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Enhancing T cell mediated immunity in DNA vaccination

av

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Abstract

For centuries infectious diseases have been one of the main causes of death in the world. With the invention of vaccine technology 200 years ago, we have been able to impede and even eradicate several dangerous pathogens. However, even with the success of modern vaccine technology, there are several diseases for which there are no effective vaccines. Cancer is the second main cause of death in the western world; where conventional treatments can cure a majority of patients, there is still no treatment for several malignancies. Thus, there is a need to develop alternative therapies to be able to salvage those patients who cannot be helped with standard therapy. One such strategy is immunotherapy - wherein specific antibodies and T-lymphocytes are raised against antigens overexpressed on the surface of the tumors by immunization. Conventional vaccines have shortcomings with regard to safety and efficacy. An alternative method is to use DNA that encodes for an antigen in a circular ring (plasmid) and injecting the DNA directly into a patient. The DNA is then taken up by cells in the body, producing the antigen *in vivo*. This methodology is important since it ensures not only long lasting expression of the antigen to continuously boost the immune system, but more importantly, it facilitates the proper processing of the antigen and presentation by the antigen presenting machinery. DNA vaccines have been able to induce immunity in both mice and men to wide selections of pathogens and tumors. However, due to low efficacy of DNA vaccines, the clinical effects have been limited and survival not significantly improved. This is despite sometimes-impressive demonstration of T and B cell responses *in vitro*. Clearly DNA vaccines must be improved for this therapeutic strategy to have a clinical relevance.

In this thesis, I have described different ways to enhance the effect of DNA vaccines, focusing on the induction of T cell mediated immunity in three different ways. Protein Transduction Domains, PTD, are short alkaline peptide segments which when fused to an epitope (small antigenic peptide) can transfer the epitope across the plasma membrane barrier. We have shown that mice immunized with PTD-epitope DNA vaccines demonstrate increased survival to a lethal infection of Lymphocyte Choriomeningitis Virus (LCMV). Furthermore, we prove that this mechanism is proteosome dependent and mediated by TAP, indicating that the effect is mediated through the normal MHC class I pathway. Protection was associated with high CD8+ T cell precursor frequency. Another mechanism by which immunity can be enhanced is by activating the secondary signal. This is usually done through a reversible covalent binding known as “Schiff-base formation”. Other amine donors in the form of a drug can mimic such Schiff base formation. Tucaresol is such a Schiff base-forming drug, which we tested in an influenza mouse model. We could see that survival was increased with the addition of Tucaresol and this was associated with higher CTL specific killing of influenza peptide pulsed target cells. An attempt has also been made in this thesis to delineate the components of the immune system that are employed in efficient clearing of pathogens or malignant cells. The model that was employed was that of HER-2/neu overexpressing tumors. HER-2/neu is a tumor-associated antigen that is overexpressed in 20-30% of all breast and ovarian carcinomas. It is expressed at very low levels or is absent in normal cells, and its overexpression in malignancies is associated with poor prognosis, thus making it an attractive target for immune therapy. Conflicting results exist regarding the role of antibodies versus T cell mediated immunity in HER-2 based immune therapy. In order to investigate this we employed B cell deficient mice, which lack the ability to produce antibodies as well as depletion of CD8+ and/or CD4+ subsets of T cells. We were able to demonstrate that immunity to a lethal challenge of tumor cells was the results of HER-2 specific T cells in the absence of antibodies. The immunity was furthermore dependent on helper T cells and their ability to produce the cytokine IFN-γ during the effector phase of the immune response. We were also able to prove that specific immunity could only be induced on co-administration of a plasmid encoding the cytokine GM-CSF.

This thesis also investigates the use of Virus-Like Particles (VLPs) as carriers of HER-2 antigen. A fusion protein consisting of the Vp2 of Murine Polyoma Virus (MyPV) with the extracellular and transmembrane domains of HER-2 neu were encapsulated by Vp1 protein from MyPV. We show that a single injection of HER-2 containing VLPs can protect from tumor challenge and tumor outgrowth in a transgenic mouse model. In summary, the efficacy of DNA vaccines can be augmented by several means; protective and therapeutic effects appear to be associated with potent T cell mediated immune responses, a lesson which can be taken to the clinic.

Keywords: DNA vaccination, CTL, T cell, IFN-gamma, anti-tumor immunity, HER-2/neu, Influenza, LCMV, Virus-Like Particles, murine models, adjuvants, immunotherapy

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