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IMMUNOLOGICAL RESPONSES IN GENITAL HPV INFECTIONS

AND

ETIOLOGY OF CERVICAL CANCER

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SUMMARY

Cervical cancer is one of the most common forms of cancers in women. Every year approximately 450,000 women are diagnosed worldwide and 200,000 die. The sexually transmitted oncogenic human papillomavirus (HPV) types are established as the major etiological agents of cancer of the cervix. Eradication of cervical cancer by vaccination against HPV has therefore become a promising approach. However, there are more than 100 types of HPV and at least 10 of these are oncogenic. Knowledge of the quantitative importance of the different HPV types in carcinogenesis is needed. As only a small fraction of ever-infected women go on to develop cancer, the role of co-factors to HPV in cervical cancer also needs to be established for evaluation of different preventive actions and validation of intermediate endpoints for such interventions. Many co-factors have been suggested such as: HPV immune response determinants, other sexually transmitted and environmental agents as well as susceptibility genes. For development, implementation and evaluation of both prophylactic and therapeutic agents against HPV and cervical cancer, knowledge about HPV-immunity and how various co-factors influence HPV persistence and carcinogenesis is required.

This thesis has investigated immunological responses and risk factors for control of HPV infection, for development of precursor stages of cancer and for invasive cervical cancer. Three studies are molecular epidemiological and longitudinal cohort studies of healthy women participating in biobank cohorts or clinical trials. The fourth study has analysed HPV immunity in a mouse model.

In **paper I**, Nordic serumbanks were used to study risk factors for invasive cervical cancer in a nested case-control study. In total, 543 prospectively occurring cases and 2675 matched controls, were identified. The serum samples were analysed for antibodies against HPV 6, 16 and 18, *Chlamydia trachomatis* and Herpes simplex virus type 2. The study found evidence of an etiological role of HPV 16 and 18 in the development of cervical cancer and suggests a co-factor role of *Chlamydia trachomatis*.

Paper II investigated the ability of a DNA vaccine to induce immunity against the HPV 16 major capsid protein L1 in mice. Although, it is well known that HPV infection can be prevented with so called virus-like particles (VLPs), vaccination with DNA coding for these particles could have practical advantages compared to VLPs. Vaccination with a modified HPV16 L1 plasmid did induce both neutralising antibodies and cell-mediated immune responses against HPV 16 in mice.

Paper III studied the concentrations of two cytokines (CXCL8 and IFN-γ) in cervical secretions in a cohort study of HPV16 DNA positive women who on follow-up either had clearance or a persistent HPV 16 infection. CXCL8 is a chemokine that attracts various immune cells in inflammation and IFN-γ is a cytokine that activates immune cells important for viral clearance. The women who cleared their infection had higher levels of both cytokines compared to women who were persistently infected.

In **paper IV**, women participating in a population-based biobank cohort who either did or did not develop precursor stages of cervical cancer (CIN) on follow-up were screened for 14 killer immunoglobulin-like receptor (KIR) genes. KIRs are expressed on natural killer (NK) cells and they can distinguish a normal cell from an abnormal. By doing so NK cells will spare healthy cells while killing the abnormal cell. More than 70 KIR genotypes were identified. One of which was associated with increased risk of CIN.

LIST OF PUBLICATIONS

I Lisen Arnheim, Tapio Luostarinen, Kristin Olsson, Steinar Thoresen, Helga Ögmundsdottir, Laufey Tryggvadóttir, Fredrik Wiklund, Gry B. Skare, Carina Eklund, Kia Sjölin, Egil Jellum, Pentti Koskela, Göran Wadell, Matti Lehtinen, Joakim Dillner: Etiology of cervical cancer. *Manuscript.*

II Erik Rollman*, **Lisen Arnheim***, Brian Collier, Daniel Öberg, Håkan Hall, Jonas Klingström, Joakim Dillner, Diana V. Pastrana, Chris B. Buck, Jorma Hinkula, Britta Wahren, Stefan Schwartz: HPV-16 L1 genes with inactivated negative RNA elements induce potent immune responses. *Virology* 2004, 322, 182-189. * These authors contributed equally to this work.

III Lisen Arnheim, the Swedescreen Steering group and Joakim Dillner: CXCL8 and INF-γ concentration levels in women with persistent and cleared Human papillomavirus type 16 infection: *Submitted for publication.*

IV Lisen Arnheim, Joakim Dillner, Carani B. Sanjeevi: A population-based cohort study of KIR genes and genotypes in relation to cervical intraepithelial neoplasia. *Tissue Antigens*, 2005, 65, 252-259

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Ab	Antibody
AC	Adenocarcinoma
Ag	Antigen
APC	Antigen presenting cell
ASC	Adenosquamous carcinoma
ASCUS	Atypical Squamous Cells of Undetermined Significance
BPV	Bovine papillomavirus
CI	Confidence Interval
CIN	Cervical intraepithelial neoplasia
CRPV	Cotton-tail rabbit papillomavirus
CTL	Cytotoxic T lymphocyte
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked immunosorbent assay
HLA	Human Leukocyte Antigen
HPV	Human papillomavirus
HSV	Herpes simplex virus
ICC	Invasive cervical cancer
IFN	Interferon
IL	Interleukin
KIR	Killer immunoglobulin-like receptor
LCR	Long control region
LSIL/HSIL	Low/High grade squamous intraepithelial lesion
MHC	Major Histocompatibility Complex
NK	Natural Killer
OC	Oral contraceptives
OR	Odds ratio
ORF	Open reading frame
PCR	Polymerase chain reaction
Rb	Retinablastoma
RNA	Ribonucleic Acid
SCC	Squamous Cell Carcinoma
STI	Sexually transmitted infection
VIN	Vulvar intraepithelial neoplasia
VLP	Virus-like particle

CERVICAL CANCER

EPIDEMIOLOGY

Accounting for 10% of all cancers in women, cancer of the cervix is the third most common malignancy in women globally, following breast cancer and colon cancer (Ferlay, Bray et al. 2001). Approximately 470,000 women were diagnosed with cervical cancer in the year 2000 and 233,000 mortalities were reported (Parkin, Bray et al. 2001). Most cases are found in the developing world and incidence peaks in the mid- to late-reproductive years (Garland 2002). Incidence rates and mortality rates are different in various parts of the world. This is probably due to different risk exposures, screening programs, therapeutic possibilities and report frequency. In the table below a small selection of cervical cancer incidence and mortality worldwide is presented (Globocan 2002):

Country	Incidence rate (100,000/year)	Mortality rate (100,000/year)
Sweden	9	2,9
Latvia	9	6,6
Haiti	90	53,5
Syrian Arab Rep.	3	1,5

Table 1. Cervical cancer incidence and mortality in four different countries.

To be able to control the spread and prevent this type of cancer understanding its etiology is of great importance. Cervical cancer is related to sexually transmitted infections where human papillomavirus (HPV) is its major cause.

ANATOMY AND PATHOLOGY OF THE CERVIX

The cervix is located in the distal part of the uterus and consists of two parts. First, the ectocervix that projects into the vagina and is covered with non-keratinizing stratified squamous epithelia. Second, there is the endocervix or the cervical canal that is lined by a single layer of mucin-producing columnar epithelium. The border where squamous and columnar epithelia meet is called the squamoucolumnar junction. The location of this junction changes with the hormonal status of the woman. It migrates onto

the convexity of the ectocervix and then back into the endocervical canal. This junction is the location of most epithelial diseases that occur in the cervix. During a woman's lifetime the columnar epithelia is retracting into the cervical canal and replaced by new squamous epithelia where columnar epithelia used to be (the transformation zone, T-zone) (Danjanov and Linder 1996).

Precursor stages of cervical carcinoma

In some women the cell replacement in the T-zone does not proceed in an orderly manner. Abnormal cells appear that resemble carcinoma cells but do not invade the stroma. Classification systems to define these dysplastic stages have been made. Cervical intraepithelial neoplasia (CIN) is a term that has been used for 25 years to describe a continuum of dysplastic abnormalities of the cervix. The diagnosis is based on histopathological examination of cervical tissue (Dillner and Brown 2004). CIN is divided into three grades for determination of dysplastic severity (Figure 1). CIN1 is the least severe grade where the lower third of the cervical epithelium is affected. Mitotic figures are not seen and koilocytes are generally present. Koilocytes are cells with enlarged, irregularly shaped nuclei, and a prominent perinuclear halo. Proceeding to the next grade, CIN2, the abnormalities have spread to two thirds of the epithelium and mitotic figures might be present. In CIN3 abnormal cells have infiltrated the whole epithelium. CIN1, 2 and 3 correspond to mild, moderate and severe dysplasia/carcinoma *in situ*, respectively, in older nomenclature. Microinvasive or invasive cancer occurs when penetration of the basal membrane has taken place (Copeland 2000).
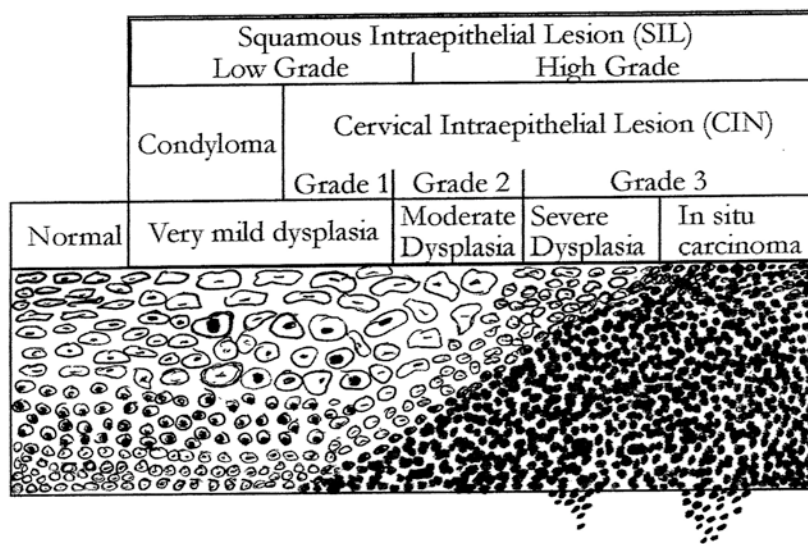

Figure 1. Cervical squamous carcinoma precursors. Schematic representation of cervical cancer precursors and the different terminologies that are used to describe them (adapted from Wright et al. 1994).

Another way of classifying the precursor stages of cervical cancer is according to the Bethesda System (Solomon, Davey et al. 2002). This classification was made with the intention to provide clearer guidance for screening and management (Richart 1990). The stages of the squamous abnormalities are: atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL). LSIL includes CIN1 lesions and koilocytotic atypia. HSIL includes CIN2 and CIN3.

The progression into cancer

The progression of cervical cancer usually takes many years (on average 15-20 years) and is a multistep process (Figure 2).
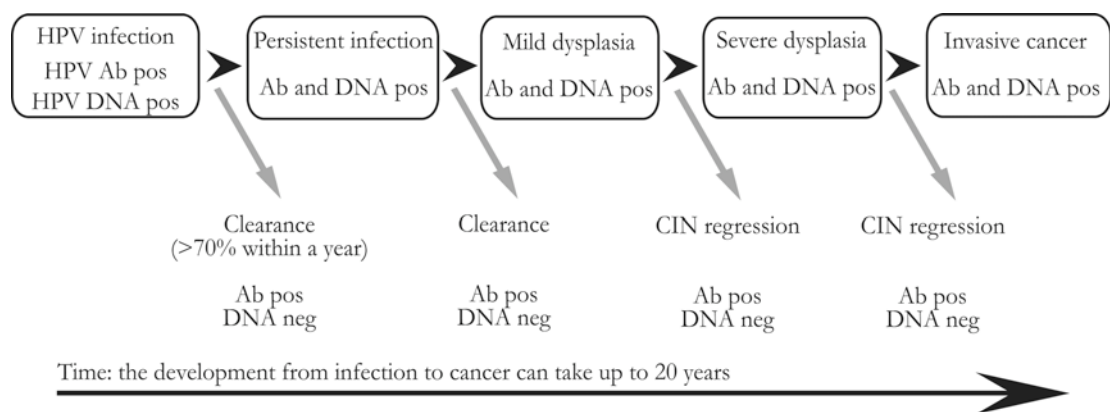
Figure 2. Natural history of cervical cancer. For each step in the disease development, clearance is more common than pathogenesis (adapted from Dillner 2001).

HPV is a necessary cause of cellular transformation, but most women will clear the virus spontaneously, probably by a competent immune response. But when the infection is persistent the cervix is prone to cellular transformation and CIN occurs, as described above. An untreated CIN can progress further to become invasive cancer. Microinvasive carcinoma happens when neoplastic epithelial cells project into the cervical stroma. The adjacent stroma is infiltrated by lymphocytes and plasma cells. The clinical staging is done according to FIGO classification. The most common type (70%) of squamous cell carcinoma (SCC) is the moderately differentiated, nonkeratinizing, large cell SCC. Less common SCC forms are well-differentiated keratinizing SCC (25%) and small cell undifferentiated carcinoma (5%). Adenocarcinoma (AC) is another form of cancer found in the cervix, which is usually derived from mucus-secreting columnar epithelium lining the endocervical canal. This cancer form is not as common as SCC but seems to have increased in incidence over the years,

both in relation to SCC and the rate in the population at risk (Smith, Tiffany et al. 2000; Hemminki, Li et al. 2002; Visioli, Zappa et al. 2004). In the 1950's and 1960's it accounted for 5-10% of primary tumours of the cervix (Hepler, Dockerty et al. 1952; Mikuta and Celebre 1969). In the 1970's and 1980's adenocarcinoma was reported to have increased to include up to 25% of the cervical cancers (Davis and Moon 1975; Devesa 1984). It is not known whether this increase is due to changes in sexual behaviour or better detection methods for this type of cancer.

Detection of CIN and cervical cancer

Before the causes of cervical cancer were known, the pathologist George Papanicolaou, introduced the Pap smear (Papanicolaou 1949). It is a cytological method that detects precancerous cells from the T-zone. Since its introduction, the Pap smear has reduced cervical cancer incidence and mortality rates by one half to two-thirds (Kurman, Henson et al. 1994).

Even though Pap smears have reduced the incidence of cervical cancer, it is a rather insensitive and unspecific method. False negative rates up to 20-30% have been reported (Burd 2003). False negative results can occur from clumping of cervical cells on the slide or contamination by other specimens e.g. bacteria, blood or yeast. A Pap smear can contain between 50,000 to 300,000 cells and if there are only a few abnormal cells on that slide it is easy to miss the cellular changes.

A woman with abnormal Pap smear is referred to colposcopy. A colposcope is a magnifying and photographic instrument used to examine the cervix. It would be possible to avoid unnecessary colposcopy procedures by testing for presence of HPV DNA in abnormal smears. In absence of HPV DNA there is little risk that high grade dysplasia will be discovered at colposcopy, whereas high grade disease is likely to be found in HPV DNA positive women (Burd 2003). HPV DNA testing could also be used to reduce screening costs by reducing screening in low risk women.

TREATMENT

Only a small proportion of mild and moderate cervical diseases develop into invasive cancer. But progression from severe cervical abnormality to invasive disease is at least 12% (Ostor 1993). The course of treatment is determined by a number of factors such as size, stage, histological features of the tumour, lymph node involvement and risk factors for complications from surgery or radiation. Cryotherapy is carried out on abnormal tissue and the surrounding 5 mm. The tissue is frozen and removed. This is a simple procedure and fertility is maintained. Another simple method is removal by a carbon dioxide laser beam. The tissue heals faster compared to cryotherapy but this method is more expensive. When invasive disease, not fully visible T-zone or glandular abnormalities are suspected, exisional

treatments are preferred. Cone biopsies are carried out on microinvasive cancers. If disease is recurrent or difficult to treat a hysterectomy, removal of the uterus, is performed.

BASICS OF THE IMMUNE RESPONSE

With this section I will discuss basic concepts in immunology that will be relevant to the understanding of the thesis and included manuscripts.

We are constantly being exposed to foreign objects such as virus, bacteria, fungi and parasites. To protect ourselves we have developed a complex array of mechanisms that will control and eliminate these microorganisms. By recognising special structures on the pathogen the immune response can distinguish foreign from host. The first barrier a microorganism will encounter is the skin, mucosal membranes or the placenta. There are also physical barriers that can stop infection such as temperature, salinity, acidity and oxygen tension. When a pathogen manages to penetrate the first line of defence it will be met by an immune response. The immune system is divided into innate and adaptive responses. These responses will stop and eliminate most microbial invaders. But there are of course some exceptions, for example: the skin does not hinder papillomaviruses; the bacterium *Helicopter pylori* do not mind acid environments and influenza virus constantly mutate to escape recognition of the immune response. Under certain circumstances the immune system overreacts and can cause diseases like allergic reactions, autoimmunity or chronic inflammation. But in general the innate and adaptive responses usually work together to eliminate pathogens.

INNATE IMMUNITY

The innate immune response is immediate, non-adaptable and usually local. This means that when a microorganism has entered the body, the innate immune response quickly reacts and recognises the microorganism as foreign without knowing which specific pathogen it is. With help from a variety of cells with different functions the innate immunity locally kills the invader at same time as it also activates the adaptive response. One important type of cell in antiviral defence is the natural killer (NK) cell. It destroys infected and malignant cells by inserting the pore-forming molecule perforin into the membrane of the target cell and then injecting it with cytotoxic granzymes. In addition to their cytotoxic function, NK cells secrete certain cytokines, which act as messengers both within the immune system and between the immune system and other systems of the body, forming an integrated network that is highly involved in the regulation of immune response. For example, at early times after infection, before the T cell response develops NK cells are the principle source of interferon-gamma (IFN-γ). Patients who lack NK cells suffer from protracted, life-threatening viral infections that cannot be cleared despite the presence of adaptive T cell immunity. Such correlations indicate that NK cells are an

essential early defence mechanism against viral infections, one that complements the activities of cytolytic T cells (Delves and Roitt 2000).

CYTOKINES AND INTERFERONS

Cytokines and interferons are proteins that can act as messengers both within the immune system and between the immune system and other systems of the body. In short, they form a network, which regulates immune responses. Some cytokines have a direct role in immune defence. For example, interferons are released by virally infected cells and by doing so hinder the infection of surrounding cells (Delves and Roitt 2000). Cytokines can be divided into two types depending on their action. Type 1 cytokines increase T cell-mediated responses and are considered to be beneficial for antitumour immunity. IFN-γ, and IL-12 are typical Type 1 cytokines. Type 2 cytokines, on the other hand, promote humoral immunity (antibodies) and inhibit Type 1 cytokines and cytotoxic T cell lymphocyte (CTL) development. Type 2 responses produce IL-4, IL-5, IL-9 and IL-10. This shift from Type 1 to Type 2 cytokines has, in cervical cancer, been seen to correlate with poor clinical outcome (El-Sherif, Seth et al. 2001; Gey, Kumari et al. 2003).

There is also another group of cytokines called chemokines. These stimulate, recruit and activate phagocytes and lymphocytes. They have a central role in inflammatory responses. For example, CXCL8 (formerly known as IL-8) is a chemokine which recruits inflammatory cells to the cervicovaginal compartment, leading to enhanced production of IL-1β and IL-6 (Al-Harthi, Wright et al. 2000).

ADAPTIVE IMMUNITY

The adaptive immune response consists of a humoral response defined by antibodies that are produced by B cells, and also a cellular response (T cells). T cells kill the infected cell and are also capable of initiating and/or terminating the immune reaction. Adaptive immunity is antigen-specific and primed by the innate response. The components of adaptive immunity specifically target, attack and eliminate the invaders that succeed in passing the first two defence barriers. The ultimate goal of the adaptive immune response, in viral infection, is to eliminate both the virus and the host cells harbouring the virus. To do so cells from the innate response (dendritic cells, monocytes and macrophages) and the adaptive response (B-cells) swallow non-self antigens and present them to different subsets of T cells (CD4^{+} and CD8^{+}). They are called antigen-presenting cells (APCs). When they have engulfed an antigen it is metabolised and presented as a small, degraded sequence (peptide) on cellular surface molecules called the Major Histocompatibility Complex (MHC). In humans this complex is termed the Human Leukocyte Antigen (HLA) (Janway, Travers et al. 2001; Murray,

Rosenthal et al. 2002). This peptide is presented to T cells that are activated and either directly kill the pathogen or activate production of antibodies, which will bind to and neutralise pathogens or prepare them for uptake and destruction by phagocytes (a cell typical of the innate response).

A key feature of the adaptive response is to produce long-lived cells that persist in a dormant state, but can re-express effector functions rapidly after repeated encounter with the same antigen. It also has the ability to adapt to an evader that is changing, for example new viral variants.

RISK FACTORS IN CERVICAL CANCER

Approximately 15% of the global cancer incidence is etiologically related to specific infections (zur Hausen 1999), viruses being the major cause e.g. hepatitis B and C are both linked to hepatocellular cancers. It is well established that infection with oncogenic HPV types is the necessary cause of CIN and cervical cancer. In the etiology of CIN and cervical carcinomas several exogenous and endogenous risk factors that might act in conjunction with HPV have been implicated. These are for example: *Chlamydia trachomatis* and Herpes simplex virus type 2, the immune response, smoking and oral contraceptives.

In this part of this work I will discuss the suggested roles for HPV and co-factors in the aetiology of cervical cancer, with particular emphasis on HPV.

HUMAN PAPILLOMAVIRUS

History and epidemiology

The oncogenic types of HPV that cause cervical carcinomas are sexually transmitted. Already in 1842, long before HPV was identified, sexual activity was correlated to cervical cancer by Rigoni-Stern (Rigoni-Stern 1842). Papillomavirus research began in the beginning of the 1900's when warts were shown, by a cell-free inoculation, to be transmitted from person to person (Ciuffo 1907). In 1933 it was confirmed that neoplasia is mediated by papillomavirus (Shope 1933). The Shope virus that was recovered from naturally occurring cutaneous papillomas in cottontail rabbits were able to produce papillomas in domestic rabbits and progress into carcinomas. The carcinogenic potential of HPV in the rare hereditary condition, epidermodysplasia verruciformis, was discovered in the 1950's (Jablonska and Milewski 1957). In the 1970's, a role for HPV in development of carcinomas of the cervix was proposed (zur Hausen, Meinhof et al. 1974; zur Hausen 1976) and in 1983 the first isolation of an oncogenic virus type (HPV 16) from cervical cancer was reported (Durst, Gissmann et al. 1983). This virus could also be detected in precursor lesions of tumours (Ikenberg, Gissmann et al. 1983) and it was demonstrated that specific viral genes were expressed in the malignant tissue (Schwarz, Freese et al. 1985). Since then epidemiological studies from all over the world have established an etiological link between HPV and cervical cancer. The first large epidemiological study of HPV DNA and cervical neoplasia came in 1987 (de Villiers, Wagner et al. 1987). At that time, HPV DNA was only detected in 30-40% of patients with CIN and invasive cancer, compared to in 10% of women with normal cytology. Since then, HPV DNA testing methods have improved and HPV DNA is nowadays detectable in almost 100% of

cervical carcinomas (Bosch, Manos et al. 1995; Walboomers, Jacobs et al. 1999).

In a recent meta-analysis of HPV DNA type-specific prevalence in cervical cancer, HPV 16 was found in 51% of all ICC cases and HPV 18 in 16.2% (Clifford, Smith et al. 2003). HPV 16 was more commonly found in SCC whereas HPV18 was more common in AC. Other common types found were HPV 45, 31, 33, 58, 52, 35, 59, 56, 6, 51, 68, 39, 82, 73, 66 and 70. HPV is considered to be the most common sexually transmitted infection in the world.

Structure and classification

Human papillomaviruses belong to the *Papillomaviridae* family. They are small, non-enveloped, double stranded DNA viruses with icosahedral symmetry. The virion has a diameter of 55-60 nm and the viral genome is approximately 7900 base pair long (Chen, Howley et al. 1982). The protein coat is composed of 72 capsomers consisting of two structural proteins: one major protein (L1) representing 80% of the total capsid. L2 is the minor protein.

Papillomaviruses are found in a wide range of animals, each infecting with specificity for a particular animal. Human papillomaviruses are therefore species specific and can only infect human skin or mucosal epithelia. Papillomaviruses can only replicate in differentiating epithelia and for that reason can't be grown in monolayered tissue cultures. Since the development of organotypic raft system it has been less difficult to study the life cycle of the virus. This is a system that is capable of reproducing the entire viral life cycle *in vitro*, allowing for the investigation of viral promoter activity, viral mRNA (messenger ribonucleic acid) expression and splicing patterns, viral DNA amplification, late gene expression and virion morphogenesis for some HPV types (McLaughlin-Drubin, Christensen et al. 2004).

To date, at least 100 HPV types have been identified (de Villiers, Fauquet et al. 2004). They are classified as genotypes and each type is given a number. The genotypes are based on the sequence homology of the L1 open reading frame (ORF) because this region is well conserved among all members of the family. If the DNA sequence differs by more than 10% from the closest known papillomavirus type it will be recognised as new type. A subtype is defined when there is a 2-10% difference in sequence homology. Less than 2% will be a variant (de Villiers, Fauquet et al. 2004).

Virtually no cross-reaction between antibodies against different HPV types have been observed, indicating that most HPV genotypes also corresponds to HPV serotypes (Konya and Dillner 2001; Dillner and Brown 2004).

HPV are grouped according to the type of epithelia they infect. The majority of HPVs infect cutaneous epithelia or skin. Approximately 40 types infect mucosal epithelia and are called genital HPVs. These types are further divided into high-risk types, inducing cell transformation and low-risk types, causing benign warts. It is suggested that at least 14 types are high-risk types (Bosch, Manos et al. 1995). The most predominant types in genital warts are HPV 6 and 11.

Genomic organisation

The genome of HPV contains approximately eight ORFs, which are transcribed from a single DNA strand (Fehrmann and Laimins 2003). In the upstream regulatory region, which is a non-coding region, sequences for viral replication are found. The gene products can be divided into two classes: early (E) and late (L) proteins (Howley 1996) (Figure 3). The early genes are primarily responsible for viral DNA replication, transcription and transformation and the late genes express viral structural proteins that are responsible for maturation and assembly of the virus particle.
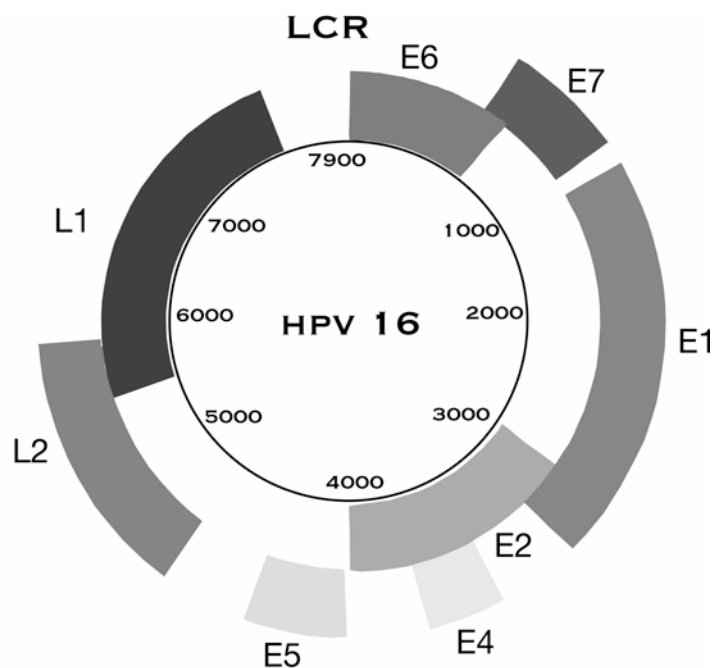

Figure 3. HPV 16 genome. Position of open reading frames (ORFs) encoding late and early genes, and the long control region (LCR) (adapted from Field Virology, 1996).

A brief description is given below of the HPV early and late proteins:

E1 is the largest ORF in the papillomavirus genome (Wilson, West et al. 2002). It is the only papillomavirus protein with defined enzymatic activity

(helicase and ATPase activity), which helps viral DNA replication to occur in an efficient manner (Lambert 1991). E1 forms heterodimers with E2, which leads to the initiation of viral replication at the viral origin (Sverdrup and Khan 1995).

The E2 protein has an important role in the life cycle of papillomavirus because it regulates viral transcription and replication. The bovine papillomavirus type 1 (BPV-1) has been used as a model to study this. The ORF of BPV-1 encodes 3 nuclear proteins that all have activity for viral transcription (Lambert 1991). E2 has been shown to induce S-phase arrest, which allows sustained synthesis of viral DNA replication, something that is essential for completion of the viral life cycle.

The ORF of E4 is found within the ORF of E2 but has a shorter reading frame. The protein is detected in productively infected cells. The E4 protein is translated from a spliced E1^E4 transcript to form a spliced E1^E4 fusion protein. The pattern of E4 distribution suggests that the E4 function might be required at all stages of the productive cycle (Knight, Grainger et al. 2004). The HPV 16 E4 protein has been shown to interact with intermediate filament of the host cell, resulting in collapse of the cytokeratin matrix and thereby release of mature virus particles (Doorbar, Ely et al. 1991; Roberts, Ashmole et al. 1993). Other proposed roles for E4 are involvement in vegetative viral DNA replication, the control of the virus maturation and inhibition of terminal differentiation of the cell in order to retain the integrity of the infected cell (Doorbar 1996).

E5 is weakly oncogenic in tissue culture assays and improves the effectiveness of the transforming activity of E7 (Bouvard, Matlashewski et al. 1994; Valle and Banks 1995). The HPV E5 protein is small, hydrophobic and located mainly at the endosomal membranes, Golgi apparatus and plasma membranes (Burkhardt, Willingham et al. 1989; Conrad, Bubb et al. 1993). The protein is probably expressed primarily during the late phase of the life cycle to modulate differentiation-induced functions like viral amplification and late gene expression (Fehrmann, Klumpp et al. 2003).

The E6 and E7 proteins are encoded by all papillomaviruses and their ORFs are located in the 5' part of the early region. These genes are the main transforming proteins of the high-risk HPV types and act by modulating the activities of the cellular proteins that regulate the cell cycle.

The E6 protein is one of the first genes expressed during HPV infection. It is about 150 amino acids in size and contains two zinc-binding domains with the motif Cys-X-X-Cys. The zinc fingers are important for protein conformation and interaction with DNA. The high-risk E6 proteins are found both in the nucleus and in the cytoplasm and it has been reported to

bind to more than 12 different proteins (zur Hausen 2002). Together with E7 from high-risk HPVs, E6 can induce cellular immortalisation of keratinocytes (Hawley-Nelson, Vousden et al. 1989; Munger, Werness et al. 1989). They do so by binding to tumour suppressor genes such as p53 and the retinoblastoma (Rb) gene. HPV E6 binds to a cellular ubiquitinin-ligase, called E6-associated protein (E6-AP), which then binds to p53. This interaction leads to p53 degradation, cell cycle disruption, and acquisition of genetic alterations contributing to malignant transformation (Fehrmann and Laimins 2003). HPV 16 E6 binds and degrades p53 two to three fold more efficiently than HPV 18 E6, while low-risk HPV 6 and 11 E6 proteins bind weakly and cannot induce p53 degradation *in vitro* (Scheffner, Werness et al. 1990).

The E7 protein is a bit shorter than E6, around 100 amino acids. E7 binds directly to the Rb gene and interferes with the ability of Rb to inhibit cell cycle arrest. This allows productive replication of HPV genes (Fehrmann and Laimins 2003). The HPV 16 E7 protein binds to Rb seven times more efficiently than the same protein of HPV 6 (Heck, Yee et al. 1992).

The general view has been that E6 is more important in suppressing cell death than E7. However, E7 has also been reported to be important in this process. By blocking HPV 16 E6 and E7 genes independently it was found that the suppressed E6 expression led to accumulation of cellular p53 protein, transactivation of the p21 cell-cycle control gene, and also reduced cell growth. When E7 was silenced, the virally infected cells were pushed into apoptosis (Jiang and Milner 2002). Two other studies revealed that E7 could be of more importance in cell transformation than earlier thought. HPV 58 E7 mutations in Chinese women were investigated. Two specific mutations were associated with CIN III and ICC (Chan, Lam et al. 2002). Another group found that Notch 1, a host cell surface receptor, in normal cells downregulates E6 and E7. But in cervical cancer cells Notch 1 is more or less absent, resulting in elevation of both E6 and E7. The downregulation of Notch 1 could play an important role in the late stages of HPV-induced carcinogenesis (Talora, Sgroi et al. 2002).

L1 and L2 proteins that make up the capsid of the virus are synthesised in the late phase of the viral cycle. The role of the capsid is to protect the genome and to target cellular surface receptors involved in infection. L1 can self-assemble into virus like particles (VLP) when expressed in eukaryotic cells (Kirnbauer, Booy et al. 1992). The VLPs are morphologically and immunologically very similar to HPV virions. VLPs are the primary candidate for prophylactic vaccination against HPV infection. One study found that L2 was not able to self assemble like L1 but when expressed together the assembly was 50-fold enhanced. It was suggested that L2 may be important in stabilisation of the capsid structure (Hagensee, Yaegashi et

al. 1993). Another suggested function for L2 is in the uncoating of virions and delivery of the viral genome to the nucleus upon infection (Roden, Day et al. 2001).

There is one part of the HPV genome that does not encode any known protein but still has an important function: the long control region (LCR). Its role is to regulate gene expression and replication.

Viral life-cycle

HPVs infect keratinocytes in the basal layer of the epithelium. To be able to complete a whole viral cycle, HPV is dependant on the differentiation of the epithelium (Stubenrauch and Laimins 1999).
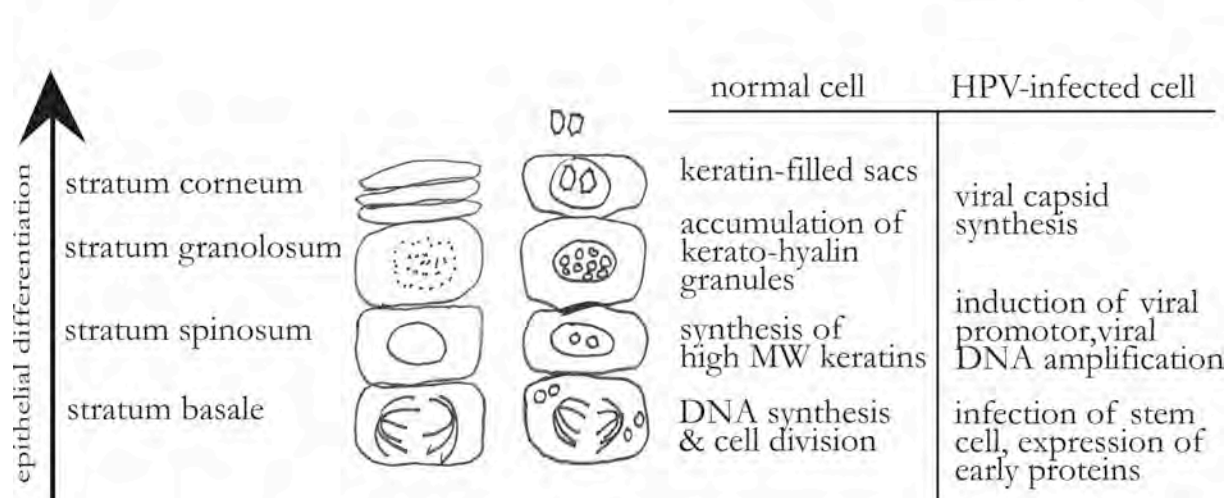
Figure 4. Epithelial differentiation in normal and in HPV infected cells. The four different stages of epithelial differentiation are shown. The left side of the figure demonstrates the normal cells and to the right, cells infected by HPV (adapted from Stubenrauch, 1999 and Fehrmann, 2003).

HPV has been reported to bind to specific cell surface receptors in order to infect cells (Muller, Gissmann et al. 1995; Volpers, Unckell et al. 1995). Heparan sulfate proteoglycans have been shown to act as a primary receptors for HPV and mediate viral attachment by interacting with the carboxyl terminus of the L1 protein (Joyce, Tung et al. 1999; Combita, Touze et al. 2001; Giroglou, Florin et al. 2001). These receptors are widely distributed on the cell surface but they may not be enough to allow viral entry. Alpha-6 integrin, expressed primarily during wound-healing, is used as a receptor for HPV 6 (Evander, Frazer et al. 1997). After attachment the virus enters the cell. It is not known how the virus penetrate the plasma membrane but recent data suggest that different HPVs use different endocytosis pathways to enter the cell (Bousarghin, Touze et al. 2003).
Once the virus has penetrated the epithelium it establishes itself in the basal layers. This is where cell proliferation begins. At this point the HPV

genome is established extrachromosomally in the nucleus and the copy number is increased to 50-100 copies per cell (Stubenrauch and Laimins 1999). When the infected cells start to divide, viral DNA is distributed in both daughter cells. One daughter cell migrates upwards to start differentiation and the other daughter cell continues to divide in the basal layer. This cell becomes a reservoir for viral DNA. This ability of HPV to remain in the nucleus allows the infection to persist for many years (Howley 1996).

Uninfected cells normally exit the cell cycle as they begin to differentiate. However, HPV-infected cells block cell cycle exit by help from the E6 and E7 proteins (as described in the section about the HPV genome). The first viral genes to be expressed are E1 and E2. They bind to the origin of replication. The binding allows unwinding of the DNA helix and initiation of replication (Seo, Muller et al. 1993). There are two modes of replication, plasmid and vegetative. In the first phase the genomes replicate an average of once per cell cycle during the S-phase (Howley 1996). In the vegetative replication, the genomes are packaged into virions in terminally differentiated epithelium. The late mRNAs of HPV 16 including L1, L2 and E4 are transcribed from the late promotor p842 and polyadenylated at the late poly (A) site (Grassmann, Rapp et al. 1996). mRNAs of L1 and L2 has inhibitory sequences (Schwartz 2000). These sequences might make it impossible for L1 and L2 expression in non-differentiating cells, whereas inhibition is overcome and mRNAs are stabilised during terminal differentiation. The capsid assembly and release of the virus takes place in highly differentiated cells close to the surface of the skin. Little is known about how the virus egresses from the cell but it has been speculated that E4 interacts with the cytokeratin network and thereby causes it to collapse which would enable newly produced viruses to exit the cell (Doorbar, Ely et al. 1991).

Detection of HPV

Testing for HPV DNA relies on molecular biology techniques. It is important that these methods can detect relevant HPV types, are easy to use and are sensitive. Conditions that affect the outcome of HPV DNA tests, are sampling quantity, quality and storage conditions. Proper extraction of the DNA is also very important for the result. The sensitivity level of an assay is usually defined as the lower detection limit or the lowest possible quantity of HPV DNA available that can be detected in a sample while specificity determines the level of accuracy of an assay (Hubbard 2003). Setting up assays where minimal false positives and negatives occur increase accuracy and leads to better scientific studies, diagnosis and patient care.

Hybrid Capture II

Hybrid Capture II assay is a commercially available HPV test. The assay is based on RNA-DNA hybridization. Genotype-specific probes are mixed in high-risk and low-risk cocktail formats and the assay is capable of detecting virtually all high-risk and low-risk groups, but a disadvantage is that the test is not able to detect specific HPV types. The Hybrid Capture II assay is also a nonradioactive, chemiluminescence method that is easy to perform and can therefore be used in most clinical laboratories. Longitudinal studies have shown that the assay has enough sensitivity to detect both high grade CIN and cancer (Schiffman, Herrero et al. 2000; Solomon, Schiffman et al. 2001). However, another disadvantage of this method is that it has been found to detect additional HPV-types not included in the assay, which are able to cross-hybridize with the probe mix (Konya, Veress et al. 2000).

HPV DNA polymerase chain reaction (PCR)

PCRs are used to amplify and detect specific DNA in either exfoliated cells or tissue samples. General or consensus primer-mediated PCR assays have been developed to screen for a broad spectrum of HPV types in clinical specimens. The general primer MY09/11 PCR in combination with the PGMY09/11 primer amplifies a 450 base pair long region in the L1 gene. A broad range of HPV types can be detected with this method (Cuzick, Sasieni et al. 1999). Another PCR method is based on the primer pair GP5+/GP6+. The region amplified is 140 bp in the L1 gene. The assay is both specific and sensitive for the prediction of high-grade CIN (Rozendaal, Walboomers et al. 1996) and can be used for a large number of samples. Specific HPV types are then detected by restriction enzyme analysis, reverse hybridisation with specific probes or nucleic acid sequence analysis (Burd 2003). There are also type-specific PCRs that are based on sequence variations present in the E6 and E7 genes. This type of PCR is primarily used for research because throughput is limited and multiple amplifications are needed for each sample (Burd 2003).

HPV serology

Measuring specific antibodies against different HPV types is a way of detecting past infection and is very suitable for epidemiological studies because serum antibodies are stable over time. Type-specific HPV serology has been important in the elucidation of the epidemiology of HPV and cervical cancer (Carter, Koutsky et al. 1996; Chua, Wiklund et al. 1996; Shah 1998; Dillner 1999; Silins, Kallings et al. 2000; Wang, Kjellberg et al. 2000). The most commonly used method is Enzyme-linked-immunosorbent-assay (ELISA) using HPV VLPs as the antigen. The standard ELISA detects anti-HPV antibodies of the IgG or IgA isotypes. As reported already by

Kirnbauer et al. in 1994, IgG anti-HPV antibodies are found in only about 60% of women testing positive for cervical HPV 16 DNA (Kirnbauer, Hubbert et al. 1994). Anti-HPV antibody detection alone can therefore not be used as a diagnostic tool on the individual level since all HPV infected women apparently don't seroconvert.

HPV and cervical cancer

Although HPV is related to a variety of cancers, researchers primarily focus on its strong association with cervical cancer. Anogenital cancers account for nearly 12% of all cancers in women (Pisani, Bray et al. 2002) and therefore have a large impact on the global health. Studies among initially virginal women have confirmed that HPV is sexually transmitted (Rylander, Ruusuvaara et al. 1994; Andersson-Ellstrom, Dillner et al. 1996; Kjaer, Chackerian et al. 2001). However, penetrative intercourse might not be necessary (Marrazzo, Koutsky et al. 2001; Winer, Lee et al. 2003). Marrazo et al. found that women who have sex with women can also be positive for HPV DNA (13% in the study). Unfortunately there are not many studies on natural history of HPV in men. The reason for this is that there is no validated method to sample DNA from male genitalia and also the fact that motivation for such a test is not so great due to the rare disease outcome in men (Schiffman and Kjaer 2003). Castellsague et al. found that men who were circumcised had lower prevalence of HPV and that their female partners were at lower risk of developing cervical cancer (Castellsague, Bosch et al. 2002).

Many sexual partners, other sexually transmitted infections (STIs), age of sexual initiation, multiparity, contraceptive methods and smoking are all reported risk factors for cervical cancer. The most important risk factor for HPV acquisition is a change in sexual partner. A study conducted in Sweden found that the risk for HPV 16 seroconversion increases with 4% for each lifetime sexual partner up to a plateau of 32% (Dillner, Kallings et al. 1996). Teenage girls with no sexual experience were not seropositive for HPV 16 or 33, whereas 54% of girls who had at least five partners did seroconvert (Andersson-Ellstrom, Dillner et al. 1996). The length of time being with a new partner before having sex is also a determinant for acquisition of HPV. Having known your partner for less than eight months before sex increases the risk of HPV infection (Winer, Lee et al. 2003).

Anogenital HPV infections luckily tend to regress spontaneously. The clearance rate after 12 months is 70% and after 18 months 80% (Hildesheim, Schiffman et al. 1994; Ho, Bierman et al. 1998). Women older than 35 years of age have lower clearance rates than younger women (Dillner 2001). Persistence is defined as the detection of the same HPV type two or more times over a period of time (Schiffman and Kjaer 2003). The period of time is not defined but is usually from a few months to a year.

A competent cell-mediated immune response is postulated to be involved in the clearance of infection. But other factors appear to play a role in the determining whether HPV should persist or not. HPV 16 tends to persist longer than other high-risk types (Franco, Villa et al. 1999; Liaw, Hildesheim et al. 2001). Variants of HPV 16 have been reported to correlate with an even greater risk in the progression to cancer (Londesborough, Ho et al. 1996; Xi, Koutsky et al. 1997; Hildesheim, Schiffman et al. 2001; Zehbe, Mytilineos et al. 2003; de Boer, Peters et al. 2004). However, in one study, HPV 16 E6 variants in cervical carcinogenis, could not be associated with risk of cervical cancer development (van Duin, Snijders et al. 2000). In a cohort study on American female university students it was found that nonwhite race and use of hormonal contraceptives were associated with an increased risk to acquire HPV 16 non-prototype-like variants (Xi, Carter et al. 2002). These types are similar to the variants commonly detected in Africa. This indicates that different ethnic groups may propagate different HPV variants. Women with high HPV 16 viral load appear to be at greater risk of developing higher grades of CIN (Ylitalo, Sorensen et al. 2000; van Duin, Snijders et al. 2002) but data are inconsistent and could be difficult to interpret delineate because viral load might be the same for long-term as for recent infections. Maybe infections of other STIs can act as independent co-factors contributing to HPV persistence.

Other HPV related diseases

Condyloma

Condylomas are transmitted through sexual contact and the most prevalent HPV types found are HPV 6 and 11 (low-risk types). These types cause more than 90% of condylomas. They are benign genital warts and one of the most common sexually transmitted diseases in the world. Painless bumps, itching and discharge are common symptoms. However, most cases are asymptomatic and transmission can occur from someone who does not appear to have warts (Dupin 2004). The incubation time can vary from weeks to months. Most frequently affected are the penis, vulva, vagina, cervix, perineum and perianal area. More than 50% of female patients with external condylomatous lesions have negative Pap smears but are positive for HPV infection (Dupin 2004).

Skin warts

Warts are benign tumours common in children and adolescents. It is estimated that 10-22 % of children will be infected during their lifetime. The warts are transmitted from person to person. Indirect contact transmission is also possible, e.g. moist floors in public showers have been reported as a route of transmission. Most warts regress spontaneously, probably due to a

cell-mediated immune response. Skin warts may differ in morphology and histology. The common warts (verrucae vulgaris) found on hands are associated with HPV 2, 4, 7 and 57. HPV 1 usually causes plantar warts. Flat warts (verrucae planae) are found on hands and face and they are usually associated with HPV 3, 10 and 41 (Silverberg 2004).

Epidermodysplasia verruciformis and skin cancer

Epidermodysplasia verruciformis (EV) is a hereditary skin disease that is rare and life-long. In patients with EV, HPV induces skin lesions that are reddish-brown macular plaques and flat warts. They are unable to clear the virus and half of the patients will develop skin cancer (Jablonska, Dabrowski et al. 1972). The EV related HPV types have also been found in normal skin of healthy people but without causing clinical symptoms (Antonsson, Forslund et al. 2000). HPV as a risk factor in skin cancer is currently being investigated. Ultraviolet (UV) radiation is the main cause of non-melanoma skin cancer. Studies have indicated that UV radiation can activate promoters on various HPV types (Purdie, Pennington et al. 1999; Ruhland and de Villiers 2001). A role of HPV in skin cancer is not established at present.

Other HPV associated diseases are squamous-cell carcinoma of the head and neck, in particular oropharyngeal cancers (Mork, Lie et al. 2001)((Shah 1998). Some studies have also found an association between HPV 16 antibodies and an increased risk of esophageal cancer (Dillner, Knekt et al. 1995; Bjorge, Hakulinen et al. 1997).

HPV IMMUNOLOGY

The majority of HPV infected women clear the virus within a rather short period of time. The immune response probably has an important role in clearance of the virus. The replicative cycle of HPV is shaped by co-evolution with its host so that the immune response might not have a chance to eliminate virally infected cells or transformed cells. Many studies have focused on the immune response to HPV infection and HPV related cancer. The immune system is complex and can vary from person to person due to different gene disposition and environmental influences. Understanding immunity to HPV is important in the development of both prophylactic and therapeutic measures against HPV infection and cancer.

Antibody responses

The study of antibody responses against HPV is a useful tool in understanding the natural history of HPV infection, the cancer association of HPV and for vaccine development. Antibodies against L1-containing VLPs are HPV type-specific and can be found both during and after

infection (Lehtinen, Dillner et al. 1996; Schiller and Hidesheim 2000). Antibodies are not responsible for clearance of the virus but are involved in protection against infection (Wang and Hildesheim 2003). Serum IgG against HPV 16 is detected in 50 to 60% of women who are positive for HPV 16 DNA (Kirnbauer, Hubbert et al. 1994; Le Cann, Touze et al. 1995; Carter, Koutsky et al. 1996; Kjellberg, Wang et al. 1999). Most women who seroconvert will do so 6-12 months after infection but 10-20% convert at the same time as HPV DNA is detectable (Andersson-Ellstrom, Dillner et al. 1996; de Gruijl, Bontkes et al. 1997; af Geijersstam, Kibur et al. 1998).

Seroepidemiological studies have found that HPV-seropositive women are at increased risk of cervical cancer (Lehtinen, Dillner et al. 1996). HPV-seropositivity has in a few studies been associated with CIN persistence and the severity of the lesion. Anti-HPV 16 antibodies were detected in approximately 30% of LSIL patients, 50% in women with HSIL (Sasagawa, Inoue et al. 1996; Bontkes, de Gruijl et al. 1999; Wideroff, Schiffman et al. 1999). HPV16 seropersistence and incidence has in one study shown to be higher in women compared to men (Thompson, Douglas et al. 2004). While the issue of whether there are sex-specific differences in the HPV antibody response needs to be studied further, it is noteworthy that the antibody response other STIs such as *C. trachomatis* is measurably different between men and women (Connor, Catchpole et al. 1997; Koivisto, Isoaho et al. 1999; Paavonen, Karunakaran et al. 2003).

The major isotypes of the antibody response against HPV are IgG1 and IgA (Wang, Kjellberg et al. 2000). The IgA response is also HPV type specific and correlates with recent number of sexual partners. These results were confirmed in a more recent study that investigated both serum and cervical IgA in women with incident HPV 16 infection (Onda, Carter et al. 2003). They observed that within 18 months of first detection of HPV 16 87.3% of the women had developed anti-HPV 16 IgA in cervical secretions. Duration of these antibodies was short, 50% had reverted to seronegativity within one year. Serum anti-HPV IgA developed more slowly but also had a short duration. Women who cleared their infection revert to seronegativity faster than women with persistent HPV 16 infection. An important factor when measuring mucosal antibodies seems to be the time of sampling. Cervical titers of anti-HPV IgA and IgG have been observed to be highest in the proliferate phase of the menstrual cycle and the lowest during the ovulatory phase (Nardelli-Haefliger, Wirthner et al. 2003).

Antibodies against non-structural HPV proteins have also been investigated. Anti-E6 and E7 antibodies can be found in cervical cancer patients but are not useful as indicators of cervical cancer prognosis (Silins, Avall-Lundqvist et al. 2002). Furthermore, they are not useful for prediction of future invasive cervical cancer. Lehtinen et al. evaluated HPV 16 and 18 E6 and E7 responses in samples taken 1-20 years before time of diagnosis.

Antibodies were detected in only 7% of women who later developed cancer (Lehtinen, Pawlita et al. 2003). Anti-E2 IgG was detected in 67% of HPV 16 DNA positive women (Rosales, Lopez-Contreras et al. 2001). Furthermore, IgA anti-E2 antibodies were found to be associated with progression of CIN. The higher stage the lower antibody levels (Rocha-Zavaleta, Jordan et al. 1997).

The roles of neutralising antibodies are to block the virus binding to cell receptors and also to inhibit uncoating of the virion (Klasse and Sattentau 2002). HPV VLP-based ELISAs have the disadvantage that they may also detect non-neutralising linear epitopes and assays based on disrupted or improperly folded VLPs may even detect antibodies that are cross-reactive between HPV genotypes, which means that HPV antibody titers is not a direct measure of the HPV neutralising capacity of HPV.

Several assays have been developed to detect antibodies capable of neutralising papillomaviruses. They rely either on neutralisation of authentic virions, pseudotype virions, pseudovirions with encapsidated reporter genes or capsids carrying a reporter gene on their surface. Pastrana et al. reported an optimised pseudovirion-based neutralisation assay that was more sensitive than an HPV 16 VLP-based ELISA (Pastrana, Buck et al. 2004) when investigating sera from women who were immunised with HPV 16 VLP vaccines and women with natural HPV 16 infection. Even though the assay is type-specific for HPV 16, it does detect all known variant strains of HPV 16 (Pastrana, Vass et al. 2001). One study has suggested that neutralising antibodies correlate with the CIN grade.Almost 86% of women with normal cervix had neutralising antibodies. Twenty-two percent of women with CIN 1 were positive and the higher the grade of lesion the fewer women were positive (Kawana, Yasugi et al. 2002), suggesting that neutralising antibodies could be used as a marker for CIN grades. However, in the study of Wang et al. (manuscript) neutralising capacity was measured in seroconversion sample, stable antibody level serum samples and cervical cancer patient serum samples with no noteworthy differences between the patient groups. Of all samples positive for HPV 16 IgG, 74% had neutralising capacity.

Cell-mediated immune responses

The cell-mediated immune response is important in the prevention of HPV infection and HPV induced neoplasm. Both skin and genital warts have an infiltration of mononuclear cells (Chardonnet, Viac et al. 1986; Coleman, Birley et al. 1994) with an approximately equal amount of $CD4^+$ and $CD8^+$ cells. Another evidence of the importance of cell-mediated immune response in the control of HPV infection is the increased prevalence of

HPV in women with HIV (Sun, Kuhn et al. 1997; Palefsky, Minkoff et al. 1999; Ellerbrock, Chiasson et al. 2000).

The bulk of work that has been done on T cell responses to HPV focus on HPV 16 E6 and E7 antigens. These proteins are involved in cellular transformation, as described earlier, and are therefore interesting targets for therapeutic vaccines. IL-2 can activate lymphocytes and NK cells. Measurement of IL-2 responses by E6 and E7 recall stimulated cells revealed that E7 responses was found earlier in women who cleared their HPV 16 infection than in the persistent group. After clearance IL-2 production was decreased but increased in the persistent group. The same study also found that E7 responses were decreased in cervical cancer patients (de Gruijl, Bontkes et al. 1998). It has also been shown that there is a disease-stage variance in CD4$^+$ responses against different HPV 16 E7 epitopes. Three epitopes were identified and all women with CIN responded against all three with Th1 response. However, women with cancer evoked only Th2 response against one of the three epitopes. (Warrino, Olson et al. 2004). IFN-γ responses against HPV 16 E6 were observed in healthy donors, indicating that a HPV 16 E6-specific Th1 memory exists. However, responses to E7 could not be detected in these individuals (Welters, de Jong et al. 2003). It is not clear why an E6 response was found and not an E7. It could be that E7 antigen presentation to T cells by Langerhans cells is difficult since high-risk E7 is only expressed in the nucleus (Guccione, Massimi et al. 2002) leading to undetected antigen in healthy individuals. The E6 protein on the other hand is expressed both in the nucleus and the cytoplasm. Furthermore, responses to HPV E2 and E6 are impaired in cervical cancer patients (de Jong, van Poelgeest et al. 2004). de Jong et al. proposed a model for HPV 16-specific CD4$^+$ T cell immunity in the development of HPV related disease where persistent HPV 16 infection is due to failed induction of Th1/Th2 immunity. HPV 16 E2-specific memory Th cells have been found in healthy donors (de Jong, van der Burg et al. 2002) and in women who cleared their infection (Bontkes, de Gruijl et al. 1999). This could correspond with the findings that E2 expression is high in low-grade CIN but decreases in high-grade CIN and cervical carcinomas (Maitland, Conway et al. 1998; Stevenson, Hudson et al. 2000) and therefore would evoke an immune response against E2 in regression of lesions.

Cytotoxic T cell lymphocytes (CTL) are the ultimate effector cells of the Th1 type activation. HPV16 E6 specific CTL seem to be important in clearance of the virus (Nakagawa, Stites et al. 2000) where a lack of these cells was observed in women with HPV 16 persistence. Memory CTL precursors are detectable in CIN lesions but seem to not be able to prevent progression into cancer (Bontkes, de Gruijl et al. 2000).

The majority of these studies observe a lack of a systemic cellular immune response against HPV in the development of cervical cancer. But it is also important to measure local immune responses, especially for evaluation of vaccine approaches because systemic and local responses might not correlate. For example, IL-10 and IL-12 levels from plasma and cervical secretions were observed not to correlate (Castle, Hildesheim et al. 2002). Few studies have focused on determining the natural levels of cytokines to see if they are predictive of disease risk among HPV-infected individuals. A switch from Th1 to Th2 type cytokines has been suggested in the development of several cancer types. This also seems to be the case in cervical cancer. It has been observed that a decrease in IFN-γ (Th1 cytokine) is associated with poor prognosis of cancer outcome (El-Sherif, Seth et al. 2001; Gey, Kumari et al. 2003). El-Sherif et al. also observed increased levels of IL-10 (Th2 cytokine) and that the higher the grade of CIN the higher the levels of IL-10. IL-6 was also related to severity of cervical neoplasia (Tjiong, van der Vange et al. 1999). Analysing phenotypic and functional characteristics of lymphocytes isolated from preneoplastic lesions or from underlying stroma revealed that there were lower numbers of T cells in stroma and that there were high levels of IL-10 in the epithelium of the T-zone (Jacobs, Renard et al. 2003). This suggests that the low number of T cells and the IL-10 production might contribute to the predisposition of the T-zone to the development of CIN and cervical cancer. Change in the Th1/Th2 patterns are also seen in HPV infection before development of cancer (Scott, Stites et al. 1999; Crowley-Nowick, Ellenberg et al. 2000; Passmore, Burch et al. 2002). The study by Crowley-Nowick found increased levels of both IL-10 and IL-12 in women who had a co-infection with HIV, HPV and other STIs. However, the association between HPV and IL-10 and IL-12 levels could not be observed in the study by Gravitt et al. (Gravitt, Hildesheim et al. 2003).

How does HPV avoid the immune system?

Several immune response pathways can be affected by HPV and this contributes to the incidence of HPV related tumours.
Examples of how HPV evades or manipulates the immune response and their consequences are listed in the table on the next page:

EVASION STRATEGY	POSSIBLE CONSEQUENCE
L1/L2 expressed late in viral life cycle	Late development of antibodies
E proteins localise in the nucleus	Poor immune recognition and response
No viral replication in APC and No cell lysis	No opportunity for APCs to engulf virions i.e. no presentation to the immune response
No blood-borne phase	Late recognition by systemic response
HPV 17 E7 blockage of interferons	No intracellular protection
Downregulation of IL-18 expression	$CD8^+$ response is hampered
Downregulation of IL-8 expression	Initial immune response prevented
HPV does not activate Langerhans cells	Initial immune response prevented
Weak binding of peptides to MHC	Poor antigen presentation

Table 2. Listing possible immune evasion strategies by HPV and their possible consequences. References for table: (Fausch, Da Silva et al. 2002; Tindle 2002)

CHLAMYDIA TRACHOMATIS

It is estimated that 89 million new cases of sexually transmitted chlamydial infections occur worldwide every year (Gerbase, Rowley et al. 1998) and it is therefore the most common bacterial cause of sexually transmitted infection. It is an obligate intracellular bacterium with prevalence rates of 3-10% among sexually active women in the general community. In some populations prevalence as high as 24% can be found (Burstein, Gaydos et al. 1998; Turner, Rogers et al. 2002). A majority of infected individuals are asymptomatic and will therefore do not seek treatment (Belland, Ojcius et al. 2004). Untreated infection can persist for several months and even years and if left untreated can cause pelvic inflammatory disease, which in turn can lead to infertility and potentially fatal ectopic pregnancies. Just like viral infections, an infection with *Chlamydia trachomatis* can reoccur (reacquisition or maybe reactivation) (Stephens 2003; Hogan, Mathews et al. 2004).

Over the years a role for *Chlamydia trachomatis* in the development of cervical cancer has been discussed. Antibodies against *C. trachomatis* have consistently been associated with an increased risk of invasive cervical cancer (Koskela, Anttila et al. 2000; Anttila, Saikku et al. 2001; Smith, Bosetti et al. 2004). A Swedish population-based prospective study investigating *C. trachomatis* DNA in women who were later diagnosed with invasive cervical cancer found a high-risk associated with chlamydial DNA

in the cervix and future development of cervical cancer (Wallin, Wiklund et al. 2002). The underlying mechanism behind this association is not known but it seems that the bacterial infection may enhance the establishment of a persistent HPV infection. Women with oncogenic HPV infection are more likely to become HPV persistent if they have had a previous
C. trachomatis infection (Silins, Ryd et al. 2005). Possible explanations for this finding could be *C. trachomatis* interference with the immune response by inhibition of apoptosis (Fan, Lu et al. 1998). The bacteria can also evade the immune response by disrupting the IFN-γ signalling pathway (Zhong, Fan et al. 1999).

HERPES SIMPLEX VIRUS TYPE 2

Herpes simplex virus (HSV) belongs to the herpesvirus family. This family contain some of the most important human pathogens, such as Cytomegalovirus, Epstein-Barr virus, Varicella-Zoster, Human herpes virus 6 and 7 and Herpes simplex virus types 1 and 2. Because HSV 2 is sexually transmitted and infects epithelial cells it was proposed to be involved in cervical cancer during the 1960's and 1970's (Rawls, Tompkins et al. 1968; Munoz, de-The et al. 1975). After the discovery of the HPV, HSV 2 was suggested to be a co-factor to HPV (zur Hausen 1982). Over the years several studies have addressed the issue of HSV and cervical cancer. HPV-positive tissues infected with HSV were able to maintain genomic copy number even though genes required for replication were repressed (Meyers, Andreansky et al. 2003) suggesting that HSV could play a supporting role in HPV replication. HPV16-immortalised epithelial cells were found to induce tumours in nude mice when they were transfected with the *Xho-II* fragment of HSV 2 DNA (DiPaolo, Woodworth et al. 1998). However, analyses of large series of cervical cancers found no evidence for presence of the *Xho-II* fragment of HSV 2 DNA (Tran-Thanh, Provencher et al. 2003). In a meta-analysis of six longitudinal seroepidemiological studies, there was no association between HSV 2 and cervical cancer (Lehtinen, Koskela et al. 2002). In contrast, a pooled case-control study did report an association of HSV 2 and cervical cancer (Smith, Herrero et al. 2002).

ENVIRONMENTAL RISK FACTORS

Smoking

The majority of reports published on effects of smoking and cervical cancer find an association, even after adjusting for high-risk HPVs (Kruger-Kjaer, van den Brule et al. 1998; Olsen, Dillner et al. 1998; Hildesheim, Herrero et al. 2001; Lacey, Frisch et al. 2001). This association could be explained by reports that long-duration smokers also have a longer duration of HPV infection (Castellsague and Munoz 2003). Several studies also report on a direct effect of cigarette smoke on HPV-induced cellular transformation.

Yang and colleagues showed that cigarette smoke condensate malignantly transformed HPV 16-immortalized human endocervical cells (Yang, Jin et al. 1996). Nicotine-derived carcinogens have also been found in cervical mucus of smokers (Prokopczyk, Cox et al. 1997).

Oral contraceptives

The use of oral contraceptives (OC) has been associated with cervical cancer in several epidemiological studies, also after adjustment for HPV DNA. A review that analysed data from 28 studies investigating use of OC as risk factor for cervical cancer (Smith, Green et al. 2003) and that included 80% of the information world wide on this topic found that the relative risk of developing cervical cancer increased with increasing duration of use. The risk was twice as high when using OC for more than 10 years compared to those who never used OC. Not much is known about the mechanism behind how OC could influence progression into dysplasia. A few *in vitro* studies have tried to explore probable mechanisms. Transcription of E6 and E7 can be stimulated by estradiol in cell lines with integrated HPV 16. Estradiol is a major oestrogen in ovarian follicular fluid (Mitrani-Rosenbaum, Tsvieli et al. 1989). Long-term use of hormones influences the cancer development in HPV 16 transgenic mice (Arbeit, Howley et al. 1996; Elson, Riley et al. 2000). Even though four of the studies in the systemic review by Smith et al. adjusted for barrier contraceptive methods, the fact remains that 80% of unmarried women who use OC are not using barrier methods (Epstein 2003). It is therefore difficult to conclude whether the association seen is due to hormonal influences, lack of condom use or other factors.

Multiparity

Multiple pregnancies increase the risk of both carcinoma in situ and cervical cancer. In a pooled analysis, the odds ratio for cervical cancer in women with seven or more full-term pregnancies was 3.8 (95% CI 2.7-5.5) compared to women who had never been pregnant (Munoz, Franceschi et al. 2002). This significantly increased risk was seen in squamous cell cancer but not in adenocarcinoma or adenosquamous carcinoma. Possible explanations include nutritional, hormonal, traumatic and immunological mechanisms (Castellsague and Munoz 2003). Results from a study on the location of the T-zone suggest that with increasing numbers of live births, the transformation zone is directly exposed for longer periods to external agents e.g. HPV involved in dysplastic lesions (Autier, Coibion et al. 1996).

IMMUNOGENETIC RISK FACTORS

Human Leukocyte Antigen complex

The role of the Human Leukocyte Antigen (HLA) system is to activate the adaptive immune response by presenting short, pathogen-derived peptides to T cells (Klein and Sato 2000). The HLA complex is found on chromosome 6 and contains more than 200 genes. Two structurally different HLA gene classes are found in this region called class I and class II. The main actors in the HLA class I genes are HLA-A, B and C. The HLA class II genes are given three letters to designate their loci on chromosome 6. The first letter D indicates the class, the second (M, O, P, Q or R) the family and the third (A or B) the α or β chain. An allelic variant is given a 4 digit number e.g. HLA-DQB1*0602, where 0602 is the variant of gene 1. Class I molecules are expressed by all nucleated cells and present peptides to and are recognised by CD8$^+$ T cells. Class II molecules are expressed by antigen presenting cells (APCs), such as B cells and keratinocytes and they present their peptides to CD4$^+$ T cells. If the immune response is going to be efficient in infectious disease, the HLA molecules must present peptides derived from foreign proteins. In addition the T cell repertoire must have clones that can be activated by such HLA-bound peptides (Hill 1998). If these requirements are not fulfilled, a person with a certain combination of HLA alleles could be more susceptible to disease compared to an individual who has another combination.

To date, HLAs have been the most extensively studied immune-related genes in relation to HPV and cervical cancer. Other genes studied in relation to cervical cancer are TNF-α and their polymorphisms, MIC-A genes and KIR genes (see below). Downregulation of HLA class I antigens is commonly found in cervical cancer (Connor and Stern 1990; Cromme, Meijer et al. 1993; Torres, Cabrera et al. 1993). A recent study found that the HPV 16 E5 protein could be involved in this downregulation by preventing the HLA-A and HLA-B in the Golgi apparatus to reach the cell surface. The protein does not seem to affect HLA-C (Ashrafi, Haghshenas et al. 2005). Individual alleles in the HLA class I complex have been associated with either increased or decreased risk of cancer. The HLA-CW*0202 gene was related to a protective effect in women with high grade and low-grade intraepithelial neoplasia (Wang, Hildesheim et al. 2002). In the same population, women who had a combination of HLA-B*07 and the HLA class II gene HLA-DQB1*0302 had an 8.2-fold increased risk for cancer/HSILs. There was also an increased risk for low-grade neoplasia (Wang, Wheeler et al. 2001). Reports on downregulation of HLA class I in CIN are not as consistent as for cancer. It has been extensively studied if HPV variants are associated with particular HLA alleles. Three studies with contradicting data have been published. One study found that women with HLA-B*44, *51 and *57 who were infected with the HPV E6 variant L83V

had an almost a four-fold increased risk for cancer compared with controls (Zehbe, Mytilineos et al. 2003). Hildesheim et al reported an association with an allele in the HLA class II region and with another variant (Hildesheim, Schiffman et al. 2001). The study by Bontkes et al. found no link between HPV 16 variants and various HLA alleles (Bontkes, van Duin et al. 1998). A series of studies have examined the relationship between HLA class II and cervical cancer and its precursors. The most consistent finding is the protective effect of HLA-DRB*13/DBQ1*0603 (Hildesheim and Wang 2002). DRB*03 and DQ6 are alleles that have been associated with an increased risk (Apple, Erlich et al. 1994; Helland, Borresen et al. 1994; Sanjeevi, Hjelmstrom et al. 1996; Helland, Olsen et al. 1998; Beskow, Josefsson et al. 2001; Lin, Koutsky et al. 2001; Ghaderi, Wallin et al. 2002). DRB1*0301 has also been seen patients susceptible to recurrent respiratory papillomatis (Gelder, Williams et al. 2003).

Killer immunoglobulin-like receptors

NK cells can provide a back-up system when cytotoxic T cells fail. Killer immunoglobulin-like receptors (KIRs) can be found on NK cells and T cells and their role is to regulate the inhibition or activation of NK cell activity.

The KIRs are a family of genes found on chromosome 19q13.4 within the leukocyte receptor complex (Trowsdale, Barten et al. 2001). Within this complex 17 KIR genes have been identified (Marsh, Parham et al. 2003) some of which have specificity for polymorphic epitopes on HLA-A, -B, -C and –G (Vilches and Parham 2002). The specific names given to these genes and their protein product are based on the molecular structure. The letter D denotes the extra-cellular Ig domain. There can be 2 or 3 domains (Andre, Biassoni et al. 2001). An L, S or a P follows the D. These stand for long (L), short (S) cytoplasmic tail or pseudogenes (P) (Vilches and Parham 2002). The long tails contain inhibitory motifs and the short tails contain, activating motifs (Bruhns, Marchetti et al. 1999). When two or more genes have very similar sequences they can be given a final letter, example 2DL5A and B (Gomez-Lozano, Gardiner et al. 2002).

The inhibitory receptors recognize HLA class I molecules. When an HLA class I molecule is absent, e.g. as a result of microbial interference or selective pressure during the carcinogenic process, the inhibitory signal is lost and the activated receptors will order the NK cell to attack the abnormal cell (Figure 5) (Delves and Roitt 2000).
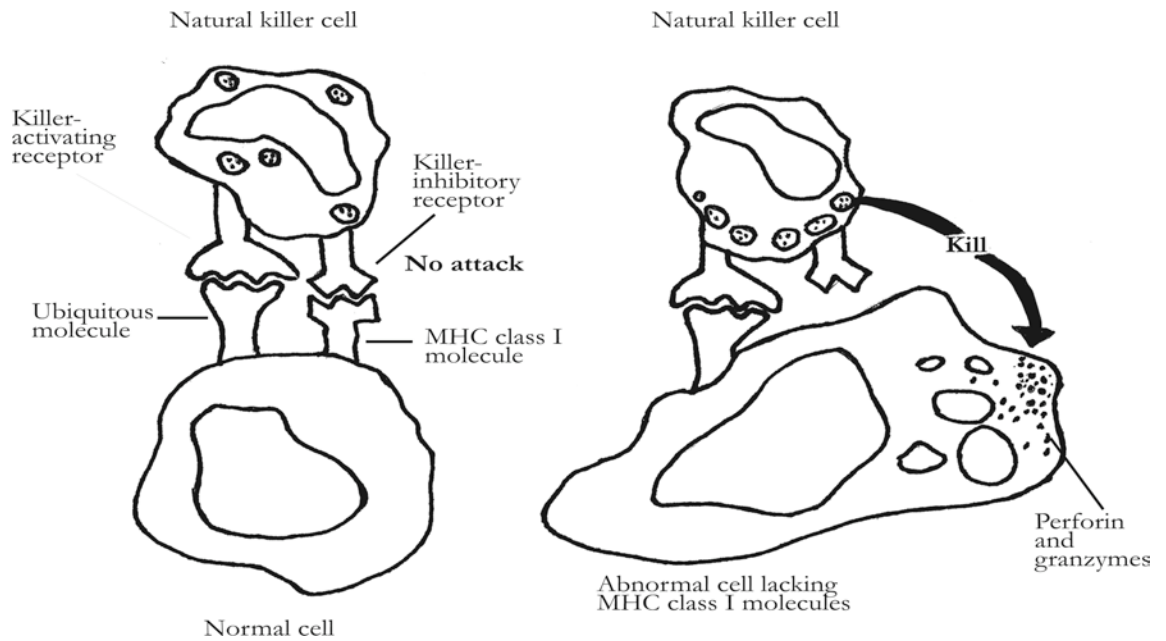
Figure 5. A system used by NK cells to recognise normal cells and cells that lack HLA class I surface molecules. Activating KIRs recognise a number of molecules present on the surface of normal cells, and in the absence of an inhibitory signal from killer-inhibitory receptors, which recognise HLA class I molecules, the receptors issue an order to NK cells to attack and kill the other cell. Cytotoxic granules of NK cells, which contain performing and granzymes, become polarised at the interface with the target cell and are released into the cell (adapted from Delves and Roitt, 2000).

The KIR region is polygenic and polymorphic. The content and number of genes varies between haplotypes (Uhrberg, Valiante et al. 1997). There are two groups of haplotypes, A and B, based on the genes present. The definition of these groups in the KIR nomenclature report 2002 says that group B haplotypes are characterised by presence of one or more of the following genes: KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5 and KIR3DS1 and the group A haplotypes are characterised by the absence of all these genes (Marsh, Parham et al. 2003).

So far, only a few studies have been published on the association of KIR and disease. Increased numbers of activating KIR genes in patients with type 1 diabetes compared with control subjects was seen in a study by van der Slik et al. The KIR2DS2 gene in combination with its putative HLA ligand was more frequent in patients than in controls. This could mean that the KIR-HLA combination in absence of inhibitory KIR-HLA ligand pairs is associated with an increased risk of disease (van der Slik 2003). KIR2DS1 and KIR2DS2 was reported to have a strong association with development of

psoriatic arthritis (Martin, Nelson et al. 2002) when the HLA ligands for the inhibitory receptors, KIR2DL1 and KIR2DL2/3 were missing.

In HIV, the presence of KIR3DS1 together with HLA-B Bw4-80Ile allele was associated with a delayed progression to AIDS. If KIR3DS1 was absent but HLA-B Bw4-80Ile present no difference in AIDS outcome was detectable. But when KIR3DS1 was present and HLA-B Bw4-80Ile absent the disease progress seemed to be more rapid (Martin, Gao et al. 2002). A weak interaction between KIR2DL3 and HLA-C1 (presence of asparagines at position 80 in protein sequence) is reported to be protective against HCV (Khakoo, Thio et al. 2004).

One study has been published regarding the association between KIRs, HLA ligands and CIN 3/cervical cancer. Carrington et al. found, just like van der Slik, that disease risk increased for each additional activating KIR gene in a woman. A strong protection was found among women who had both HLA-C Group 2 and Bw4 ligands but not KIR2DS1 and KIR3DS1 compared to women who had these two KIRs but not the HLA ligands suggesting a role of the innate immunity and natural killer cells in cervical neoplasia (Carrington, Wang et al. 2005).

PREVENTION OF HPV AND CERVICAL CANCER

SCREENING AND HPV TESTING

Since the Pap smear screening was introduced in the Nordic countries mortality rates have fallen with 76% in Iceland, 73% in Finland and 60% in Sweden between 1986-1995 (Sigurdsson 1999). Norway didn't introduce organised screening until 1995. In all the countries, the incidence rates have decreased with a world standardised rate from 19.4 between 1958-1962 to 8.9 per 1000,000 in 1993-1997 (Moller, Fekjaer et al. 2002). Different countries recommend different time intervals for when a woman should have her Pap smear taken. According to the European Guidelines for Quality assurance in cervical cancer, every 3-5 years is recommended. In Finland and the Netherlands they screen every 5 years. In USA one Pap smear/year is recommended. The screening programme in Finland costs less than the expenses saved by the prevention of cancer and associated treatment and care costs (Hakama and Hristova 1997).

Even though Pap smears have reduced cancer mortality, these tests are not always sensitive enough to distinguish between healthy and sick patients. The risk that a woman with CIN will have a false-negative diagnosis on cytology has been estimated to be about 25% (Boyes, Morrison et al. 1982). By testing for oncogenic HPV it is possible to identify the women who are actually at risk for developing CIN. The optimal age for HPV testing is likely to be between 30-40 years of age, i.e. before the risk of cervical cancer starts to increase. By 35 years of age the population-based prevalence with oncogenic HPV types in Western countries is 1-8% and the spontaneous clearance rate is lower than that of younger people (Chua, Wiklund et al. 1996; Rozendaal, Walboomers et al. 1996; Kjellberg, Wiklund et al. 1998; Cuzick, Beverley et al. 1999; Forslund, Antonsson et al. 2002). HPV positive women over 35 years of age have a high-risk of CIN or cancer. It has been postulated that protective effect of a negative HPV test would be longer than the one of a negative Pap smear (Kjaer, van den Brule et al. 2002). The protective effect of a negative Pap smear lasts about 3 years, whereas the incubation time of an HPV infection is much longer (Dillner 2001). If this is the case also in practice, which is currently being evaluated, women with negative HPV tests do not need as many Pap smears. Thus, resources could be saved and it is possible that the attendance rate of a screening program could increase when fewer visits are required. Insufficient treatment of cervical neoplasia increases the risk for residual or recurrent disease. The risk to develop invasive cancer after treatment of CIN is increased up to 5 times up to 8 years after treatment. The current follow-up procedure is colposcopy and Pap smear. Several studies have shown that recurrent disease is associated with persistent HPV infection (Chua and Hjerpe 1997;

Kjellberg, Wadell et al. 2000). This suggests that HPV DNA testing could also be used in follow-up after treatment (Söderlund-Strand et al. in press J Clin Micobiology).

VACCINATION

Virus-like particles

As HPV is the major risk factor for cervical cancer, a prophylactic vaccine against HPV infections would be expected to reduce the incidence of cervical cancer. All prophylactic vaccine candidates undergoing clinical trials today are based on HPV VLPs (virus like particles). Vaccinations with virus-like particles have been shown to be both safe and highly protective in animal models. Rabbits that were immunised with cotton-tail rabbit papillomavirus (CRPV) VLPs were protected upon CRPV challenge and neutralising antibodies were detected in most of these studies (Breitburd, Kirnbauer et al. 1995; Jansen, Rosolowsky et al. 1995). VLP vaccines have also been found to be safe and induce a strong antibody response in humans. In a double-blind, placebo-controlled, dose-escalation trial with HPV 16 VLP L1, all study subjects seroconverted and neutralising antibody titers correlated with ELISA titers. Serum antibody levels did not differ between groups who received vaccine with or without adjuvant (Harro, Pang et al. 2001). Two other phase I trials investigated the immunogenicity of HPV 11 VLP L1. The vaccine was well tolerated and induced high titers of neutralising antibodies (Brown, Bryan et al. 2001; Evans, Bonnez et al. 2001). Cellular responses cross-reactive between HPV types were observed in a lymphoproliferative assay. Although the importance of these responses or clearance is not established, the results indicate that the possibility that VLP immunization might be able to induce protection or improved clearance against other HPV types should be considered.

In the first phase II clinical trial that was published 2392 women were given HPV 16 L1 vaccine or a placebo. A 100% efficacy against persistent HPV infection as well as against HPV 16 associated CIN lesions was found (Koutsky, Ault et al. 2002). A more recent study evaluated the efficacy and safety of a bivalent vaccine candidate using a HPV 16/18 L1 VLP (Harper, Franco et al. 2004). They saw a 91.6% efficacy against any infection (transient or persistent) and 100% against persistent HPV 16/18 infection.

Although the VLP vaccine has been proven to be safe and efficient, there are many issues to be solved regarding their use. At what age should immunisation take place? Studies have shown that women become infected quite soon after sexual debut (Kjaer, van den Brule et al. 2002). Should a vaccine also be administrated to children or only during adolescence? Should there be "catch-up" vaccination in older ages? Should the vaccine be given to men as well as women? How susceptible are men to HPV

infection? Which HPV types should be included in a vaccine? If the aim is to reduce cervical cancer incidence by 90%, 8-10 HPV types would have to be included in the cocktail, under the assumption that each type represented in the cocktail would be 100% effective (Dillner and Brown 2004). *In vitro* and animal studies have demonstrated cross-reactivity when using L2 protein for immunisation. The L2 protein induces a cross-neutralising antibody, which recognises a broadly neutralising epitope (Kawana, Yoshikawa et al. 1999; Roden, Yutzy et al. 2000; Kawana, Kawana et al. 2001). Maybe these findings could lead to vaccines that do not need to contain as many as 10 types. However, the L2-induced neutralising response has much lower titers than the type-specific neutralising response induced by VLPs. Evaluation of the use of low-risk HPV types in a vaccine is ongoing. HPV 6 and 11 do not cause high-risk dysplasia, but they are responsible for 90% of genital condyloma lesions. Another factor that is important in the design of a vaccine rationale is the duration of the antibody responses against the antigen. The levels of cervical antibodies appear to fluctuate during the menstrual cycle in women who were not using oral contraceptives (Nardelli-Haefliger, Wirthner et al. 2003), raising the possibility that protection might be less efficient during certain stages of the menstrual cycle.

Who should receive a HPV VLP vaccine? The vaccines are likely to be expensive and the countries that could afford vaccination campaigns are probably already providing cervical cancer screening. Most cases of cervical cancer occur in developing countries where screening is rare and where a vaccine would be expected to have an enormous health impact. The issue of whether it is absolutely necessary to maintain a cold store chain, which could be difficult in a poor country, has not been fully investigated.

DNA vaccination

Because VLP production and purification is costly, an alternative would be immunisation with DNA expressing L1, possibly also L2. DNA immunisation is a rather new vaccination strategy that involves the direct introduction into the host of plasmid DNA encoding the desired antigen. With these types of vaccines it would be easier and cheaper to produce cocktails against many HPV serotypes. The L1-specific humoral immune responses generated by L1 DNA vaccines have led to preventive effects in animal models (Donnelly, Martinez et al. 1996; Sundaram, Tigelaar et al. 1997). Several studies have investigated the immune response to HPV DNA vaccines in mice. Three of them are studies where they have immunised with HPV 16 L1 DNA by different routes of administration (Dupuy, Buzoni-Gatel et al. 1999; Kowalczyk, Wlazlo et al. 2001; Rocha-Zavaleta, Alejandre et al. 2002). All three studies observed serum antibodies against HPV 16. Local IgA was also found in two of them. Dupuy et al. did not find high titers of either IgG or IgA, but found $CD4^+$ and $CD8^+$ responses.

Rocha-Zavaleta's study found that after oral immunisation the local IgA was higher than after immunisation by the intramuscular route. However, the local IgA response was not as long lasting as the serum IgG response. This is in contrast to a study where mice immunised vaginally with a DNA vaccine against HPV 6 L1 developed a long-lasting IgA resonse (Schreckenberger, Sethupathi et al. 2000).

The expression of L1 antigens after immunisation using DNA plasmids coding for the L1 sequence is commonly low or undetectable. Various strategies such as codon optimisation and inactivation of RNA elements have been used to overcome this problem (Leder, Kleinschmidt et al. 2001; Cheung, Cheng et al. 2004; Mossadegh, Gissmann et al. 2004; Rollman, Arnheim et al. 2004). Mossadegh et al. replaced all codons by those more frequently used in mammalian genes, to increase expression level of HPV 11 L1. Mice immunised with the humanised gene induced high levels of HPV 11 antibodies, compared to the wild type (wt) gene. This study is similar to the one of Leder et al. who humanised the gene of HPV 16 L1. Another approach is the one by Collier et al. who showed that the first 514 nucleotides of the L1 coding region contain multiple inhibitory elements that act independently of one another and that the major inhibitory element is located within the first 129 nucleotides of the L1 gene. Introduction of point mutations in the inhibitory elements in the 5' end of the L1 gene, which altered the RNA sequence without affecting the protein sequence, specifically inactivated the inhibitory elements and resulted in production of high levels of human papillomavirus type 16 L1 mRNA and protein in human epithelial cells (Collier, Öberg et al. 2002). The immunogenicity of these mutant plasmids was investigated in mice. Neutralising antibodies and cellular immune responses were found, whereas the wt plasmid failed to induce humoral responses (Rollman, Arnheim et al. 2004).

Regarding the safety aspect, genomic integration of DNA vaccines has not yet been demonstrated. It is estimated that the frequency of integration is much lower than that of spontaneous mutations.

Therapeutic vaccination

Another approach to vaccination is to eliminate already ongoing HPV infection or intraepithelial neoplasia or cancer. The expression of HPV oncogenes in cervical tumours provides a good target for this kind of treatment because they are expressed in all stages of epithelial differentiation. Various strategies have been tested in mouse models using peptides, proteins, DNA, viral vectors or a combination. The aim of these vaccines is to express E6 or E7 antigens that will mount an immune response and clear the dysplasia or cancer.
E7 peptides have been tested in phase I/II clinical trials immunising women who were diagnosed with CIN or VIN (vulvar intraepithelial neoplasia)

(Steller, Gurski et al. 1998; Muderspach, Wilczynski et al. 2000). Cinical trials have also been carried out using HPV proteins. Proteins can have the advantage over peptides that they may be presented by many HLA haplotypes. These trials have shown that protein vaccines are safe but that the immunogenicity and clinical outcome varies between individuals (Goldstone, Palefsky et al. 2002; Santin, Bellone et al. 2002; Santin, Bellone et al. 2003). Since HPV E6 and E7 are oncogenic, a DNA vaccine containing these genes is likely to cause malignancy. Attempts to develop therapeutic vaccines containing E7 have been made e.g. engineered constructs diverting E7 away from the nucleus to other parts of the cell such as the endosomal and lysosomal compartments or mutated the E7 gene so that the transformation potential is inactivated (Moniz, Ling et al. 2003). One small clinical study with therapeutic DNA vaccine encoding HPV 16 E7 in patients with anal HPV infection has been published (Klencke, Matijevic et al. 2002). The study was so small that no conclusion on immune protection could be made but an immune response was observed. Viral vector vaccines constitute another approach that is currently being evaluated. The results are encouraging because specific immune responses can be demonstrated. However, just as with the other approaches no clear correlation between immunogenicity and clinical outcome can be observed (Davidson, Boswell et al. 2003; Davidson, Faulkner et al. 2004; Smyth, Van Poelgeest et al. 2004).

AIMS OF THE INVESTIGATION

The overall aim of this thesis is to contribute to the understanding of natural history of HPV infection and progression to cervical intraepithelial neoplasia. It also aims to demonstrate the usefulness of prospectively followed biobank-based studies for immunological and virological studies on the natural history of cancer.

I. To study risk factors in the etiology of cervical cancer, in particular by studying immunological markers of infections in women who later develop invasive cervical cancer.

II. To study the immunogenicity of a modified HPV 16 L1 DNA vaccine in mice.

III. To study local cytokine levels, in particular CXCL8 (IL-8) and IFN-γ, in women who either had a persistent or a cleared HPV 16 infection.

IV. To investigate the presence of killer immunoglobulin-like receptor (KIRs) genes and genotypes in Swedish women and whether the presence of certain genes and genotypes would be associated with CIN risks.

MATERIAL AND METHODS

Paper I, III and IV are epidemiological studies, where I have studied causes of cervical cancer and HPV infection within a prospectively followed population. These studies were nested case control studies. In studies I and IV cases were identified using registry linkage to identify cases of cancer or CIN occurring during follow-up. Eligible controls were women who had not developed disease at the time of diagnosis of the matched case. Development of cervical cancer is a long process and time from enrolment of a woman into a cohort until she becomes a case can take decades. An advantage of the studies in paper I and IV is that all cases and controls were identified through a cancer registry linkage with data from the biobanks. A biobank is a research project that involves storage of biological material, such as serum, DNA and tissues. Biobanks can also be built within the health care system for administrative or clinical diagnostic purposes. The same personal identifiers that identify the specimens are used in all biobanks and in all health data registries, thereby enabling retrieval of data and identification of cases and controls during many years of follow-up and obtaining specimens also from several biobanks.

In paper II we have used mice to investigate the immunogenicity of a potential prophylactic DNA vaccine against HPV 16.

PAPER I

The study base comprised four population-based biobanks in Finland, Norway, Iceland and Sweden. More than 1,000,000 residents had donated blood between 1973-1997. Cases and controls were identified by record linkage between the cancer registries and serumbank databases. The linkage was done on the basis of personal identification numbers. Women diagnosed with invasive cervical cancer and who had donated a serum sample at least one month before diagnosis was considered a case. Five controls per case were individually matched for sex, age at serum sampling (within 2 years), storage times (within 2 months) and for region. In total 543 cases and 2675 controls were identified and included in the study. The majority of cases were classified as having squamous cell carcinoma (SCC) (n=408), 109 cases were diagnosed with adenocarcinoma (AC) and 21 with adenosquamous carcinoma (ASC). The serum samples were analysed for antibodies against HPV 6, 16 and 18, *Chlamydia trachomatis* and Herpes simplex virus type 2.

The statistical analyses were made with conditional logistic regression. Paraffin embedded biopsies and histopathological slides from cases were also retrieved and cancer diagnosis confirmed by a single expert pathologist.

DNA is extracted from the biopsies and analysed for presence of HPV DNA. Diagnostic confirmation, HPV DNA and cotinine (a marker for nicotine use) analysis are currently ongoing.

PAPER II

A eukaryotic expression plasmid encoding the HPV 16 L1 DNA had been mutated in such a way that higher protein expression is yielded. The modified and a wild type plasmid were used for immunisation of mice to evaluate its potential as a prophylactic vaccine in mice. The wild type plasmid (pCL1wt) contains inhibitory sequences, which makes expression impossible in human monolayered cell cultures. By inserting point mutations at the 5' end of the gene it is possible to inactivate the inhibitory sequence without altering the protein sequence. Two mutated plasmids were used in the study. The difference between them is that pCL1MUT has introduced point mutations only at the 5' end whereas pCL1MUTDP has been further modified at position 570 and 573, where an intragenic polyA signal was destroyed.

Four groups of C57Bl/6 mice were immunised intra muscularly (i.m.) with the three plasmids described above or with an empty plasmid (pKCMV). One group of mice remained naïve. The adjuvant used for i.m. immunisation was pegylated murine granulocyte-macrophage colony-stimulating factor (PEG-GMCSF). A gene gun protocol was also set up where 10 animals were immunised with a mixture of all three plasmids, together with 3 control animals (pKCMV). The skin of the gene-gun immunised mice was pre-treated with imiquimod, an immune response modifier. All mice were immunised 3 times at weeks 0, 4 and 20. Sera were collected 10 days after the second and third immunisation. All animals were sacrificed on day ten after the third immunisation. Spleenocytes were collected for analysis of cell-mediated immune responses.

An L1VLP-based ELISA and a pseudovirus neutralisation assay measured antibodies. T cell responses were analysed by measuring IFN-γ responses in an ELISpot assay. CD8^{+} T cells were depleted to determine which fraction of T cells that secreted IFN-γ.

PAPER III

Cervical smears collected in a Swedish, population-based study were analysed for cytokine concentration. The study enrolled 12,527 women. Of these women, 67 were found to be HPV 16 DNA positive at enrolment. At follow-up, on average 23 months later, 32 were still HPV 16 DNA positive but 35 had cleared the infection.

An additional 19 women, randomly selected among HPV DNA and seronegative women, were included in the study as controls. Serum samples were analysed for *Chlamydia trachomatis*, HSV 2 and HPV antibodies. The women also filled in a questionnaire about their choice of contraceptives, smoking habits, lifetime partners and number of pregnancies.

Cytokine kits from Biosource and a Luminex 100^{TM} were used to measure concentration of CXCL8 and IFN-γ. Significant concentration differences between sample 1 and 2 were determined by a ranking test. Covariate effects on cytokine concentration changes were analysed by a Mann-Whitney test.

PAPER IV

In this nested case-control study, 65 women diagnosed with CIN and 150 healthy control women were analysed for KIR genes and genotypes.

Sequence-specific primer PCR performed the KIR typing. Primers for 14 KIR genes and 6 additional primer mixes for KIR2DL5-subtyping were used to detect KIR genes in the study population.

All samples had also been analysed for HPV 16 and 18 antibodies, MICA genes and HLA DQA1, DQB1 and DRB1 genes.

RESULTS AND DISCUSSION

RISK FACTORS IN INVASIVE CERVICAL CANCER (PAPER I)

In this large-scale prospective seroepidemiological study 543 cases with invasive cervical cancer and 2675 matched controls were analysed for specific antibodies against HPV6, 16, 18, *Chlamydia trachomatis* and HSV-2. The risk of developing invasive cervical cancer was more than twice as high for women who were HPV16 seropositive than for those who were seronegative. Women who were HPV18 positive had a small increased risk of developing cancer. However, when investigating risk factors associated with histological type, HPV18 had a clear risk associated with developing adenocarcinoma (AC). HPV18 DNA is known to be preferentially found in adenocarcinoma (Clifford, Smith et al. 2003), but prospective evidence indicating that this histological subtype-specific association is of etiological significance has not been available before. HPV 16 on the other hand was associated with the highest risk of developing squamous cell carcinoma (SCC).

An indication of possible antagonism between different HPV types was described by Evans et al, who showed a protective effect on cervical cancer by a history of condyloma accuminata (genital warts) (Evans, Bond et al. 1992). Genital warts are usually caused by HPV6/11 and they are one of the most common sexually transmitted infections. In the seroepidemiological field this issue was raised when results from two studies found that protection against cervical cancer was seen in women who were seropositive against certain broadly cross-reactive HPV antigens (Dillner, Lenner et al. 1994) and by the finding that the cervical cancer risk associated with HPV 16 seropositivity differed substantially between populations at low risk and high risk for STIs (Dillner, Lehtinen et al. 1997). A highly elevated risk was observed among HPV 16 seropositive women in the low risk population, whereas there was no excess risk in the high risk population. This has prompted investigation as to whether an interaction between HPV 16 and other HPVs might exist. An interaction can be defined as that the joint effect of two or more different exposures for disease development is more or less than expected than if the exposures are independent. We found an antagonistic interaction between HPV 16 and 6, meaning that women who were seropositive for HPV 16 alone was at higher risk of developing cervical cancer than those women who were positive for both HPV types. Our findings confirm two previous studies on antagonistic interaction between HPV 16 and 6 (Luostarinen, af Geijersstam et al. 1999; Silins, Wang et al. 1999). The mechanism behind this antagonism is not known. It cannot be due to prevention of infection since seroconversion to both HPV types is observed. Conceivably prior infection with HPV 6 may affect whether an infection with HPV 16 becomes persistent or not. The study of

interference between HPV types is important in understanding HPV-induced carcinogenesis and for design and evaluation of vaccines. Further studies are needed to clarify this potential interaction.

Both *Chlamydia trachomatis* and HSV 2 have been reported to be risk factors in the development of cervical cancer. We found in the present study that history of *Chlamydia trachomatis* was clearly associated with an increased risk of cervical cancer in women with SCC, but also with AC and ASC, although the significance was not as strong as for SCC. HSV 2 was associated with a small excess risk (of borderline significance) of developing cervical cancer, but did not have any association with a specific histological type. The association between *C. trachomatis* and cervical neoplasia has been investigated in several studies. It appears that *C. trachomatis* is not involved in the progression or persistence of cervical neoplasia, but possibly in the persistence of HPV. The data on HSV 2 as a co-factor in cervical cancer development is less consistent between different studies than those of *C. trachomatis*. The weak association observed in the present study could be due to residual confounding.

Some of the serum samples were taken as long as 30 years ago. To study the possibility that antibody decay had occurred we investigated seroprevalences and odds ratios for ICC and SCC related to HPV 16 seropositivity by three serum sampling periods. Seroprevalences were stable over time. Thus, we have no evidence of significant antibody decay over time. Also, it appears that the epidemic spread of HPV 16 has been stable during the 1970's to 1990's.

Overall, the present study provided further evidence of the etiological role of HPV 16 and 18 in cervical cancer. It has also confirmed previous data indicating that there is an antagonistic interaction between HPV 6 and 16 and that *C. trachomatis* is a possible co-factor in the development of cervical cancer.

PROPHYLACTIC DNA VACCINATION AGAINST HPV 16 L1

(PAPER II)

Modification of the HPV 16 L1 gene results in expression of L1 protein in HeLa cells (Collier, Öberg et al. 2002). The silent mutations of the pCL1MUT were made within the first 514 nucleotides of the L1 gene. Even higher levels of L1 were observed *in vitro* when the gene had been further modified by altering the polyA signal (plasmid pCL1MUTDP). We aimed at investigating the capacity of these plasmids to induce immune responses in experimental animals.

41

The animals were immunised at 3 different time points, either by intramuscular immunisation or by using a gene gun. The adjuvant used for i.m. vaccination was PEG-GMCSF, a cytokine that induces differentiation and local recruitment of professional antigen-presenting cells. This adjuvant has been shown to elicit humoral responses when using plasmid DNA vaccination (Rollman, Hinkula et al. 2004) (Leachman, Tigelaar et al. 2000). We tried in one experiment to immunise animals with DNA in the absence of adjuvant but immune responses were not observed (data not shown). For the gene gun immunisation the mice were treated with imiquimod cream. This cream is used as treatment against genital warts and has been shown to be effective as an adjuvant in DNA immunisation (Zuber, Brave et al. 2004). All mice immunised with the mutated plasmids had both binding and neutralising IgG antibodies against HPV 16. The responses were observed in both i.m. and gene gun immunised animals (see Table 6). The wild type plasmid did not induce any humoral responses. Other studies that used wild type HPV 16 L1 plasmids report the presence of both binding and neutralising antibodies but titers are relatively low and other adjuvants and immunisation schemes were used which makes comparison quite difficult (Dupuy, Buzoni-Gatel et al. 1999; Kowalczyk, Wlazlo et al. 2001; Rocha-Zavaleta, Alejandre et al. 2002). Because cervical IgA may be important in the protection against HPV, it would have been interesting to investigate whether our plasmids could induce such antibodies in the cervix. Local IgA responses were seen in two studies with DNA immunisation administrated orally (Rocha-Zavaleta, Alejandre et al. 2002) and vaginally (Schreckenberger, Sethupathi et al. 2000).

We also wanted to investigate cell-mediated responses (CMI) because of its suggested role in clearance of HPV (De Bruijn, Greenstone et al. 1998). Spleenocytes from sacrificed mice were restimulated with HPV 16 L1 VLPs and IFN-γ responses were measured. We observed that 8/10 animals had detectably increased numbers of IFN-γ secreting T cells. The gene gun immunisation was not as successful in raising CMI compared to i.m. immunisation. Only 3 animals out of ten had detectable IFN-γ secreting T cells. There are several possible reasons for this. Induction of T cell responses by gene gun immunisation has been reported to be difficult. The amount of DNA administered by gene gun is much lower than with i.m. immunisation. Maybe this amount is not enough for induction of cellular immunity, but only for antibody responses. Another explanation is suggested by the report of Fausch et al. who observed that VLPs are taken up by Langerhans cells but they do not become activated (Fausch, Da Silva et al. 2002). The fact that the gene gun immunisation used a mix between wild type and mutated plasmids might also have contributed to the low responses observed. When CD8$^+$ T cells were depleted from the spleenocyte population IFN-γ responses were reduced, demonstrating that the cellular immunity consists of both CD4$^+$ and CD8$^+$ T cells. A separate

peptide mapping experiment revealed T cell epitopes in multiple regions of the L1 immunogen.

	plasmid (no of mice)	no of animals positive for detectable ELISA IgG	no of animals positive for detectable neutralising IgG	no of animals positive for INF-gamma in all splenocytes	no of animals positive for INF-gamma after CD8 depletion	no of animals positive for INF-gamma to 3 peptide pools
intra muscular	naive (4)	0	0	0	0	0
	pKCMV (3)	0	0	0	0	0
	PCL1wt (5)	0	0	3 (60%)	0	0
	pCL1MUT (5)	5 (100%)	5 (100%)	5 (100%)	2 (40%)	1 (20%)
	pCL1MUTDP (5)	5 (100%)	5 (100%)	3 (60%)	2 (40%)	3 (60%)
gene gun	pKCMV (3)	0	0	0	0	0
	plasmid mix (10)	7 (70%)	5 (50%)	3 (30%)	1 (10%)	0

Table 6. Number of animals positive in each assay performed, for each plasmid used.

We did not observe any significant difference in immune responses against HPV 16 between the two mutated plasmids. We can therefore not conclude from the experiments we performed that one should be better than the other in vaccination against HPV 16.

It has been demonstrated that VLP L1 immunisation in humans induces robust but declining serum IgG titers (Koutsky, Ault et al. 2002). It would therefore be interesting to compare IgG durability in mice between VLP and DNA immunisations. It could also be of interest to compare the immunogenicity of our DNA plasmid with codon optimised HPV 16 L1 plasmids, such as the one of Leder et al. (Leder, Kleinschmidt et al. 2001) in the same experimental set up.

As mentioned earlier in this thesis, different routes of administration give rise to different kinds of immune responses. How our plasmids should be administrated should be evaluated in a future study. A combination of i.m. immunisation that will enhance systemic responses, with a type of immunisation that will enhance local responses should be investigated.

The advantages of using DNA immunisation as a prophylactic vaccine against HPV are that a DNA vaccine would probably be easier to distribute and store under third world conditions. Also, DNA immunogens are easier to manufacture, especially multivalent vaccines, protecting against many HPV types is desired.

SUGGESTIVE ROLE FOR CXCL8 AND IFN-γ IN CLEARANCE OF HPV 16 (PAPER III)

The role of the immune response in HPV infection and progression to cancer is not completely understood, but is believed to be important in HPV persistence and clearance because immunocompromised patients (such as renal transplant recipients and HIV-1 infected patients) have SIL lesions that progress more rapidly (Sillman, Sentovich et al. 1997; Ferenczy, Coutlee et al. 2003). We therefore wanted to investigate local immune responses in women with HPV 16 persistence or clearance. The concentration levels of CXCL8 and IFN-γ were measured in cervical secretions taken at two different time points (enrolment and follow-up). The levels of CXCL8 and IFN-γ were both significantly increased in women who cleared their HPV 16 infection, on average 23 months after enrolment, but not in those who were HPV 16 persistent. A non-significant increase in concentration levels of the cytokines was also seen in the HPV negative women. Our findings of increased levels of IFN-γ are in line with earlier observations that IFN-γ is decreased in women with HPV 16 related CIN (El-Sherif, Seth et al. 2001). The relation between CXCL8 and HPV 16 clearance and persistence has, to our knowledge, not been studied before. Increased levels of CXCL8 in women with a cleared infection could possibly be explained by the work of Huang and McCance who reported that HPV 16 E6 and E7 downregulate CXCL8 production by human keratinocytes (Huang and McCance 2002).

Because several co-factors have been associated with cervical cancer we wanted to investigate whether any of those had any affect on cytokine levels. The co-factors tested for in this study were *C. trachomatis*, HSV-2, smoking, use of oral contraceptives, number of sexual partner and ever being pregnant. We could not see any significant effect of any of these co-variates on cytokine concentration change but there was a tendency for inverse association with *C. trachomatis*. This tendency is well in line with our previous findings where *C. trachomatis* was observed to be a co-variate for HPV clearance (Silins, Ryd et al. 2005).

Our findings suggest a role for CXCL8 and IFN-γ in the clearance of HPV 16 infection but because of the extensive redundancy and pleiotropy in cytokine action it is difficult to distinguish whether these cytokines or other covariate immune mediator(s) may be important in control of HPV 16 infection.

KIR GENES AND GENOTYPES IN WOMEN WITH CERVICAL INTRAEPITHELIAL NEOPLASIA (PAPER IV)

KIRs on NK cells regulate the inhibition and activation of NK cell activity (see figure 5) and can therefore be involved in disease progression or

protection. The aim of this study was to compare the presence of KIR genes and genotypes in women with CIN and healthy women to see if certain KIR genes are more common in women with disease. To do so we performed KIR typing for 14 specific KIR genes by PCR. We could not detect any significant difference in frequency of individual KIR genes between cases and controls. The gene of KIR2DL5 exists in four allelic variations and three of these are not transcribed. We therefore wanted to investigate the frequency difference between these alleles. We found that one of the non-transcribed versions of KIR2DL5 was significantly more frequent in controls than cases. We don't know if this has any importance in prevention of CIN, but it would be interesting to investigate.

Based on the gene content, two KIR haplotypes have been identified (Uhrberg, Valiante et al. 1997). The B haplotype contains various combinations of KIR2DS1, 2DS2, 2DS3, 2DS5, 3DS1, and 2DL5. The group A haplotype is characterised by the absence of all these genes (Marsh, Parham et al. 2003). We were not able to identify haplotypes in our cases and controls, but we investigated their corresponding genotypes. The frequencies of haplotypes A and B are roughly equal in the Caucasian population, but on the basis of the gene content, haplotype B displays a much greater variety of subtypes. In our material we found that only 19% of all the individuals had genotypes corresponding the A haplotype. Only four of the genotypes corresponded to an A haplotype, the rest were genotypes corresponding to B haplotypes. But 29% of cases had genotypes of the A-type and only 15% of controls. To have a genotype corresponding to an A haplotype increases the risk of developing CIN two times (OR 2.3 95% CI 1.1-4.6) (results not presented in the paper). We also observed that a specific genotype corresponding to a group A haplotype was associated with increased risk of disease (OR 6.7 95% CI 1.7-26.3; P = 0.04 after correction for multiple comparisons).

To study only the presence of KIR genes present two problems. The presence of a gene does not necessarily reflect the gene product and the presence of the gene or protein does not mean that its corresponding HLA ligand is present. It is therefore important to investigate not only the KIR genes but also their ligands, something that we were not able to do in this study. Even though the functional aspects of KIR in relation to CIN are difficult to cover in this work, our study does suggest that future studies may be rewarding. Such studies should include a larger independent material and should also include HLA class I typing.

SAMMANFATTNING PÅ SVENSKA

VAD ORSAKAR LIVMODERSHALSCANCER?

Livmoderhalscancer eller cervixcancer är en av kvinnans vanligaste cancerformer. Ca 450.000 fall upptäcks varje år världen över och omkring 200.000 kvinnor dör. Majoriteten av fallen är kvinnor i tredje världen. I Sverige drabbas ca 500 kvinnor och 150 dör varje år.

Olika riskfaktorer i utvecklingen av cervixcancer har rapporterats. Redan på 1840-talet kopplade man sexuellt beteende med cervixcancer, men det var först på 1970-talet som man förstod betydelsen av humant papillomavirus (HPV) i utvecklingen av livmoderhalscancer. Sedan dess har många studier bekräftat att HPV är en förutsättning för att man ska kunna få denna cancertyp.

HPV är ett vanligt virus som de flesta har haft någon gång i livet. Över 100 olika HPV-sorter har identifierats. Dessa sorter kan ha olika spridningsvägar och orsaka både vanliga vårtor och ett flertal cancrar. Ett trettiotal sorter är sexuellt överförbara och kan orsaka både kondylom och livmoderhalscancer. De vanligaste sorterna som man finner i biopsier från cervix är HPV 16, 18, 33 och 45, där HPV 16 är allra vanligast. Eftersom sannolikheten för en HPV-infektion är direkt proportionell mot antalet sexualpartners under en livstid ser man väldigt sällan infektion hos barn eller personer utan sexuell erfarenhet. Efter sexualdebuten ökar förekomsten av HPV i livmoderhalsen snabbt med åldern och når en topp mellan åldrarna 20-25 innan den avtar igen. Även om en HPV-infektion är vanlig läker den ofta ut av sig själv. Efter ett år har 70% av de infekterade blivit av med viruset och efter 18 månader 80%. Hos en majoritet av de kvinnor där viruset inte läker ut utvecklas så småningom förstadier till cancer. Cancer utvecklas dock hos < 1 % av alla kvinnor som någon gång under livet har fått en HPV-infektion. Eftersom så få utvecklar cancer förstår man att en HPV-infektion inte är tillräcklig och därför finns det nog flera riskfaktorer som bidrar i utvecklingen av cancer. Till exempel, om man är smittad med klamydia samtidigt som HPV 16 är risken större för att få livmoderhalscancer än om man bara är infekterad med HPV 16. Ännu har man inte kunnat förklara detta orsakssamband men en teori är att klamydia manipulerar immunförsvaret så att det inte kan hitta viruset. Andra riskfaktorer som föreslagits är bland annat rökning och användandet av p-piller.

VARFÖR ORSAKAR HPV CANCER?

HPV består av ett antal olika proteiner som kan delas in i tidiga (E) och sena (L). De tidiga proteinerna är viktiga för virusets förökning och de sena

för dess struktur. När HPV har lyckats ta sig in i en värdcell använder den sig av cellens maskineri för att kunna föröka sig. Men denna process sker på bekostnad av värdcellens egen förmåga att föröka sig. Två av HPVs tidiga proteiner stör den normala cellcykeln, som vanligtvis gör så att cellen delar sig kontrollerat. Men med HPV på plats försvinner kontrollen och celler börjar dela på sig ohämmat. Cellerna tappar sin vanliga funktion vilket leder till att de odödliggörs och blir cancerceller.

VAD DEN HÄR AVHANDLINGEN HANDLAR OM

HPV har huvudrollen i utvecklingen av livmodershalscancer och olika birollsinnehavare har nominerats. I den här avhandlingen har vi i tre kliniska projekt försökt att komma lite närmre svaren på de frågor som ställs kring olika riskfaktorers biroll i processen. En av anledningarna till att vissa kvinnor får cancer är att immunsvaret, som eliminerar både virusinfektioner och cancerceller, är väldigt individuellt eftersom den genetiska dispositionen varierar. Men också för att vi alla utsätts för olika yttre riskfaktorer, som rökning och andra vanliga sexuellt överförbara infketioner. I två av studierna (artikel III och IV) har vi undersökt olika komponenter av immunsvaret. Vi ser att de verkar ha en viss roll i att HPV inte läks ut och i utvecklingen av cancer.

I den första artikeln i den här avhandlingen (I) har vi bland annat undersökt klamydia och herpes roller i utvecklingen av cancer. Våra fynd stärker hypotesen att klamydia är en viktig birollsinnehavare. Vi ser också att klamydiainfektion är vanligare hos kvinnor som utvecklar cancer i körtelepitelet, jämfört med kvinnor som får skivepitelcancer i livmodershalsen. Skivepitelcancer är den vanligaste formen av cervixcancer och är starkt kopplad till HPV 16-infektion.

I artikel II har vi undersökt om en nu DNA-baserad kandidat till ett förebyggnde vaccin mot HPV 16 ger upphov till ett immunsvar i möss. Vi såg att de immuniserade mössen fick ett specifikt immunsvar mot HPV 16. Nästa steg är att ta reda på om vaccinkandidaten är säker och om den har någon effekt i människa. Vi tror nämligen att den vaccinkandidat som vi har använt skulle kunna vara enklare att tillverka och lättare att distribuera än de proteinbaserade vaccin som redan befunnits säkra och effektiva.

Varför måste man studera alla dessa små delar av immunsvaret och olika bidragande riskfaktorer när man vet att det är HPV som orsakar cervixcancer? Jo, för vi behöver lära oss att förstå hur HPV påverkar kroppen, och vad som påverkar HPV, för att kunna utveckla och utvärdera nya medel att både förebygga och behandla HPV-infektioner och dess följdsjukdom, cancer.

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