Immunotherapy based on influenza virosomes and recombinant Semliki Forest virus

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Chapter 9

Discussion and future perspectives
Introduction

This thesis describes the pre-clinical development of two virus-based systems for immunotherapy: influenza virosomes and recombinant Semliki Forest virus (rSFV). It demonstrates the potency of influenza virosomes for the induction of cytotoxic T lymphocyte (CTL) responses against a model antigen (OVA) and against E7, a tumor-specific antigen derived from human papillomavirus (HPV), the etiological agent of cervical cancer. The research described in this thesis furthermore shows that CTL induction by virosomes is not hampered by influenza virus-specific immunity. Likewise, it is shown that the induction of anti-tumor CTL by rSFV, as described in detail previously [1-5], is not impeded by SFV-specific immunity in homologous prime-boost protocols. Importantly, in this particular part of the study a crucial involvement of T cell competition in vector-specific immunity was discovered. Finally, this thesis presents research on heterologous prime-boost strategies with virosomes and rSFV. Such strategies are found to result in higher numbers of target antigen-specific CTL but did not result in improved anti-tumor immunity compared to a homologous protocol with rSFV. In the present chapter, the implications of the research described in this thesis for the development of virosomes and rSFV as immunotherapeutic agents, particularly for the treatment of (pre)malignant cervical disease, will be discussed.

Virosomes as immunotherapeutic agents

The immunization studies in this thesis demonstrate that virosomes are efficient inducers of CTL activity and anti-tumor responses. A strong CTL response could be mounted by immunizing mice with less than 1 µg of virosome-encapsulated antigen. In contrast, other antigen delivery systems usually require up to a 100-fold higher doses of antigen [6]. Influenza virosomes are such potent inducers of target antigen-specific CTL responses because they actively deliver the antigen to the cytosol of antigen presenting cells (APC) [7;8]. Active delivery involves binding of the virosome to its cellular receptor via hemagglutinin (HA), receptor-mediated endocytosis, delivery of the virosomes to acidic endosomes, and ultimately, low-pH-induced fusion of the virosomal membrane with the endosome membrane mediating the introduction of virosome-encapsulated antigens into the cytosol of the cell. Upon cytosolic delivery, the antigens are processed for major histocompatibility complex (MHC) class I presentation [9;10]. Interestingly, the fusion activity of virosomes, which was found to be indispensable for delivery of encapsulated antigen to the cytosol of dendritic cells (DC) in vitro [11], was not essential
for CTL induction in vivo. Indeed, immunization with fusion-inactivated E7-virosomes or E7 protein mixed with empty virosomes also induced CTL activity and resulted in a delay in tumor growth, although the levels were significantly lower than with fusion-active virosomes. It can therefore be concluded that virosomes function not only by actively delivering antigen to the cell cytosol, but they may act also as adjuvants “per se” [12]. Furthermore, fusion-inactivated virosomes may also facilitate uptake of the target antigen by APC via receptor-mediated endocytosis [13]. As fusion-inactivated virosomes are unable to actively deliver their contents to the cytosol, they must then depend on the intrinsic capacity of DC to shuttle exogenous antigens into the MHC class I pathway of antigen presentation [14]. However, while this process of cross-presentation does enable CTL induction, it appears to be much less efficient than CTL induction via active cytosolic delivery of antigens by fusion-active virosomes.

The effect of neutralizing antibodies on CTL induction by virosomes or recombinant Semliki Forest virus

A major part of the research described in this thesis focuses on the effect of delivery-system-specific or vector-specific immunity on CTL induction by virosomes and rSFV. Interestingly, it is shown that virosomes and rSFV are both capable of inducing strong CTL responses in the presence of neutralizing antibodies directed against the virosomes or SFV respectively. In general, such antibodies are thought to be the most important mechanism by which pre-existing or prime-induced immunity may hamper the effect of pathogen-derived delivery systems or viral vectors [15].

In the presence of influenza virus-specific antibodies, the mechanism of CTL induction by virosomes is most likely altered. Influenza virosomes still induce strong CTL responses but most likely not via sialic acid receptor-mediated endocytosis. Chapter 5 discusses the possibility that influenza virus-specific antibodies obstruct the normal uptake of virosomes by blocking receptor binding, but at the same time facilitate CTL induction via a different mechanism of virosomal cell entry. Virosomes opsonized by influenza virus-specific antibodies may be targeted to cells expressing Fc-receptors such as professional APC. These APC might subsequently take up the virosomes via internalization of these Fc-receptors. After subsequent delivery to endosomes, the low pH within the endosomal lumen may cause detachment of the antibodies and trigger membrane fusion thereby allowing antigen delivery to the cytosol. Accordingly, in previous studies, it has been shown that opsonization of influenza virus by antibodies against HA and neuraminidase (NA) augmented virus uptake by Fc-receptor expressing cells and...
enhanced infection. This phenomenon is called antibody-dependent enhancement of infection (ADE) [16-19].

In Chapter 7 we show that the mechanism of CTL induction by rSFV is not altered in the presence of SFV-specific antibodies although the levels of expression of the transgene antigen are substantially reduced under those conditions. Clearly, CTL induction by rSFV is not limited by the antigen concentration in the dose range tested. Under those conditions, rSFV remains capable of infecting cells and induces enough transgene expression to allow strong transgene-specific CTL induction. Chapter 5 and 7 thereby demonstrate two distinct mechanisms by which antigen delivery systems or vectors can evade pre-existing antibodies.

The involvement of T cell competition in CTL induction by rSFV and virosomes

The research in Chapter 7 demonstrates that there is a previously overlooked mechanism of vector-specific immunity that may, under specific conditions, interfere with CTL induction by rSFV in pre-immune animals. This mechanism is T cell competition. T cell competition is a phenomenon that normally plays a role in establishing the immunodominance of epitopes presented on the same APC at the level of the responding T lymphocytes. It implies that different T lymphocyte clones compete for activating signals from professional APC to the exclusion of other clones [20;21]. T cell competition and other mechanisms involved in establishing immunodominance probably play an important role in vector-specific or delivery system-specific immunity. And, while several studies show means of circumventing problems associated with pre-existing immunity [22-29], it remains to be determined whether the effects of T cell competition and immunodominance can be circumvented.

One potential way of circumventing T cell competition may be increasing the immunodominance of the target antigen. During T cell competition, vector epitopes and epitopes of the target antigen are both presented on the same APC. Modification of these epitopes, for instance by amino acid substitutions, may stimulate the presentation of target antigen epitopes and/or suppress the presentation of epitopes of the vector. For example, it has been shown that translation of HPV16 E7 from a plasmid made up of codons recombinantly optimized for expression in human cells is up to 100-fold higher than translation from the wild type HPV16 E7 gene [30]. Furthermore, processing of epitopes for MHC class I presentation has been demonstrated to be dependent on the nature of the flanking amino acids [31;32] and MHC class I binding has
been shown to be amenable to modification through alteration of the MHC anchor residues of a peptide [33]. Thus, modification of the vector or target antigen epitopes may facilitate their presentation. In that way immunodominance of target antigen epitopes over vector epitopes may be achieved, which in turn may enable the immune system to focus the response on the target antigen instead of on antigens of the vector.

Interestingly, we found few indications that CTL induction by OVA-virosomes is hindered by T cell competition in mice with pre-existing immunity. Perhaps, the influenza virus antigens are less effectively processed for MHC class I presentation than the OVA epitopes and therefore less immunodominant. This feature may be capitalized on for future immunizations by selecting an influenza virus strain with poorly immunogenic proteins.

As it is of critical importance that the immune response is focused on the target antigen instead of on the vector, it is conceivable that a single epitope vaccine is better suited to circumvent T cell competition than a vaccine comprising multiple epitopes such as proposed for instance by Vazquez Blomquist et al [34]. Immunization with a multi-epitope vaccine would disperse the desired response, thereby perhaps allowing vector-specific T lymphocytes to outcompete target antigen-specific T lymphocytes. A combined prophylactic and therapeutic vaccine against cervical cancer as proposed by Scheurer et al [35] will therefore probably be less effective in patients with vector- or delivery system-specific immunity than a single epitope vaccine. On the other hand, inclusion of a T helper epitope will most likely not hinder immune focusing on a CTL epitope of the target antigen as there is likely no competition between CD4+ and CD8+ T lymphocyte clones [20]. In that regard, inclusion of a T helper epitope may be considered a desirable addition to vaccines.

Importantly, in our study, T cell competition did not hinder CTL activation in homologous prime-boost immunization protocols. This is most likely due to the fact that a prime immunization with rSFV expressing E6,7 induces SFV-specific and E6,7-specific CTL responses of comparable strength that do not compete to the exclusion of E6,7-specific CTL during a subsequent booster immunization. Nevertheless, there is a theoretical possibility that during a homologous immunization protocol with rSFV expressing a very weak antigen, T cell competition would help to establish an SFV epitope as the sole dominant epitope, thereby hampering CTL induction against the transgene. Furthermore, treatment with one rSFV vector will most likely severely hamper the efficacy of a subsequent immunization with rSFV expressing a different transgene.
Increasing the effectiveness of immunization strategies based on virosomes and rSFV

The same mechanisms that, in pre-immune animals, focus the immune system on an SFV epitope instead of on a transgene epitope probably also play a role in establishing a transgene epitope as the sole immunodominant epitope in heterologous prime-boost strategies [36-39]. Indeed, as shown in Chapter 8, a heterologous prime-boost immunization protocol with virosomes and rSFV resulted in increased numbers of specific pCTL. Surprisingly, such a heterologous prime-boost regimen did not result in improved functional responses. Further research into this matter may result in development of heterologous prime-boost regimens that also enhance the functional activity of the CTL response.

Finally, the potency of immunotherapeutic strategies based on virosomes and/or rSFV may be increased by combining them with other forms of treatment. Co-administration of agents that deplete or inhibit regulatory T helper lymphocytes [40;41] may result in stronger specific CTL induction. Furthermore, chemotherapy, inducing tumor cell death and inflammation at the tumor site could enhance the effectiveness of immunotherapy [41]. Radiation therapy may modulate the peptide repertoire of tumor cells and may enhance peptide presentation in the context of MHC class I molecules [42], thereby making tumors more susceptible to immunotherapy.

The development of an immunotherapeutic strategy against (pre)malignant cervical disease based on virosomes and/or rSFV

HPV-induced cervical disease is a particularly attractive candidate for immunotherapy. Due to the relatively slow progression of the disease from HPV infection to low-grade CIN and from low-grade CIN to high-grade CIN or cervical carcinoma, there is ample time to identify patients before they develop advanced cervical carcinoma. Screening programs are essential in this respect and their implementation in national healthcare programs means that patients with HPV infections often present themselves with CIN instead of cervical carcinoma. Many other cancers do not have clear pre-malignant stages, nor are there screening programs in place for the majority of these cancers. Therefore, patients with malignancies other than cervical cancer often present with advanced disease. An established tumor may be hard to eradicate with immunotherapy due to the bulkiness and the immunosuppressive activity of such tumors [43]. CIN lesions, on the other hand, are comparatively small and may not have suffered from
the genetic alterations that reduce the immunogenicity of advanced tumors such as cervical carcinomas [44], making them perhaps more sensitive to immunotherapy.

This thesis and previous studies [2-4] specifically demonstrate the effectiveness of virosomes and rSFV for the induction HPV16-specific immune responses in a murine model. Combined with the fact that cervical cancer is a particularly attractive candidate for immunotherapy as stated above, the results obtained in his thesis and previous studies represent an excellent basis for future clinical trials into therapeutic immunization strategies based on virosomes or rSFV against HPV-induced CIN. Based on the results obtained so far, immunization with rSFV appears to induce more potent immune responses than immunization with virosomes and would therefore be the most attractive candidate for evaluation in a clinical trial. However, virosome-based vaccines are already approved for use in humans, whereas currently rSFV is not and the further development of virosomes may therefore be more feasible.

Some may argue that, since the recent introduction of prophylactic vaccines against HPV (Gardasil® and Cervarix™), there is no longer a need for the development of a therapeutic vaccine for cervical cancer. However, while these prophylactic vaccines have great potential for reducing CIN and cervical cancer rates, several issues concerning the ultimate effectiveness and benefit of these vaccines remain unresolved. It is at this stage unknown whether the protection induced by the vaccines is long-lasting. In addition, the only high-risk HPV types incorporated in the vaccines are types 16 and 18. Therefore, the current prophylactic vaccines only offer protection against about 70% of cervical cancers [45-47]. Furthermore, due to the high costs, these prophylactic vaccines will not be readily available in developing countries, while these countries would benefit most from these vaccines as cervical cancer already has a strongly reduced incidence in developed countries due to extensive HPV screening programs. Therefore, prophylactic vaccination against HPV may contribute comparatively little to a future decrease in cervical cancer rates. Most importantly, as the prophylactic vaccines have no therapeutic potential, those already infected with HPV will not benefit from vaccination. So, even if massive population-wide prophylactic vaccination was instituted today, it would take decades before lower incidences of HPV-induced pre-malignant lesions and invasive cervical cancer would be observed. Therefore, there remains a window of several decades in which therapeutic vaccination could be an important addition to our arsenal of anti-cervical cancer treatments.
Conclusion

In conclusion this thesis describes two potent systems for CTL induction that are not or only marginally hindered by delivery system-specific or vector-specific immunity. This sets virosomes and rSFV apart from many other systems for immunotherapy. Therefore, the research described in this thesis is additional support for the notion that virosomes and especially rSFV are very promising systems for anti-tumor immunization strategies in general and immunotherapy against (pre)malignant cervical disease in particular.

Reference List


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