Therapeutic Immunization strategies against cervical cancer
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Chapter 7

General discussion and future perspectives
General discussion

The immune system plays an important role in the control of human papillomavirus (HPV) infection and HPV-induced premalignant precursor lesions of cervical cancer called cervical intraepithelial neoplasia (CIN lesions) [Welters 2003, Ferenczy 2003]. Two of the early proteins of high-risk HPV types, E6 and E7, interact with the cell cycle regulation proteins p53 and pRb and can cause immortalization of cells. E6 and E7 are constitutively expressed in cervical cancer cells and this expression is required for maintenance of the malignant phenotype. This makes these proteins attractive targets for an immunotherapy directed against HPV-induced CIN lesions and cervical cancer.

In the research project described in this thesis, insight was gained in the mechanisms and efficacy of two distinct immunization strategies against cervical cancer. Both immunization strategies aim at the induction of a cellular immune response against infected and/or transformed cells. The first strategy is a genetic immunization approach based on the use of recombinant Semliki Forest virus (rSFV) encoding recombinant protein antigens. The second strategy is a protein-based immunization approach exploiting the property of virosomes to deliver entrapped protein antigen to the cytosol of antigen-presenting cells (APC).

As the ultimate goal of our studies is to develop an immunotherapeutic treatment of HPV-induced cervical cancer and CIN lesions, the immunizations were directed against an oncoprotein expressed by HPV-transformed tumor cells. Yet, to unravel the mechanisms involved in the delivery and processing of protein antigens in both strategies, studies were performed using model antigens including ovalbumin (OVA) and the influenza virus nucleoprotein (NP). In this chapter, the two immunization strategies are described and their potential application for therapeutic immunization against CIN lesions or cervical cancer is discussed.

Recombinant Semliki Forest virus

Recombinant SFV (rSFV) encoding the HPV type 16 (HPV16) early proteins E6 and E7 (rSFVE6E7) was used successfully for induction of cytotoxic T lymphocyte (CTL) responses against tumor cells expressing these oncoproteins, as described in Chapter 2. Moreover, immunization with rSFVE6E7 protected 40% of mice against challenge with an HPV16 E6E7-expressing tumor [Daemen 2000, Chapter 2]. These results indicated that the use of rSFVE6E7 represents a promising immunization strategy for therapeutic vaccination against CIN lesions or cervical cancer.
Further immunization studies with rSFV vectors encoding HPV protein antigens

The study with rSFVE6E7 described in Chapter 2 was performed and published several years ago. Since that time our group proceeded with this research line to further improve the efficacy of this rSFV-based immunization strategy. We indeed succeeded in optimizing the efficacy by generating a novel rSFV construct. This construct encodes a fusion protein of HPV16 E6 and E7. In addition, to stimulate the amount of protein produced, a translational enhancer is included [Daemen 2002]. Infection of BHK cells with this virus, rSFVenhE6,7, results in much stronger and more stable expression of E6 and E7 than infection with rSFVE6E7. Moreover, mice immunized with rSFVenhE6,7 virus develop a stronger HPV-specific CTL response than mice immunized with rSFVE6E7. A single immunization with rSFVenhE6,7 is sufficient to induce CTL responses and after two immunizations strong CTL responses persisting for 3 months are detected, whereas rSFVE6E7 requires three immunizations for a strong response. Tumor challenge experiments with rSFVenhE6,7 showed that more mice are protected against tumor development upon immunization with rSFVenhE6,7 virus than upon immunization with rSFVE6E7.

Therapeutic immunization against established HPV16 E6- and E7-expressing tumors with rSFVenhE6,7 turned out to be extremely effective [Daemen 2003]. For therapeutic immunization studies, mice are injected with tumor cells and treated three times with rSFVenhE6,7 particles starting at different time points after tumor inoculation. When this treatment is started two days after inoculation all mice clear the tumor. When treatment is initiated one week after inoculation, the majority of the mice resolve the tumor. When treatment is started two weeks after inoculation of the tumor cells, tumors grow very fast in the beginning but upon treatment even large tumors regress to undetectable levels and one third of the mice remain tumor-free at 3 months after inoculation. In CTL assays at up to 340 days after immunization, performed with spleen cells of mice that remained tumor-free, all mice show strong specific lysis.

The route of immunization with rSFVenhE6,7 is important in induction of precursor CTL and in the efficacy of tumor treatment [Daemen in press]. Mice immunized intravenously or intramuscularly show higher precursor CTL frequencies and better tumor clearance than mice immunized via intraperitoneal or subcutaneous routes. In this study, again, tumors as large as 500 mm³ completely resolve upon immunization with rSFVenhE6,7 demonstrating the potential of this vaccination.

Breaking of immunological tolerance by rSFV

The level of HPV-specific CTL activity is generally low in cervical cancer patients [Ressing 1996, Evans 1997], suggesting that they have mounted a certain degree of immunological tolerance or ignorance for the HPV-derived antigens. In an effort to test the potential of rSFV in a situation closely resembling this condition in cancer patients, immunization studies using K10HPV16-E6/E7 transgenic mice were performed
[Riezebos-Brilman, manuscript submitted]. These transgenic mice constitutively express HPV16 E6 and E7 under the control of the keratin 10 promoter in the suprabasal layers of the epidermis [Ewuarakul 1994, Borchers 1999]. The HPV-specific CTL tolerance is extremely strong in these mice and earlier attempts to break this tolerance by immunizing with protein or DNA had been unsuccessful [Borchers 1999, Michel 2002]. In contrast, upon immunization with rSFV enhE6,7 K10HPV16-E6/E7 transgenic mice did develop HPV-specific CTL activity showing that CTL immunological tolerance can be broken by immunization with rSFV.

**The mechanism underlying CTL induction by rSFV: direct priming?**

Direct priming has been described as a mechanism of induction of immune responses for several viral vectors [reviewed in Norbury 2003]. In direct priming, APC are infected with the viral vector followed by production of the recombinant protein within the cytosol of the APC itself, such that these antigens have direct access to the MHC class I presentation pathway. Direct priming has been reported for vaccinia vectors and poxvirus vectors [Shen 2002, Bronte 1997]. Adenoviral vectors have even been specifically altered to more effectively target dendritic cells (DC) by modification of the fiber knob of the virus or by redirecting the virus to DC via antibodies to CD40 [Worgall 2004, Tillman 1999]. Direct infection of DC due to these modifications indeed results in enhanced immune responses against the encoded antigen.

In an effort to elucidate the mechanism behind the successful immunization with rSFV we performed infection studies in murine and human dendritic cells (DC) [Huckriede 2004, Chapter 3]. DC are the most potent professional APC [Banchereau 1998, Théry 2001] and we reasoned that direct infection of these cells could result in high level production of rSFV-encoded recombinant protein [Liljeström 1991]. Since this protein would be produced in the cytosol of the APC, it would be processed for MHC class I presentation. Moreover, the presence of viral RNA would likely result in maturation of the DC enabling effective presentation of antigenic peptides by these infected DC [Lanzavecchia 1999]. Although the infection of DC would ultimately lead to apoptosis, presentation of peptides could be possible beforehand since infected cells can remain viable for up to 72 hours [Hardy 2000].

However, upon infection of murine or human DC cultures with rSFV encoding the reporter gene β-galactosidase (LacZ), the percentage of DC expressing LacZ was extremely low (0.15% at an MOI of 1000). Since these primary DC cultures also contain low levels of other cell types it is possible that the observed infected cells are not even DC [Fields 1998]. These observations indicate that direct infection of DC by rSFV is very inefficient. Since, \textit{in vivo}, as little as 100 rSFV particles are able to induce a CTL response against an encoded antigen, direct infection of DC is most likely not the mechanism of immune response induction [Zhou 1995]. A later study by Navas et al.
confirmed our observations indicating that indeed human DC do not support rSFV infection [Navas 2002].

Direct priming is not the only mechanism through which viruses or viral vectors mediate antigen presentation. Cross-priming of viral antigens or antigens encoded by viral vectors has been described and sometimes both mechanisms occur in the same experimental system [reviewed by Norbury 2003].

Or cross-priming?
Since the effective induction of cellular immune responses after rSFV immunization is not due to direct priming of APC, and since rSFV induces apoptosis in infected cells, we next hypothesized that in vivo the following events might result in the observed CTL responses: (i) upon administration of rSFV to mice a number of different cell types are infected, (ii) cells infected with rSFV produce high levels of recombinant protein, (iii) after about 48-72 h infected cells die via apoptosis and release apoptotic bodies [Glasgow 1998, Murphy 2000], (iv) APC take up these apoptotic bodies and present this exogenous antigen via cross-presentation pathways. Apoptotic bodies have been described to be an excellent source of antigen for cross-priming [Albert 1998a, b].

If cross-presentation by APC of recombinant protein in apoptotic bodies is the mechanism behind rSFV immunization, the level of expressed protein would be important for the level of CTL induction against rSFV encoded proteins. Accordingly, in Chapter 3 we studied CTL induction after immunization with rSFV encoding the influenza virus nucleoprotein (NP) as a model antigen. Indeed, when three rSFV constructs resulting in different expression levels of the model antigen (NP versus enhNP) and availability of antigenic peptides (NP versus ubNP) were compared in vivo, there was a marked difference in induction of NP-specific CTL activity. The rSFV construct encoding for enhanced NP induced by far the highest levels of NP-specific CTL, followed by the construct encoding unmodified NP. A construct encoding ubiquitin-NP indeed resulted in accelerated degradation of NP, the ubiquitin moiety targeting the protein for rapid degradation by proteasomes. This rSFVubNP construct induced moderate induction of CTL, whereas rapid antigen degradation should be beneficial in case of direct priming by rSFV [Whitton 1999]. These studies indicated that indeed availability of high levels of antigen is important in the mechanism of CTL induction by rSFV consistent with a mechanism of cross-priming [Huckriede 2004, Chapter 3].

These observations are supported by our CTL and tumor challenge studies using constructs encoding HPV16 E6 and E7. In these studies the construct generating large amounts of a fusion protein of E6 and E7 was superior in induction of CTL and antitumor responses in comparison with the regular construct which generates considerably less, non-fused E6 and E7 [Daemen 2002, 2003 and Chapter 2]. The main differences between these two constructs is the stronger and more stable
expression of the proteins in the case of rSFV\textsubscript{enh}E6,7 compared to rSFV\textsubscript{E6E7}, supporting the cross-presentation hypothesis.

**Potential of rSFV as a therapeutic vaccine**

Taken together, the studies with rSFV encoding the HPV16 proteins E6 and E7 indicate that rSFV is a very powerful vaccine for induction of CTL responses. The fact that even established tumors can be treated effectively and that immunological tolerance in mice can be broken by immunization with rSFV\textsubscript{enh}E6,7 shows that this vector is extremely promising as a therapeutic vaccine for CIN lesions or cervical cancer. The mechanism underlying this successful immunization strategy is most likely not direct priming through infection of APC, but rather cross-presentation of proteins acquired from other infected cells.

**Influenza virosomes as a delivery system for protein antigens**

As a second immunization strategy influenza virosomes containing protein antigens were used [Chapter 4-6]. Influenza virosomes are reconstituted viral envelopes which retain the cell entry and membrane fusion characteristics of native influenza virus [Almeida 1975, Stegmann 1987, 1993, Bron 1993]. During the reconstitution protocol the genetic material of the virus is removed and protein antigens (such as OVA or E7) may be added resulting in the formation of virosomes containing the protein antigen involved [Bungener 2002, Bungener in press, Chapter 4-6]. Influenza virosomes prepared according to this protocol enter cells through receptor-mediated endocytosis and deliver encapsulated protein antigen to the cytosol of cells resulting in processing and presentation of this antigen in the context of MHC class I [Bungener 2002, Chapter 4]. The influenza virus hemagglutinin (HA) mediates the fusion of the virosomal membrane with the endosomal membrane resulting in this cytosolic delivery of encapsulated antigen. HA remains in the endosome upon fusion and is processed for presentation in MHC class II. Since not all of the virosomes will fuse with the endosomal membrane, some will continue in the endo-lysosomal pathway resulting in degradation of the proteins to peptides which can enter the MHC class II presentation pathway. Influenza virosomes are thus expected to deliver encapsulated protein antigen for presentation in both MHC class I and II resulting in optimal activation of the immune system.

The studies described in Chapter 4 of this thesis confirmed this hypothesis *in vitro*. Incubation of murine DC with OVA virosomes resulted in strong MHC class I and II presentation of OVA peptides at picomolar concentrations of OVA. In addition, the virosomes induced upregulation of MHC class I and II and a number of costimulatory molecules on the DC. As expected, fusion activity of the virosomes was required for
MHC class I, but not MHC class II presentation of OVA peptide in these in vitro experiments.

Subsequent immunization experiments with OVA and E7 virosomes [Bungener in press, Chapter 5 and 6] demonstrated that virosomes containing protein antigens are effective inducers of CTL and antitumor responses against these antigens. In vivo, the requirement for fusion activity of virosomes appears to be less stringent since fusion-inactivated virosomes were still capable of inducing CTL responses although to a significantly lower extent than fusion-active virosomes. The ability of fusion-inactive virosomes to deliver protein antigens for MHC class I presentation is most likely due to escape of this exogenous antigen from the endosomes of APC and presentation of the antigen by MHC class I molecules in a process called cross-presentation.

In contrast to rSFVE6E7 immunization, virosomes were able to induce production of antibodies against the virosoome-associated proteins E7 and OVA [Chapter 6 and Bungener unpublished observations].

The effect of pre-existing antibodies on the outcome of immunization with influenza virosomes
An important question when virosomes are to be employed as vaccines in humans is if influenza virus-specific antibodies have a positive or a negative effect on the outcome of the immunization. For viral vectors, like adenovirus, inhibition of responses by pre-existing antibodies has been described [Papp 1999]. To circumvent this problem, different adenovirus serotypes with low prevalence, like adenovirus 35, can be used for immunization [Vogels 2003].

The role of pre-existing antibodies to influenza virus is of importance since the majority of the human population has been infected with influenza virus and therefore has influenza HA-specific antibodies [reviewed in Couch 1983]. Furthermore, a substantial proportion of the population in western countries is vaccinated yearly with influenza vaccine containing antigens to three influenza virus strains currently circulating [Johansen 2004]. In the Netherlands about 3 million people are vaccinated yearly [http://www.degrotegriepmeting.nl]. The vaccinated part of the population will likely rise in coming years since discussions on the health and economic benefits of influenza vaccination for non-high-risk groups are underway. Thus, when influenza virosomes will be used in patients to vaccinate against pathogens other than influenza or against tumor antigens, these patients will have pre-existing antibodies against one or more strains of influenza virus. Since the predominant influenza protein in virosomes is HA, this discussion will focus on the effects of HA-specific antibodies on influenza virus and virosomes.
Interaction of HA-specific antibodies with influenza virus at the natural site of infection results in virus neutralization

During a natural infection with influenza virus, the virus enters the host via the epithelium of the lungs. Binding of the virus to respiratory epithelial cells is mediated by HA. The cellular receptors for influenza virus are terminal sialic acid residues on glycoproteins and glycolipids [Skehel 2000, Fleury 1999].

A previous infection with the same subtype of influenza virus or vaccination against this subtype results in pre-existing HA-specific antibodies. Upon secondary infection with the same influenza virus, these HA-specific antibodies will bind to the influenza virus HA. These antibodies are directed against the receptor-binding site of HA and binding of such antibodies to HA results in neutralization of the virus by prevention of binding to sialic acids residues on the cells.

Interaction of HA-specific antibodies with influenza virosomes: effect of booster immunization

For immunization aiming at inducing immunity against a pathogen, booster vaccinations are often applied to ensure a good immune response. The principle of a booster vaccination is the augmentation of the immune response by a repeated encounter of the antigen by the immune system. More specifically, antibodies induced by the first immunization will bind to the same antigen used during the booster immunization. These pre-existing antibodies prevent binding of the antigen to its natural receptor. Instead, the specific antibodies target the antigen to immune cells like DC, monocytes, macrophages, B cells, neutrophils and granulocytes via binding of the Fc part of the antibody to Fc receptors present on these cells. The antigen is taken up by the immune cells via receptor-mediated endocytosis and processed for antigen presentation. In fact, so called immune complexes of antigen and antibodies are used for effective immunization against various antigens (Rafiq 2002).

In other words, pre-existing antibodies are essential in augmenting the immune response to pathogens. The action of pre-existing antibodies is what makes a booster immunization so effective. It is generally accepted that these pre-existing antibodies do not inhibit responses against antigen but rather boost the response. However, one of the questions that is often posed during a discussion on immunization with virosomes is if pre-existing antibodies will completely abolish the effect of the immunization.

As indicated above, the logical answer to this question is “no”. Pre-existing antibodies to HA will prevent binding of the virosomes to sialic acid residues, the natural cellular receptors for HA. Instead, the Fc portions of HA-specific antibodies bound to virosomes can bind to the Fc receptor of APC. Thus, by preventing binding to the natural receptor but mediating binding to Fc receptors, pre-existing antibodies to HA will target influenza virosomes to APC.
Indeed, vaccination with the commercially available virosomal vaccines Epaxal® and Inflexal® is not hampered by pre-existing antibodies against influenza. Inflexal® is a virosomal influenza vaccine consisting of virosomes prepared from three influenza subtypes. Epaxal® is a vaccine against hepatitis A consisting of influenza virosomes with inactivated hepatitis A virions attached to the surface of the virosomes. Cytosolic delivery of the hepatitis A virions is not likely and not necessary in the case of Epaxal®. The assertion that pre-existing antibodies to influenza do not hamper the immune response to Epaxal® and Inflexal® can be deduced from the facts that (i) the volunteers in clinical trials for these virosomal vaccines were not screened for influenza exposure, (ii) influenza antibody prevalence is above 50% for this age group (Infectious Agents Surveillance Report, Japan) and (iii) these vaccines are effective in all volunteers in the executed clinical trials [Conne 1997, Glück 1992].

Effect of pre-existing high affinity antibodies to HA on the immune response against antigen encapsulated in influenza virosomes

The effect of pre-existing antibodies during a booster immunization with a regular subunit vaccine or with a virosomal vaccine containing an antigen on the outside of the virosomal particles is described in the preceding part of this discussion. When the antigen against which the immune response has to be directed is present in the lumen of the virosome the situation is more complicated.

Our hypothesis is that antigen on the inside of virosomes enters the cytosol of the cell and thus the MHC class I processing and presentation pathway via fusion of the virosomal membrane with the endosomal membrane. When pre-existing antibodies to HA are present, the virosomes will be targeted to the Fc receptor on APC. As a consequence, the virosomes will end up in the endo-lysosomal pathway of the APC.

The next step would be fusion of the virosomal membrane with the endosomal membrane, mediated by the fusion peptide present on HA2. Since antibodies to HA are directed against the receptor-binding moiety of HA, HA1, fusion of the virosomes may well still take place in the presence pre-existing antibodies [Skehel 2000]. Furthermore, antibody-mediated enhancement (ADE) has been described for influenza virus.

ADE was originally described by Hawkes in the 1960s for some members of the Flaviviridae family [Hawkes 1967]. Since these original observations, ADE has been described for a large number of viruses including influenza virus, human immunodeficiency virus (HIV), Ebola virus, yellow fever virus, dengue virus and other Flaviviruses [Ochiai 1988, 1992, Robinson 1988, Takeda 1988, Takada 2003a, Schlesinger 1981, Daughaday 1981, reviewed in Takada 2003b]. In ADE, antibodies target the virus to the Fc receptor but do not inhibit fusion of the viral membrane with the endosomal membrane. Thus, in ADE pre-existing antibodies do not inhibit infection with the virus.
As indicated above, Fc receptor-mediated ADE has been described for influenza virus [Tamura 1991]. Moreover, the existence of subtype cross-reactive antibodies to influenza virus enhancing the uptake, but not neutralizing virus of a different subtype was described [Ochiai 1992, Tamura 1993, 1994]. Cross-reactive antibodies also mediate uptake and infection via ADE. The affinity of the HA-specific antibody could play an important role in the ability of HA to mediate fusion inside the endosome of cells that have taken up virus-antibody complexes via their Fc receptor [Schofeld 1997]. If the affinity of the antibody is low, it may release HA upon lowering of the pH in the endosomal compartment enabling HA-mediated fusion. However, as mentioned above, antibodies directed against HA do not bind to the fusion peptide on HA2. Thus, even in the presence of high affinity anti-HA antibodies, fusion of the virosomal membrane with the endosomal membrane and delivery of virome-encapsulated antigen may well take place.

The precise role of pre-existing high affinity antibodies to influenza HA in induction of cellular immune responses to influenza virome-encapsulated antigens is still under investigation. Preliminary immunization experiments performed with OVA virosomes in mice pre-immunized with influenza subunit vaccine of the same strain indicate that pre-existing antibodies against influenza membrane proteins could indeed be beneficial to the CTL response to encapsulated protein antigens [Bungener unpublished observations].

In conclusion, there are compelling indications that pre-existing antibodies (high or low affinity or cross-reactive) against influenza virus HA will not hamper the immune response to influenza virosomes or antigens encapsulated in these virosomes.

Therapeutic vaccination against precursors of cervical cancer

In this thesis two immunization strategies are described that could be exploited for therapeutic immunization against HPV-induced CIN lesions or cervical cancer. We have demonstrated that immunization of mice with rSFV encoding HPV16 E6 and E7 proteins results in high levels of specific CTL which are capable of eradicating established E6 and E7-expressing tumors [Daemen 2000, 2002, 2003, in press]. Moreover, rSFVenhE6,7 is capable of breaking CTL tolerance in HPV16 E6E7-transgenic mice [Riezebos-Brilman, manuscript submitted]. The immunization studies presented in Chapter 5 and 6 show that immunization with protein-containing virosomes also results in induction of powerful CTL against encapsulated protein antigens [Bungener in press and Chapter 6]. Initial tumor protection studies in mice vaccinated with E7 virosomes show good protection rates against tumor outgrowth [Chapter 6]. Both rSFV and virosomes therefore represent promising therapeutic vaccine candidates against CIN lesions and cervical cancer. When choosing the most optimal vaccine for
this purpose a number of advantages and disadvantages of the two immunization strategies need to be considered.

**Superiority of rSFV to virosomes in tumor treatment**
First, based on the results obtained so far, immunization with rSFV appears a more powerful approach than immunization with influenza virosomes. The first results of tumor protection studies with OVA virosomes [Bungener, unpublished observations] and E7 virosomes [Chapter 6] suggest that immunization with rSFV is more effective for eradication of tumor cells in vivo than vaccination with virosomes in their current form.

A quantitative comparison based on the same amount of E6 and E7 protein for each vaccination strategy has not been made yet. The levels of protein present in vivo after immunization with rSFV are difficult to estimate for the constructs we used since levels vary per construct. Furthermore, an enhancer sequence cloned before NP or E6E7 dramatically changes the expression levels of the recombinant proteins. In vitro infection and pulse labeling studies comparing rSFVenhNP with rSFVNP and rSFVubNP indicate that the differences in protein production can be 10-fold. Upon intraperitoneal immunization with $5 \times 10^7$ rSFV-luciferase particles about 20 ng of luciferase protein is present in the collected organs (peritoneal lining, spleen, liver and lungs) 24 hours after immunization [Klimp 2001]. The amount of produced protein after an immunization will be larger since production will continue for about 72 hours. However, these results indicate that the levels of protein present after rSFV immunization are in the ng range whereas immunization with virosomes requires µg quantities of protein to be effective [Bungener in press, Chapter 5 and 6], again underlining the superiority of rSFV to virosomes.

**Other considerations in choosing the ideal therapeutic immunization**
For the practical issue of designing a therapeutic immunization strategy against CIN lesions or cervical cancer other factors are important. A number of virosomal vaccines are already approved for use in humans (Epaxal®, Inflexal®, and Invivac®) whereas rSFV is not yet in the phase of clinical trials. Up to now, three clinical trials using rSFV as an immunization strategy have been announced. Two trials among patients with glioblastoma multiforme or stage III/IV metastasizing melanoma or renal cell cancer have been announced [Ren 2003]. The third announced trial will be performed by the European Vaccine Effort Against HIV/AIDS (EuroVac); this network is developing rSFV vectors encoding HIV-1 subtype C gag, pol, nef and env genes for use in human clinical trials. The fact that, so far, rSFV has not been applied in humans will make a trial with rSFV among patients with CIN lesions difficult, since these lesions can already be treated effectively, be it with considerable morbidity. Depending on the results of the announced trials it will become easier to start new trials but currently, for treatment of CIN lesions, a trial with virosomes is likely to be more feasible.
Feasibility of therapeutic vaccination

In patients with cervical cancer, activation of specific CTL capable of clearing the entire tumor will probably be very difficult. A second problem in cervical cancer patients is tumor immune evasion by MHC class I allele downregulation [Ritz 2001, Bontkes 1998]. Treatment of cervical cancer by immunizing with rSFV encoding HPV antigens or virosomes containing HPV proteins will therefore probably not be feasible unless other immune-modulating features are added. On the other hand, in patients with CIN lesions spontaneous regression within 1 year is reported for 12-37% of patients depending on the severity of the lesion [Nobbenhuis 2001]. The immune system plays an important role in the clearance of CIN lesions and in these patients a cellular immune response against the E6 and E7 proteins can be detected [Nakagawa 2000, Kadish 2002]. These observations indicate that in CIN patients therapeutic immunization may well be a feasible strategy. For these patients, vaccination may be preferable to the sometimes aggravating treatments that are currently available.

Future perspectives

This thesis focuses on the development of an immunotherapeutic immunization to treat CIN lesions or cervical cancer. Next to therapeutic vaccination against cervical cancer, there is extensive ongoing research in the area of prophylactic vaccines against HPV. These vaccines aim at prevention of HPV infection by induction of antibody responses against HPV capsid proteins. Virus-like particles (VLP) of HPV are very promising prophylactic vaccines with up to 100% protection from HPV infection and dysplasia [Jansen 2004]. These prophylactic vaccines, containing the late capsid proteins (L1 and L2), will likely become available in the coming years making prevention of infection with some of the high-risk HPV types possible. For treatment of non-vaccinated women who will develop lesions in the next decades, continuing efforts to develop therapeutic immunizations for HPV-induced CIN lesions are needed. Furthermore, a prophylactic vaccine containing all of the high-risk HPV is not likely to be cost-effective indicating that therapeutic immunization may well remain a viable option for the future.

The results presented in this thesis provide a basis for future research into immunization against precursors of cervical cancer. Both virosomes and rSFV are able to induce CTL and antitumor responses against encapsulated or encoded protein antigens. One possible way to further improve immunization responses with virosomes and rSFV is the prime-boost strategy [Woodland 2004]. For this strategy priming of the immune response occurs through immunization with one vaccine regimen and the immune response is boosted by immunization with another. The resulting immune response is often more powerful than the immune response after immunization and
booster with the same vaccine. Since both rSFV and virosomes are effective vectors by themselves a combination of the two might be even more promising for induction of a useful immune response against HPV antigens. To investigate this hypothesis, a prime-boost study with both immunizations will be performed in mice in the near future. Also, the issue of the effect of pre-existing antibodies against influenza virus (of the same or different viral subtypes) on the outcome of immunization with virosomes will be investigated further in the coming months.

Further improvements of virosome efficacy could perhaps be accomplished by targeting virosomes specifically to APC or by incorporation of adjuvants in the virosome formulation. Mastrobattista et al. have described targeting of influenza virosomes to ovarian cancer cells by incorporating poly(ethylene glycol) (PEG)-derivatized lipids into the virosome membrane and coupling Fab’ fragments of anti-epithelial glycoprotein-2 to the distal ends of PEG lipids [Mastrobattista 2001]. It would be interesting to investigate if these or similar targeting techniques work as effectively in vivo.

In conclusion, both rSFV and virosomes are promising immunization strategies for treatment of CIN lesions. Efforts in the next years will elucidate if these strategies will become part of the treatment protocol for CIN lesions.
References


