Immunotherapy of Cancer by Vaccination with Long Synthetic Peptides

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Introduction
Preventive vaccination is the most successful medical intervention of all times. Therapeutic vaccination is not yet a recognized treatment modality in any disease, let alone cancer. Nevertheless many attempts at cancer immunotherapy have been conducted.1,2 Particularly in melanoma, occasional impressive clinical responses have been noted in a small minority of patients, but the overall results were disappointing.2 Our group has argued for some time that perhaps the mode of vaccination is essential for achieving therapeutic results in established disease.2,5 Most preventive vaccines are based on the induction of neutralizing antibodies to the surfaces of viruses or bacteria. Therapeutic vaccines, in contrast, require induction of robust cell-mediated immunity, capable of attacking and eliminating abnormal antigen-bearing cells. It is precisely in this area that our group has made substantial progress in recent years in pre-clinical mouse6 and rabbit7 models, which has culminated in clinical trials8,9.

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A very popular approach to therapeutic cancer vaccination has been vaccination with exact MHC-(HLA-) binding peptides derived from the sequence of tumor-associated antigens.2,5 Our group has reported more than 10 years ago that such a vaccination approach is far from optimal, because it can lead to immunological tolerance towards the immunizing antigen, rather than immunity.10-12 The only mode of immunization by which tolerance induction by exact MHC class I binding peptides could be avoided was *ex vivo* peptide loading on dendritic cells (DC), followed by injection of the peptide-loaded dendritic cell.13 Our finding that vaccination with greater than 15 Amino Acids-long peptides has much better therapeutic results and is in fact capable of eradicating established palpable tumors in mice,6 is linked to our earlier observations with *ex vivo*-loaded DC. We have now shown that injection of 8-10 Amino Acids long peptides leads to the exogenous loading *in vivo* of MHC class I molecules on all cells that have such molecules, including T-cells and B-cells.14 These B-and T-cells, moreover recirculate and therefore also arrive in lymph nodes throughout the body in the absence of immunostimulatory adjuvants. This unavoidably leads to immunological tolerance, because T- and B-cells, in contrast to properly activated DC, lack the costimulatory surface molecules required for appropriate CTL effector cell generation. Upon immunization with long peptides (> 15 amino acids long), in contrast, antigen presentation to CD8 T-cells was found to occur only by DC in the lymph nodes draining the vaccination site.14 This is due to the fact that only DC, the professional antigen processing and presenting cells in our body, are equipped to efficiently process long peptides into the 8-10
amino acid long fragments presented by MHC class I molecules.15

**Immunopharmacology**

Traditionally, vaccines have not been subjected to pharmacodynamics and to other pharmacological considerations. Empiricism ruled their design and application. This is hardly surprising, considering the complexity of traditional preventive vaccines that are mostly composed of killed or attenuated microorganisms. It is not possible to follow the fate of all of the components of such vaccines in vivo and therefore most of the attention was focused on the protective action of the vaccines and the lack of side effects, not on their pharmacological mode of action. This is not a problem when the only goal of preventive vaccination is induction of neutralizing antibodies. Therapeutic vaccines, however, require intense research of the active ingredients and their mode of action, because the goal of such vaccines is the induction of robust cell-mediated immunity, requiring optimal interplay between DC, other cellular components of the innate immune system, CD4 helper cells and CD8 Cytotoxic T Lymphocytes (CTL).2,16 Our results with synthetic long peptides indicate that it is highly valuable to investigate the immunopharmacology of such peptides and the added or coupled ingredients that cause optimal antigen processing and presentation by optimally activated DC. We postulate that eventually all traditional vaccines will be replaced by rationally designed synthetic vaccines for precisely the reason that such vaccines contain only immunostimulatory components that can be easily followed by immunopharmacology, including pharmacodynamics and do not contain components that strongly down-regulate cell mediated immune responses in traditional vaccines such as viral vectors.

**Preclinical Results in Therapeutic HPV16 Vaccine in Mice**

In a mouse model with a transplantable HPV16-positive tumor we systematically compared the efficacy of different peptide vaccine modalities to eradicate established palpable tumors. Vaccination with long peptides together with the TLR ligand CpG showed the best performance. About 75% of mice with palpable HPV16-positive tumors was permanently cured by vaccination with a long peptide containing the immunodominant CTL epitope and an overlapping helper peptide in combination with the TLR ligand CpG.6 A much lower percentage of mice was cured by vaccination with the exact MHC class I binding peptide. Long or short peptides in another adjuvant were even less efficient.

**Preclinical Results in Therapeutic CRPV Model in Rabbits**

Based on the positive results with a long peptide in the mouse HPV16 tumor model we wanted to test the long peptide vaccine concept in a real virus infection model. To this end we induced skin warts in rabbits with a vaccine consisting of all overlapping peptides of the E6 and E7 proteins of Cottontail Rabbit Papilloma Virus (CRPV). Established warts treated with this vaccine in Montanide ISA 51 adjuvant (a mineral oil) eradicated most of the warts and prevented progression towards malignancy. Moreover virus could no longer be detected in most sites of original wart lesions.7

**Clinical Results**

Encouraged by these results we embarked on a phase clinical vaccination study in end stage cervical cancer patients with a vaccine consisting of 27-35 amino acid long overlapping peptides of the complete sequence of the HPV16 E6 and E7 oncogenic proteins. The vaccine was well-tolerated and induced robust T-cell responses against multiple CD4 and CD8 epitopes of HPV16 E6 and E7. One out of 35 patients achieved a complete and lasting regression of all disease. Five additional patients achieved stable disease lasting up to two years as of this writing.8,9 We then went on to conduct a phase II vaccination study in patients with Vulvar Intraepithelial Neoplasia grade III (VIN III), a premalignant condition. In this study 20 patients with VIN III were vacci-
nated 4 times with the same vaccine consisting of 13 overlapping long peptides of the E6 and E7 oncoproteins of HPV16. Four (20%) of these patients achieved complete regression of all lesions, other patients showed partial regression of the lesions with strong relief of symptoms. These results are encouraging, because further improvement can be expected from co-formulation of this vaccine with a potent TLR ligand.

References