 1.6 (CI: 1.1 – 2.3) in South Africa.

In 1993 the Centres for Disease Control and Prevention (CDC) labelled invasive cervical cancer as an AIDS-defining illness based on limited data. From this time onwards, this issue has remained controversial. However, in 1998 the CDC Sentinel Hospital Surveillance System for HIV infections reported that the prevalence of invasive cervical

cancer for HIV-infected women to be 10.4 cases per 100 000 women compared with 6.2 cases per 100 000 HIV non-infected women (RR=1.7; 95% CI 1.1 – 2.5) (Chin et al, 1998). Similar elevated increases were reported by other studies (Fordyce et al, 2000; Franceschi et al, 1998). Franceschi et al (1998) in an Italian-based study reported a relative risk of 15.5 (95% CI 4.0 – 40.1) for cervical cancer amongst women with HIV/AIDS. In contrast Phelps et al (2001) reported a rate of invasive cervical cancers to be 5 per 1000 person-years amongst HIV-infected women compared with 0 per 1000 person-years amongst HIV non-infected women. This large multicentre study was conducted in the course of the HIV Epidemiology Research Study (HERS) and followed 871 HIV-infected women between 1993 and 2000 and reported five cervical cancers compared with no cancers amongst HIV non-infected women. Massad et al (2004) reported only one case of cervical cancer in the Women's Interagency HIV Study (WIHS) of 1661 HIV-infected and 8260 HIV non-infected women-years of follow-up.

Since the HIV epidemic has reached epidemic proportions in the developing world where invasive cervical cancer is also most prevalent, the relationship between HIV/AIDS and cervical cancer should be most apparent in these parts of the world. However, reports from African countries such as Kenya, Rwanda and Cote d'Ivore have not confirmed any positive link (Gichangi et al, 2002; Newton et al, 2001 and La Ruche et al, 1998). Although the prevalence of the HIV infection had increased threefold in Kenya over a period of a decade, there was no increase in the number of cervical cancers compared with that of other gynaecological cancers (Gichangi et al, 2002). Newton et al (2001) reported a link between HIV infection and non-Hodgkin's lymphoma but no increase in

invasive cervical cancer in Uganda. Parkin et al (1999) also did not find any increase in cases of cervical cancer in Uganda. It is postulated that the mortality amongst women from competing HIV/AIDS-related illnesses in the developing world is responsible for the short lifespan of women who do not live to an age to manifest with cervical cancers. Moodley et al (2001) reported that HIV-infected women presented at least 15 years earlier than HIV-negative women. Boccalon et al (1996) postulated that the HIV virus may influence the pathogenesis of HIV associated cervical pathology by molecular interaction between HIV and HPV genes as a result of up-regulation of the *E6/7* oncogenes by the HIV virus. Infection with both HIV and HPV may result in dysregulation of hormonal and cellular components of the immune system, leading to progression of the disease (Clark and Chetty, 2002). Studies have also reported high failure rates of preinvasive cervical lesions with standard therapy in HIV-infected women compared with HIV non-infected women (38-62% versus 15-18%, respectively) (Chirenje et al, 2003).

2.0 THE HUMAN PAPILLOMAVIRUS AND ITS LINK TO INTRAEPITHELIAL AND INVASIVE CERVICAL NEOPLASIA

2.1 HISTORICAL PERSPECTIVE

In 1907 the viral aetiology of common warts was established and first described by Giuseppe Ciuffo. It was only in the 1970s with the advent of molecular technology when HPV was studied in detail (Ciuffo, 1907; Meisles and Fortin, 1976). The transmission of the virus from man to man was determined by innoculation with a cell-free extract of wart tissue. Warts may infect several sites in the human body and can also infect several animal species. Rous and Beard (1935) reported the link between benign papillomas in rabbits and certain cancers. As molecular technology advanced it became possible to study the molecular link between papillomaviruses and cancer. Meisels and Morin (1981) further described the high prevalence of HPV and cervical dysplasia. De Villiers et al (1981) cloned the first genital human papillomaviruses. Zur Hausen (1982) also described the link between papillomaviruses and other lesions. HPV types 6 and 11 were described in association with benign condylomata acuminate. Subsequently, together with other investigators (Durst et al, 1983 and Boshart et al, 1984) HPV types 16 and 18 were cloned by zur Hausen and others from cervical cancers and the transcription of these HPV types in cervical cancers was confirmed. Since then many studies confirmed the link between papillomaviruses and cervical dysplasia or cancers. In 1988 zur Hausen reported that HPV types 16 and 18 were present in about 70% to 80% of high grade squamous intraepithelial and invasive cervical neoplasias. With the advent of

recombinant DNA technology and molecular cloning, many HPV types were discovered. To date more than 150 HPV types have been reported (zur Hausen, 2000).

2.2 EPIDEMIOLOGICAL EVIDENCE LINKING HPV AND CERVICAL NEOPLASIA

The highest rates of HPV infection is after the onset of sexual activity usually in the 20-30 year age interval. In most women HPV infection is self-limiting and as much as 90% of women will clear HPV infections over a period of time. The median duration of high-risk HPV infections is about one year and few months for low-risk infections (Schiffman and Kjaer, 2003). In populations at high risk of HPV infection, a second peak of HPV infection has been reported among postmenopausal women (Herrero et al, 2000). Of the women who develop persistent infections only 2-3% will develop cervical dysplasia (Clarke and Chetty, 2002). Results from epidemiological studies showed a consistent link between cervical cancers, pre-cancers and papillomaviruses. These studies included case series, case-controlled and cohort studies. Of the more than 100 HPV types described, about 40 have been shown to infect the genital tract (Woodman et al, 2007). Since some of the early studies utilized non-amplified DNA hybridization techniques the results were inconsistent. The point prevalence of HPV infection ranges from 14% to 35% (Ho et al, 1998). Munoz et al (1988) reported a wide range of 15% to 92% of the presence of HPV DNA in cervical tumour specimens. However, with the advent of the highly sensitive

polymerase chain reaction (PCR) technology, there emerged strong molecular evidence for the link between HPV and cervical neoplasias (Schiffman et al 1993; Munoz et al, 1992). The largest study of the prevalence of HPV in cervical cancers was the International Biological Study on Cervical Cancer which studied over 1000 women from many countries and utilized PCR technology (Bosch et al, 1995). This study reported a prevalence of 93%. A re-analysis of the specimens was performed and with the use of different primers the prevalence of HPV in cervical cancers increased to 99.7%. This high prevalence is consistent across the world even in areas of varying prevalence of cervical cancer and was demonstrated in both retrospective and prospective studies (Bosch, 2002).

Based on information pooled from 11 case-controlled studies (Munoz et al, 2003), it has been established that there are 15 high-risk HPV types which are oncogenic to the epithelium of the anogenital tract (16, 18, 26,31, 33, 35, 39, 45, 51, 52, 53,56, 58, 59, 66, 68, 73 and 82). Twelve types are regarded as low-risk HPV types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108). The oncogenicity of all other HPV types is unknown. HPV types 16 and 18 have been identified in about 70% of cervical carcinomas (Clifford, 2003). Across the world HPV types 16 and 18 predominate in cervical carcinomas (Clifford, 2005).

The sequence of events leading to the development of invasive cervical carcinomas was subsequently established. It became evident that HPV infection was a necessary factor followed by the development of intraepithelial lesions and then cervical carcinomas. Herrero et al (2000) demonstrated that HPV infection was acquired after sexual activity

in young women. The prevalence of HPV infection then declined after the age of 30 years. Smith et al (2008) reported the age-specific prevalence of HPV in a meta-analysis of 346 160 women. It was noticed that HPV prevalence decreased with increasing age from peak prevalence in women younger than 25 years of age. In women between 25 and 35 years, the HPV prevalence differed in different geographical regions of the world from 15% in Northern Europe to 20% in Africa. In older women, there were inconsistent trends with a decrease or plateau noticed in most studies. Low-grade cervical squamous intraepithelial lesions (LGSIL) were commonest around the age of 29 and were associated with many HPV types. Its prevalence then declined after the age of 30 years. Low-risk HPV infection is associated with transient HPV types, whereas high-risk HPV types are associated with more persistent infections (Franco et al, 1995). High-risk HPVs are also associated with a greater risk of progression from atypical cells of undetermined significance (ASCUS) to high grade lesions and greater duration of infection compared to low-risk HPVs (Schlecht et al, 2003). High-grade cervical squamous intraepithelial lesions (HGSIL) were noted commonly between the age of 30 and 40 years. High-risk HPVs notably types 16 and 18 were dominant in HGSIL lesions and cervical cancers. The odds ratios described were 320 for HGSIL and 710 for cervical carcinomas. Sun et al (1997) reported that HIV-infected women have a higher incidence of high-risk HPV types in low-grade squamous intraepithelial lesions (LGSIL). Although about 60% of low-grade intraepithelial lesions regress in HIV non-infected women, this regression rate decreases to about 27% in HIV-infected women (Petry et al, 1994; Maiman et al, 1993). Langerhans cells are the antigen-presenting cells of the cervix. Spinillo et al (1993)

reported a significant reduction in Langerhans cells in CIN lesions among HIV-infected women compared to a matched control group.

2.3 CLASSIFICATION AND STRUCTURE OF PAPILLOMAVIRUSES

Previously the Papillomaviruses belonged to the Papillomavirus genus and with the Polyomavirus genus, constituted the Papovaridae family. They are now grouped independently as the papillomavidae family and are unrelated to polyomaviruses and SV40. The size and genomic organization of the two genera are different. Animal papillomaviruses are species-specific and have a predilection for epithelia at specific sites. These viruses share structural and functional similarities, including the ability for proliferation and transformation of the host epithelium. They are associated with dysplastic and neoplastic processes. The HPVs exist in a number of types called genotypes since their classification is based on the nucleotide of which about 40 infect the anogenital epithelium.

The Papillomaviruses are classified according to the DNA sequence homology in certain genes, especially the *L1* gene, which codes for the viral capsid. This classification has been ratified by the International Committee on the Taxonomy of Viruses (de Villiers et al, 2004). For a new HPV type to be confirmed, the total DNA from the virus must be cloned and the DNA sequence must be obtained from the *E6*, *E7* and *L1* genes. The *L1*, *E6* and *E7* genes of any type should have less than 90% identity with any other known type. A subtype is established if new isolates have a homology between 90 – 98% with

any known type. A variant is established if a new isolate has more than 98% homology. Papillomaviruses which share a 60% homology in the L1 region are grouped together in the α and π genera. The α genus includes all high-risk HPVs, low-risk HPVs, whereas the β papillomaviruses include viruses associated with Epidermodysplasia verruciformis. The genera are further sub-divided into species e.g., α9 includes 16, 31, 33, 35, 52 and 58. Depending on their tropism, they are divided into mucosal and cutaneous types. The phylogenetic tree representing the family of papillomaviruses is represented in Figure 1.

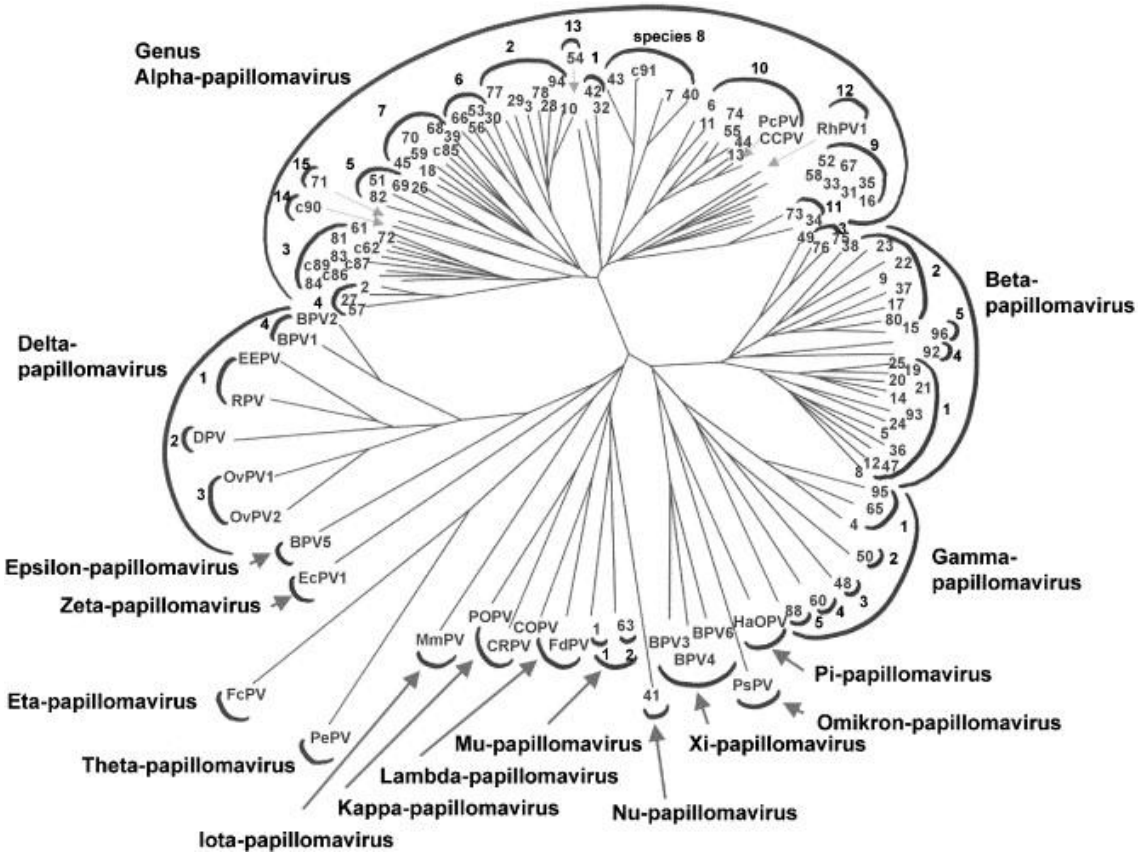


Figure 1: Phylogenetic tree of the HPV family of viruses

2.4 STRUCTURE OF THE HUMAN PAPILLOMAVIRUS

Human Papillomaviruses are small, double-stranded DNA viruses of about 55nm with an icosahedral protein capsid containing 72 capsomers. It has a circular genomic structure containing 7500 to 8000 base pairs.

The capsid has two structural proteins:

- The L1 protein which makes up 80% of the total viral protein with a molecular mass of 53 000 – 59000 daltons. This protein is the major capsid protein encoded by the *L1* gene.
- The L2 protein is a minor protein with a molecular weight of 70 000 daltons and is encoded by the *L2* gene.

2.5 THE HPV NUCLEIC ACID/GENOME

Within the capsid is the circular double-stranded supercoiled DNA genome of approximately 8 kilobases (Kb) in length with a molecular weight of 5×10^6 daltons. Only one strand of this genetic material serves as a template for DNA transcription or open reading frames (ORFs) (Cole et al, 1987). The genome is divided into early (E), late (L) and non-coding regions. The open reading frames are the coding regions and are classified as “early” or “late” depending on when gene function occurs in a specific time period in the life-cycle of the HPV infection. Early genes are expressed at the onset of the

infection and mediate specific gene functions which control viral DNA transcription, replication and cellular transformation. The E1 and E2 genes play a role in viral replication and maintenance of the genome. The linear representation of the HPV genome is represented in Figure 2.

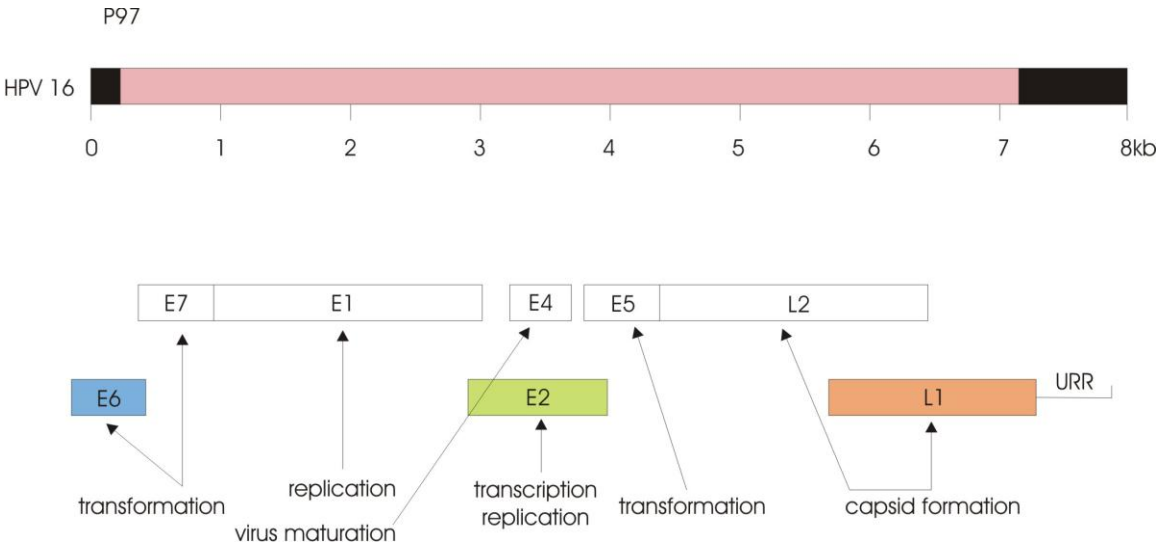


Figure 2: Linear arrangement of HPV 16 genome

The open reading frames (ORFs), E and L are preceded by a non-coding region, also referred to as the upstream regulatory region or long control region. Many early ORFs have been identified. The genome of the open reading frame (ORFs) is divided into:

2.5.1 ORFS WITH ONCOGENIC PROPERTIES

THE HPV E6 ONCOPROTEIN

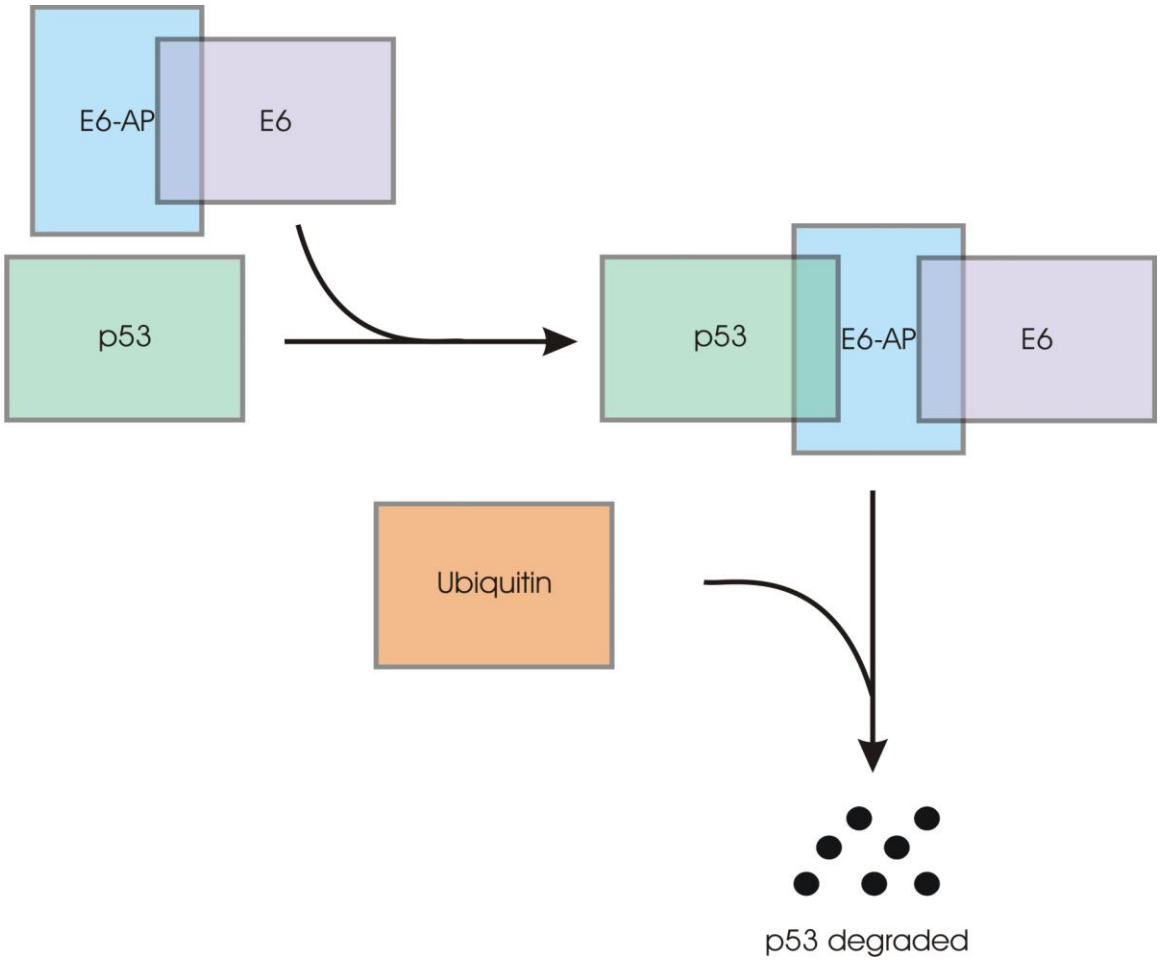
The E6 protein has a molecular weight of 16kD and is composed of 150 amino acids All papillomaviruses encode an E6 ORF downstream of the non-coding region. The E6 ORF encodes for a small protein of about 150 amino acids producing a product of 16-18kDa. Due to alternative splicing of the E6 transcripts, E6*I and E6*II proteins are produced. The full-length E6 protein has a zinc binding motif (cys-x-x-cys) which when bound to zinc ions is capable of binding to DNA. Co-operation with the E7 protein is necessary for its full oncogenic role. One of the first genes expressed during HPV infection is the E6 gene (Fehrmann & Laimins, 2003).

The high-risk HPVs are integrated into the host cell genome in cervical cancers. These genes therefore play a role in the initiation and progression of tumours. The *E6* gene of the high risk HPV virus deregulates the cell cycle via its interaction with the p53 tumour-

suppressor gene. The E6 protein product binds to the p53 gene product and forms a complex which then results in proteolysis of the p53 gene product (Scheffner et al, 1990).

The degradation occurs via an ubiquitin pathway that results in the reduction of the half-life of the p53 from 3 hours to 20 minutes. The shortening of the half-life of the p53 reduces its function. The first protein which was shown to interact with E6 is E6-associated protein (E6AP), which is an E3 ubiquitin ligase. The E6 degradation of p53 requires the cellular protein referred to as E6AP, which in combination with E6 serves as an E3 ubiquitin ligase (Scheffner et al, 1993; Huibregste et al, 1993). A separate process independent of the E6AP activity is the down regulation of p53 transcriptional control by high-risk E6 proteins. This occurs via a p53 co-activator CBP/p300 by E6. CBP/p300 regulates various signal-modulating events and plays a role in cell cycle inhibition and differentiation (Giles et al, 1998). The E6-E6AP has also been reported to promote degradation of the src family kinase Blk (Oda et al, 1999). These tyrosine kinases are important in signal transduction in proliferating cells and it is thought that E6 de-regulates these pathways affecting cell growth. Shai et al (**2008)** recently documented that K14Crep53 mice treated with oestrogen developed cervical cancer due to inactivation of the *p53* gene in he presence also of the HPV *E6* and *E7* oncogenes. The authors concluded that in hormone-responsive tissues, *p53* inactivation in association with HPV oncogenes is necessary for carcinogenesis, including mammary tumours.

Figure 3: HPV 16 *E6* oncogene and *p53* gene degradation



Many other protein-protein interactions of E6 are described e.g., ERC 55 and paxillin may affect vital areas of cellular function, the disruption of which may promote malignant transformation (Chen et al, 1995; Tong and Howley, 1997). Rapid tumour

formation has been shown to occur in p53 null mice, providing evidence for its tumour suppressor role. Key host regulatory factors such as cyclins, cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (CDIs) (Southern et al, 1998; Thomas et al, 1998). The cell cycle arrest at the G1/S point occurs as a result of cyclin/kinase inhibition by p21 CDI. Loss of functional p53 due to E6 binding results in failure of G1/S arrest (Southern et al, 2000). The E6 gene product is also capable of activating the enzyme telomerase which is responsible for counteracting the shortening of the chromosome's telomeres. This natural shortening occurs in the process of aging and the E6 interaction prolongs the lifespan of the affected cell (Mantovani & Banks, 2001). The E6 protein also modulates immune function by regulating the transcription of genes involved in innate immunity. High-risk E6 interacts with two proteins involved in the innate response to viral infections: interferon regulatory factor-3 (IFR-3) and Toll-like receptor 9 (TLR9) (Ronco et al, 1998; Hasan et al, 2007). Viral infection or dsRNA activates IRF-3 leading to the transcription of interferon-β (Hiscott, 2007). Viral or bacterial dsDNA activates TLR9 which induces cytokine production involved in cellular defence (Muller et al, 2008). Loss of function of IRF-3 and TLR9 has been demonstrated in cell lines indicating that such HPV 16 E6 expression is involved in HPV-related cervical carcinogenesis. Both E6 and E7 can independently immortalize human cells.

THE HPV E7 ONCOPROTEIN

The E7 ORF encodes for a small protein of about 100 amino acids which weigh about 10kDa and is well conserved among different HPVs. The E7 oncogene is a key oncogene which exerts its activity by binding to the cellular proteins of the retinoblastoma (*pRB*) gene family. The *pRB* gene is regarded as a tumour-suppressor gene. The pRB protein is a phosphoprotein which in the phosphorylated state is required for the limitation of cell proliferation and suppresses the oncogenic properties of different HPV types (Brookstein & Lee, 1991). In conjunction with the E2F group of transcription factors, the *pRB* exerts control over cell replication (Boyer et al, 1996). The hypophosphorylation of the *pRB* results in uncontrolled release of transcriptionally active E2F, cell cycle progression into the S-phase of the cell cycle and loss of cell cycle-dependent regulation of E2F responsive genes (Chellapan et al, 1992; Weinberg 1995). Although the E7 protein is capable of transformation and immortalization in vitro, it requires E6 for HPV transformation and immortalization (Barbosa and Schlegel, 1989). The E7 protein interacts with various proteins involved in the regulation of cell-growth, especially the transition from G1 to S-phase of mitosis. The E7 protein de-regulates the cell cycle leading to increased cell proliferation, immortalization and transformation. This is achieved by the interaction of E7 with the proteins of the retinoblastoma tumour suppressor family (Rb, p130, histone deacetylases (HDAC), AP-1 transcription factors, TATA box proteins, cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors). The role ole of HPV 16 *E7* oncogene and *pRb* tumour-suppressor gene in cell-cycle regulation (infected cell and uninfected cell) is illustrated in Figures 4a and 4b.

Figure 4a: Role of HPV 16 *E7* oncogene and *pRb* tumour-suppressor gene in cell-cycle regulation (Uninfected cell)

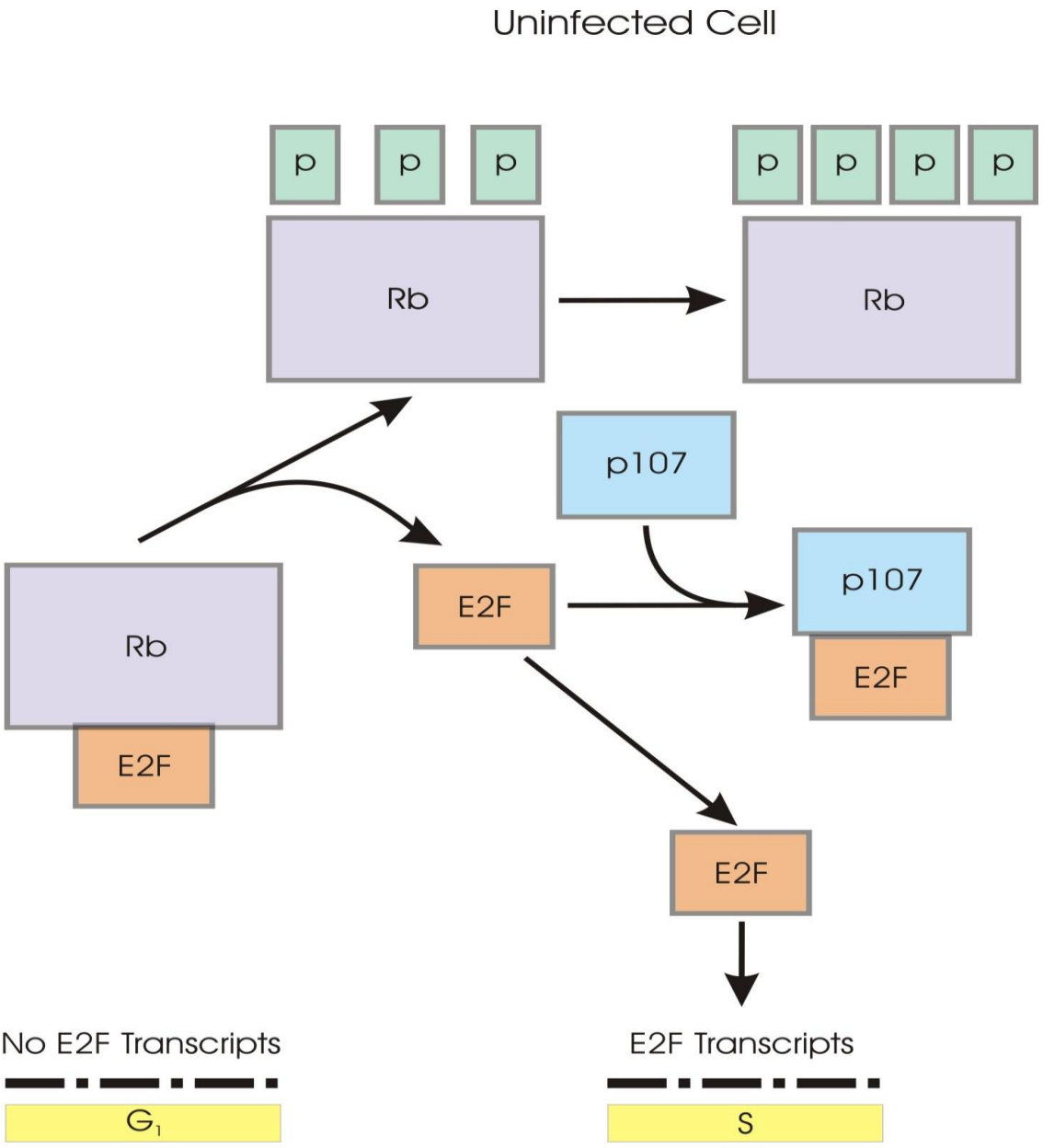
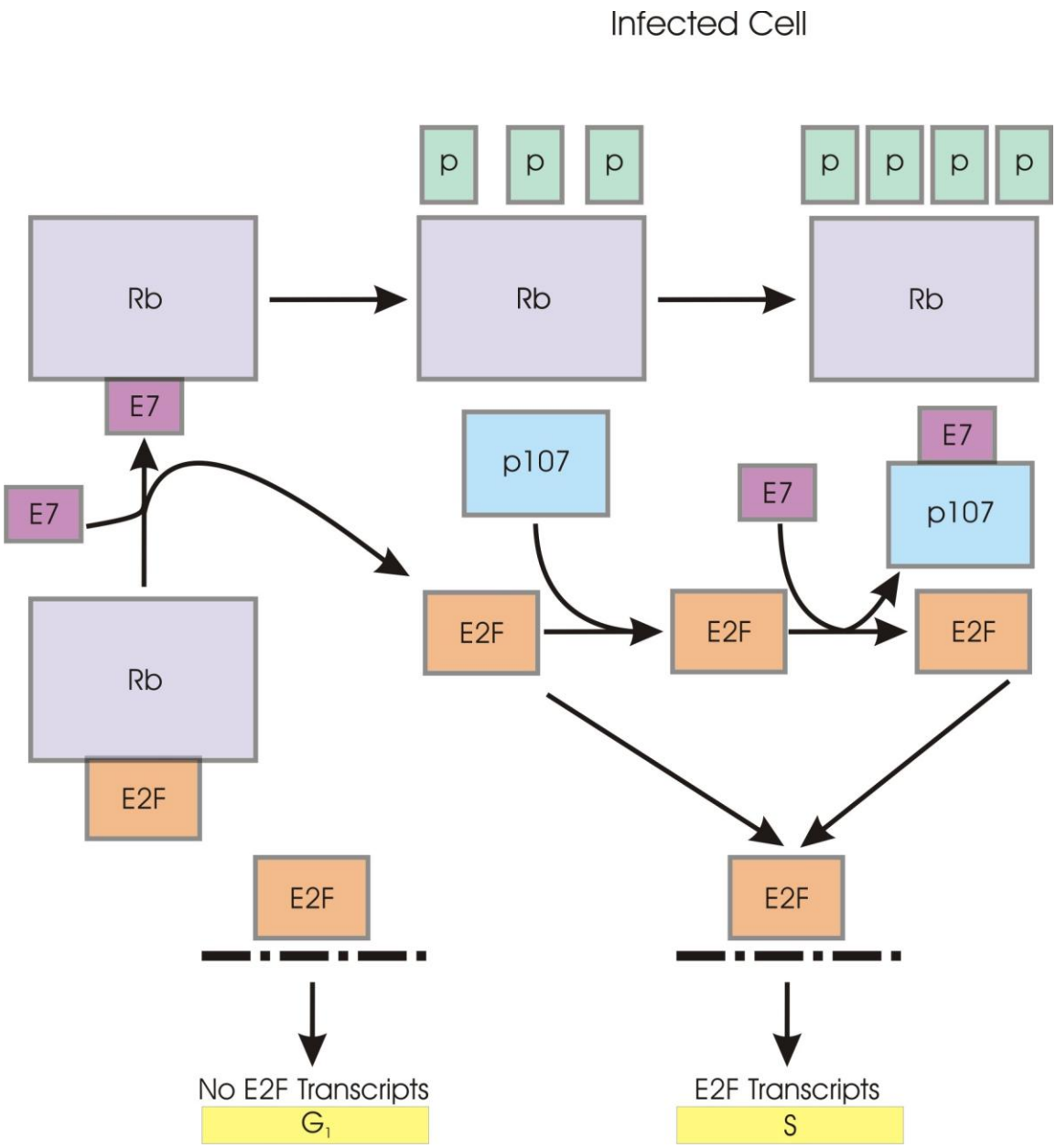


Figure 4b: Role of HPV 16 *E7* oncogene and *pRb* tumour-suppressor gene in cell-cycle regulation (Infected cell)



When the cell progresses from G0 to S-phase, Rb becomes hyperphosphorylated by G1 cyclin-CDKs, releasing E2F, which in turn activates genes involved in DNA synthesis and cell-cycle progression (Dyson, 1998). Binding of E7 to hypophosphorylated Rb

induces cells to enter premature S phase by disrupting Rb-E2F complexes (Patrick et al, 1994). The E7 gene product can also overcome G1/S cell cycle arrest induced by either p21 or p27 by binding to both of these proteins or with cyclins A and E (Demers et al, 1996; Hickman et al, 1994; Morozov et al, 1997). In contrast to the E6 gene product of low-risk HPVs which do not bind *p53*, the E7 gene product of the low-risk HPVs bind to p53 at a reduced affinity (Elbel et al, 1997). Human papillomaviruses play a role in the initiation of oncogenic events. Transcripts encoding for the E6 and E7 are initiated in the Upstream Regulatory Region (URR) of the viral genome.

Transcriptional activity of both E6 and E7 is under control of p97 which is suppressed mainly by the viral E2 product (Romanczuk et al, 1990). Transcription of the HPV 16 E6/E7 ORFs produces three different splice products due to alternate splicing using a common donor site at nucleotide 226 and two different splice acceptor sites at nucleotides 409 and 526. The full length transcript encodes for the functional E6 protein, whilst the E7 protein is most likely encoded by the E6*1 and E6*11 splice products (Smotkin & Wettstein, 1986; Smotkin et al,1989; Cornelissen et al, 1990). The major splice product is the E6*1 (Smotkin et al, 1989). Using specific primer pairs designated S3 and S4 in the reverse transcriptase-polymerase chain reaction (RT-PCR) to detect these splice products yields the following fragments: full-length product consisting of 525 base pairs (E6), E6*1 consisting of 343 base pairs and E6*11 consisting of 226 base pairs. Further testing using the S1/S2 primers in a nested system (nRT-PCR) with the products of the S3/S4 primers yield the following products: an E6 full-length fragment with 395 base pairs; E6 *1 fragment consisting of 213 base pairs and E6*1 consisting of

95 base pairs (Sotlar et al, 1998). It is thought that the detection of the E6*1 and E6*11 splice products are unequivocal proof of HPV 16 E6/E7 oncogene transcription (Cornelissen et al, 1990). The presence of the E6*1 spliced product is thought to correlate with lesion severity from cervical scrapes (Sotlar et al, 1998).

The amplification products of the HPV 16 genome in relation to the spliced products produced with the S3/4 and S1/2 primers and their respective positions is illustrated in Figure 5.

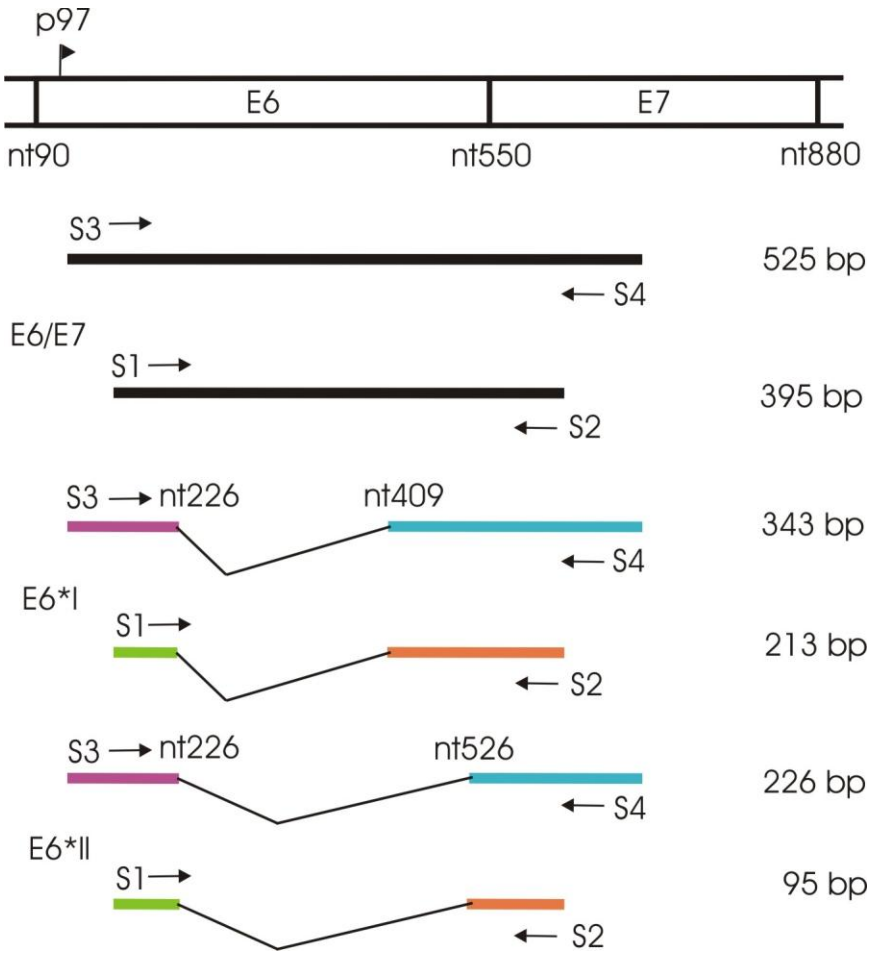


Figure 5: HPV 16 amplified products. Spliced products produced with the S3/4 and S1/2 primers and their positions

In vitro experiments have demonstrated that HPV16 E7 targets insulin-like growth factor binding protein-3 (IGFBP-3) which regulates the mitogenic activity of IGF-1. The IGFBP-3 is a product of the p53-inducible gene which blocks cell proliferation and induces apoptosis (Zwerschke et al, 2000). The induction of apoptosis by the IGFBP-3 is blocked by HPV E7 which facilitates proteolytic cleavage of IGFBP-3.

The E6 and E7 genes play a vital role in cervical cancer genesis as is evidenced by the constant observation that in HGSIL and cervical cancers, the HPV genome is integrated into host chromosomes (Daniel et al, 1995). The E6 and E7 genes are always retained and the proteins expressed (Cone et al, 1992). Both the E6 and E7 transcripts are expressed in low levels in basal cells with an increase in expression in the more differentiated upper cell layers of the epithelium. In high grade lesions and invasive cancers the E6 and E7 transcripts are expressed in all layers of the epithelium (Stoler et al, 1992). The pathological pathway of HPV infections depend on the physical state of HPV DNA. High-risk HPV are more likely to integrate within the human genome, whereas low-risk HPVs are usually maintained as extrachromosomal circular episomes (Arends et al, 1998).

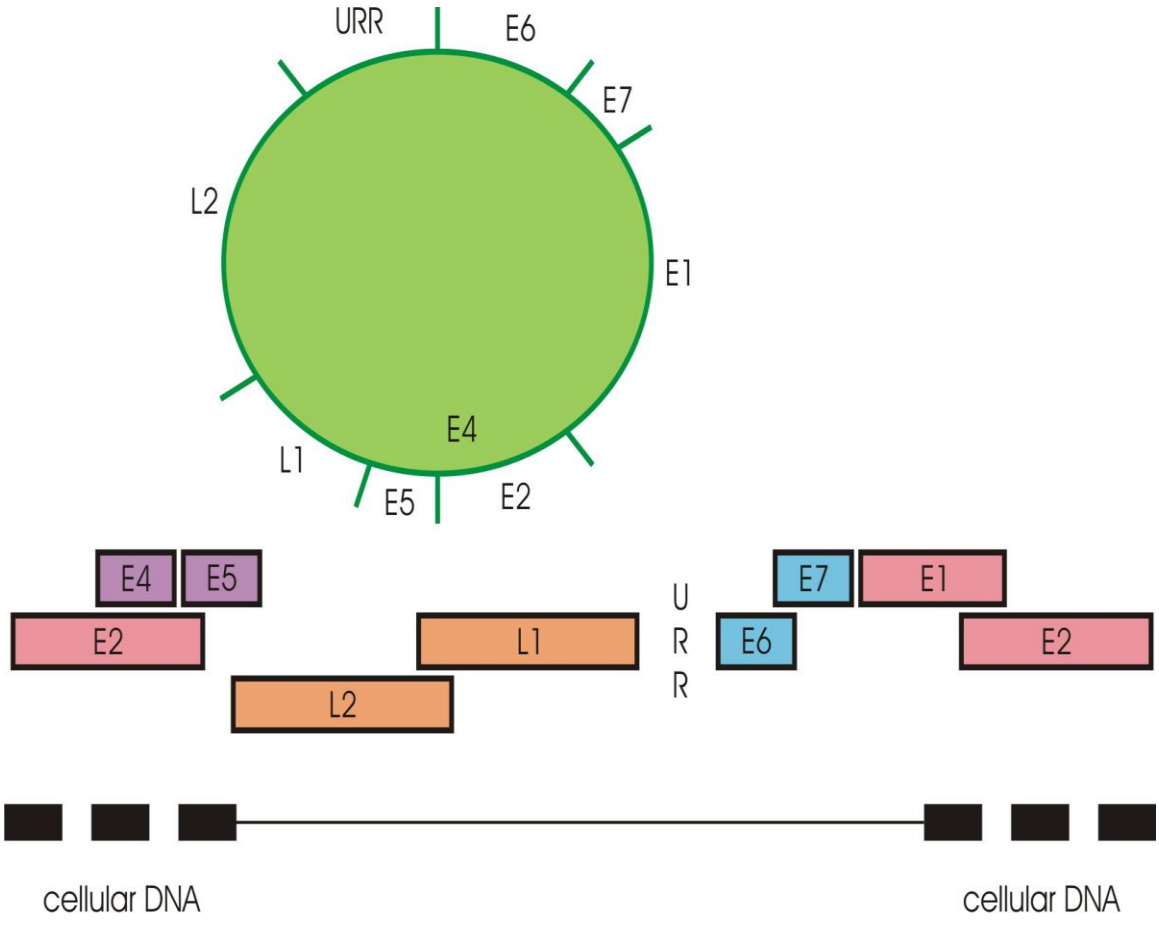
ROLE OF VIRAL INTEGRATION IN TUMOUR GENESIS

There are two forms of the HPV with regards to its physical state: the episomal form and the integrated form. The virus initiates infection as an episome with low DNA copy number in the basal cells. High copy number is associated with replication. With differentiation and viral replication, high viral copy numbers are found in the superficial

epidermis (Bedell et al, 1991). In precursor lesions of the cervix, the HPV exists as an episomal form. However, in invasive cancers HPV integration into the host genome has been reported (Matsukura et al, 1989) (Figure 6).

Viral integration may take place at various sites such as chromosomal break points, cellular oncogenes or fragile sites. The HPV viruses seem capable of recognizing specific DNA sequences in the genome and integrate in the human DNA, making surrounding areas of the genome unstable and allow genes in these regions access to mutagenic agents. Although integration may be random, disruption of the cellular HPV is consistently within the E1 or E2 ORF, resulting in loss of one or both genes (Choo et al, 1987). During this integration process, the URR and E6 and E7 transforming genes remain conserved/intact with increased transcription of these genes reflective of integration and loss of E2. The replication cycle of HPV is depicted in Figure 6.

Figure 6: A linear representation of the genome of HPV 16 E1 and E2 ORF following HPV integration



However, some HPV-associated cancers contain only episomal forms, whereas other cancers contain a mixture of episomal and integrated forms (Kristiansen et al, 1994; Matsukura et al, 1989). Transcription of the episomal form can regulate HPV function of the integrated virus disrupted in the E1 and E2 genes. From in vitro studies it has been demonstrated that 62% of tumours have integrated HPV, 16% have episomal forms and 22% have both episomal and integrated forms. Almost all tumours with episomal or mixed forms with HPV 16 DNA had intact E1 and E2 genes. In contrast, 50% of tumours

with integrated HPV 16 DNA had disrupted E2 significantly associated with decreased disease-free survival rate (17% versus 70.4%). Szostek et al (2008) recently reported on the physical state of HPV 16 among 42 women with SIL and 23 women with invasive cervical cancer. In the cancer group, the free episomal viral genome was not detected. Twenty six percent of samples from this group contained a mixture of the episomal and integrated forms of viral DNA. In women with SIL, the free episomal form was predominant.

2.5.2 HPV REGULATORY PROTEINS

THE E1 AND E2 PROTEINS

Viral HPV DNA is integrated into the host cell genome (Cullen et al, 1991). The E1 nucleotide gene sequence is a highly conserved region amongst various HPV types. The E1 ORF codes for many proteins of molecular weights ranging from 68 to 85kDa. The E1 and E2 genes play a role in viral replication and maintenance of the genome. The E1 gene has helicase and adenosine triphosphatase (ATPase) activity which catalyses the unwinding of the DNA structure and also brings the DNA polymerase to the origin of the replication where E1 is complexed with the E2 protein to initiate replication (Ustav et al, 1998). The E1 binding site is located between two A+T-rich sequences, which demonstrate variability in length and little sequence conservation between HPV types. These sequences are in between three binding sites for the E2 protein whose purpose is to stabilize the binding of E1 and promote viral DNA replication (Wilson et al, 2002).

The E2 ORF codes for proteins which are necessary for viral DNA replication and regulation of transcription. It therefore acts as a transcriptional regulator by binding to a specific DNA sequence (ACCGNNNNNNCGGT) in the viral URR which constitutes the E2 binding site (Dostatni et al, 1988; McBride et al, 1989). The disruption of the E1 and E2 genes results in de-regulated expression of the viral E6 and E7 (Munger & Phelps, 1993). This is achieved by preventing the formation of the complex of transcription at the p97 promoter. Increase in E6 and E7 expression produces HPV transformed cells which are less likely to undergo programmed cell death/apoptosis (Sanchez-Perez et al, 1997). The E2 proteins modulate the viral enhancer and the E6 promoter. A separate E2 protein comprising a fusion of the E8 ORF with part of the E2 protein has been reported by Zobel et al, 2003. This complex is able to repress transcription and DNA replication and therefore plays a role in maintenance of the latent state observed in the basal epithelial cells.

THE E4 ORF

The exact role of the E4 is unknown. The E4 ORF resides in the early region of the genome, but is expressed in the late stage of the cell cycle. The E4 ORF encodes a protein localized in the cytoplasm in cutaneous warts (Breitburd et al, 1987). It is postulated that the E4 protein may play a role in virion assembly (Doorbar et al, 1998). The E4 ORF in conjunction with kearatin may facilitate collapse of the cytokeratin

network and viral release or may play a role in G2 arrest and HPV DNA replication. The E4 protein is regarded as a 'late' protein localized to the upper layers of the epithelium.

THE E 5 ORF

The E5 gene product is a small protein of about 10kDa localized mainly within the intracellular compartments such as Golgi apparatus. Its main function is thought to be mitogenic stimulation of cells by interacting with signal transduction pathways. The E5 proteins of HPV types 6 and 16 increase the proliferative capacity of human keratinocytes via interaction with epidermal growth factor receptor (EGFR) resulting in stimulation of DNA synthesis. In a recent study of the role of the E5 gene using immortalized human keratinocytes (HaCaT cells), it was demonstrated that E5 was necessary for the formation of bi-nucleated cells which is a common precursor of precancerous lesions (Hu et al, 2008). Co-expression of HPV 16 E6/E7 enhanced the proliferative capacity of the E5-induced bi-nucleated cells.

2.5.3 CAPSID ANTIGEN GENES

The mature HPV particle has an icosahedral outer capsid coat composed of two proteins: the L1 protein and the L2 protein.

The L1 protein is regarded as a major protein and comprises 80% of the total viral protein. It has a molecular mass of 53 000-59 000 kDa. The L2 protein is a minor component with a molecular mass of 70 000 kDa. *In vitro* studies have shown that there

is cross reactivity between the L1 regions of HPV 6, 16 and BPV. In contrast there is less reactivity in the L2 region. Part of the L2 region, namely 210 amino acids from the N-terminus and 30 amino acids from the C-terminus, are conserved. The L1 and L2 proteins have a nuclear target signal and move from the cytoplasm to the nucleus where capsomeres are synthesized and virions are assembled (Zhou et al, 1990). The L1 protein serves as a major target for vaccine research and has resulted in the successful development of two commercially available vaccines (Gardasil ® & Cervarix®). These two vaccines are based on the development of virus-like particles (VLP) against the HPV 16 and 18 L1 proteins.

THE UPSTREAM REGULATORY REGION (URR)

The URR is also known as the long control region or the non-coding region of HPVs. It is present between the early and late regions. This region contains regulatory sequences and the origin of DNA replication, two transcriptional start sites and promoter elements for DNA polymerase II. This element has variation in length amongst the papillomaviruses. The URR also has enhancer elements which mediate transactivation of transcription and also contains glucocorticoid receptor complex elements. The promoter site viz. p97 transcription initiator of HPV 16 is located in the URR. In experiments designed to analyse the activity of isolated regulatory sequences, it has been shown that glucocorticoid hormones are significant activators of the promoter (von Knebel Doeberitz et al, 1991). Binding of progesterone and glucocorticoids to this region increases E6 and E7 transcription (Crook et al, 1991). The URR also contains keratinocyte-enhancer

elements by which HPV 16 are thought to be tropic for squamous epithelia (Cripe et al, 1987). The HPV type 16 enhancer localized to a 232 bp fragment is epithelial-specific. This fragment contains binding sites for AP-1 (transcription factor-binding site), nuclear factor 1 (NF-1) and transcriptional enhancer factors (TEF-1 and TEF-2) (Chong et al, 1991). The AP-1 factor confers cell-type specific transcription of HPV genes.

2.6 REPLICATION CYCLE OF THE HUMAN PAPILLOMAVIRUS

The infectious cycle of the human papillomavirus parallels that of the target cell which in the case of the cervix is the squame. Papillomaviruses replicate and assemble exclusively within the nucleus. Viral growth is dependent upon a cycle of keratinocyte differentiation. The HPV enters the epithelium probably via microabrasions in the epithelium and infects the basal cells. HPV genomes are established as extrachromosomal elements within the nucleus and replicate in synchrony with cellular DNA. After cell division, the daughter cells migrate towards the supra-basal compartment, within which the uninfected keratinocytes initiate terminal differentiation. The HPV-infected cells enter the S-phase of the cell cycle with resultant amplification of viral replication products (Munoz et al, 2006). In the lower layers of the epithelium, cell proliferation and episomal maintenance occurs. Early stages of viral growth include amplification of viral copy number from 1-10 to 50-100 virus episomes/cell (Middleton et al, 2003). Thereafter the virus and cell replicate without the amplification of viral number. The HPV encode for only one DNA replication enzyme, E1 and is otherwise fully dependent on host

cellular machinery. However, this replication can only occur in mitotically active cells. To overcome this problem, the HPV reactivates cellular DNA synthesis in non-dividing cells, inhibit apoptosis and inhibit the program of cellular differentiation, allowing for viral replication. There is strict control of the HPV E6 and E7 oncogenes with very little E6/E7 levels detectable. Viral gene expression is limited to the keratinocyte or cells which have the capability for squamous maturation. High levels of viral protein expression (E6/E7 and late genes) occurs only in the upper layers of the stratum spinosum and granulosum of the squamous epithelium (Middleton et al, 2003). In the upper layers, viral gene expression is up-regulated resulting in the release of thousands of viral episomes. Here, the late viral proteins L1, L2 and E4 are synthesized in conjunction with viral assembly. Disintegration of the epithelial cells as a result of natural turnover facilitates release of infectious virions (Fehrmann & Laimins, 2003). The time from viral infection to release is about three weeks which is about the time taken for differentiation of the basal cell.

2.7 IMMUNOLOGY OF HPV INFECTIONS

Not all women with HPV infection will develop disease. It has been noted that persistent HPV infection is necessary for the development of neoplastic cervical lesions (Chakrabarti and Krishna, 2003). Both the innate and adaptive immune systems are required for the defence against HPV infections. The innate system comprises phagocytes, cytokines and complement. The adaptive system comprises antibodies and

cytotoxic effector cells (T and B cells). T-cells are capable of recognizing antigens processed into short peptides and bound to the major histocompatibility complex (MHC) proteins and are presented as membrane-bound receptor complexes on the surface of cells. The two major subsets of T-cells are the CD8 and CD4 cells. CD8 T-cells recognize antigens presented by the class II MHC and CD4 cells recognize antigens presented by the class I MHC. The activation of the CD4 cells results in the production of cytokines. There is reduction in expression of HLA class I molecules in cervical cancer associated with a decrease in CD8 T cells (Hilders, et al 1994). Genital warts which do not regress do not have immune cells at the site of infection. The first line of defence is the innate system which interfaces with the pathogen and destroys it without the development of any memory. However, the innate system activates the adaptive system which produces effector cells to maintain memory of the pathogen. Whilst antibodies clear the pathogen, the cell mediated responses clear the viral-infected cells. Unlike many other micro-organisms which are destroyed by the human body before disease manifests, the HPV is adept at evading the host immune mechanisms. At the onset of initial infection, mucosotropic virions remain at the site of infection and do not induce lysis of the infected epithelial cells and hence there is no inflammation. The antigenic capsid proteins are not expressed until differentiation has reached the superficial layers of the epithelium. The innate immune system is down-regulated by interactions of the E6 and E7 early HPV genes and the innate immune system, in respect of interferon and nuclear factor-κß signals (Nees et al, 2001; Kanodia et al, 2007). Type 1 interferons, namely IFN-α and IFN-β have anti-viral, anti-proliferative, anti-angiogenic and immunostimulatory functions which serve as a bridge between the innate and adaptive immunity (Le Bon and

Tough, 2002). There is poor recruitment of effector cells to the cervical epithelium. Systemic viraemia does not occur. HPV-infected keratinocytes are also resistant to lysis by natural killer cells although they can be destroyed by cytokine-activated NK cells and macrophages. HPV infection is associated with the failure of antigen presentation to the MHC I and II complexes. Only about fifty percent of women exposed to HPV infection will develop an immune response (Stanley, 2003). Failure to induce a satisfactory immune response is due to deficient activation of the innate immunity and ineffective priming of the adaptive mechanisms resulting in viral persistence. Thus the basic mechanisms to trigger the immune response in cervical epithelium are non-existent (Stanley, 2006).

Human papillomavirus infections are predominantly an intraepithelial phenomenon and therefore the Langerhans cells, which are the antigen-presenting cells of the squamous epithelium, should be able to detect the HPV virus. Antigen presenting cells have special properties to prime tumour-specific T cells in the T cell-dependent areas of the lymph nodes (Paglia and Guzman, 1998). It seems as if the Langerhans cells are not activated by the viral capsids. This is in contrast to the stromal dendritic cells which are activated by viral capsids. Since the HPV virus remains within the epithelium, this activation does not occur. Although the innate immune response is immediate, antibody conversion against high-risk HPVs takes almost 6 months to 1 year. Antibody levels, however, remain stable over many years and it has been demonstrated that innoculation with viral like particles result in a rapid IgM and IgA antibody response followed later by appearance of stable IgG antibodies which can be detected in cervical mucus.

Antibody production prevents the spread of infection and repeat infections (Stanley, 2001). In natural infections, cell-mediated immune responses result in clearance of genital warts and intraepithelial lesions (Coleman et al, 1994). This occurs with sero-conversion and the formation of antibodies to major capsid proteins. However, in humans the seroconversion is either low or does not occur at all. The majority of women clear HPV infection and this clearance is dependent on whether the HPV is of the high-risk or low-risk type. High-risk HPV 16 takes about 8-14 months to clear (Giuliano et al, 2002). In 10% of women, HPV infection is not cleared and persistent infection develops. Of this 10%, about 2-3% of women develop SIL and progression to invasive cervical cancer (Stanley, 2008). The development of such neoplastic cervical lesions is associated with the expression of the HPV E6 and E7 proteins at cellular level and resistance to innate and adaptive immunity. Although interferon- β is capable of clearing episomal HPV, it cannot clear integrated HPV (Pett et al, 2006). Immune defence mechanisms against HPV E2 and E6 are deficient in cervical neoplastic lesions even if cytotoxic T-cells are produced (Kobayashi et al, 2004).

In summary: the HPV virus evades the innate immune system and delays the activation of the adaptive immune system. The dendritic cells are exposed to low levels of viral proteins for a lengthy period of time resulting in lack of local defence mechanisms and the establishment of viral infection. The establishment of persistent HPV infection results in the expression of the E6 and E7 proteins and the lack of a cell-mediated response favouring the progression to SIL and invasive cancers.