Peptide vaccines: the future of immunotherapy?

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It is widely accepted that vaccines are valuable preparations for prevention against life-threatening diseases such as polio, measles and smallpox and their utilization led to control and/or eradication of aforementioned infectious diseases. Already at the end of 18th century, Edward Jenner invented the smallpox vaccination using cowpox virus as the immunizing agent, which is less virulent than small pox and thus not hazardous to humans but leads to immunization.[1] However, it was not until Louis Pasteur and later Robert Koch revolutionized the field of vaccination in late 1880´s in which they expanded the scope of vaccines against other infectious diseases such as anthrax and rabies [1]. Following these discoveries, within the last century, seminal advances were made in microbiology and vaccine development.

According to Oxford Dictionary, a vaccine is described as:" *a substance used to stimulate the production of antibodies and provide immunity against one or several diseases, prepared from the causative agent of a disease, its products, or a* ***synthetic substitute****, treated to act as an antigen without inducing the disease*.".[2] However, there are some drawbacks using the infectious agent or whole proteinogenic antigens in those formulations. On one hand, the microbe expresses not only the antigen of interest but also a number of other antigenic proteins, which may lead to allergic reactions. On the other hand, when using whole antigens, these proteins usually not only contain one but multiple epitopes. Hence, design of vaccines carrying the epitope of interest that are immunogenic enough to raise a T-cell response without being allergenic is required. One class of biomolecules, namely peptides, are adequate substitutes to show this desired effect and therefore represent an emerging field in vaccination biology. Moreover, they are easily accessible via SPPS in quantitative amounts.

Peptide vaccines usually consist of 20-30 amino acids and an adjuvant to enhance immunogenicity of peptide epitopes, which are normally weak antigens and could therefore not always initiate an immune response. However, these adjuvants involving chemical substances and biomolecules (ligands, proteins etc.), might cause allergic reactions, similar to those of whole antigens. Based on this background, I will discuss two recent examples of peptide vaccines with covalently bound adjuvants, referred to as self-adjuvating vaccines. On one hand, self-adjuvating properties of TLR ligands in peptide vaccines will be discussed [3], on the other hand it will be demonstrated how serendipity influences vaccine design exemplified by self-adjuvating (TLR-7 ligand) peptoid vaccines.[4]

SELF-ADJUVATING PEPTIDE VACCINES BEARING T_N ANTIGENS

The first publication discussed herein describes the elegant combination of chemoselective ligations and organic synthesis to obtain peptide vaccines with covalently attached, lipidated adjuvants.[3] Richard Payne and coworkers succeeded with the synthesis of a self-adjuvating peptide vaccine carrying the T_N antigen (Figure 2a) of Mucin 1 (MUC1) variable number tandem repeat (VNTR) domain (Figure 2b). MUC1 is a mucin, a class of proteins that are heavily O-glycosylated and are involved in host-defense against bacteria and other microbes. Like other mucines, MUC1 is expressed on the surface of epithelial cells. Interestingly, aberrant glycosylation of MUC1 (truncation of large O-glycans) results in formation of new epitopes carrying the so-called tumor associated carbohydrate antigens (TACAs), T_N and T antigens respectively (Figure 2a). Both overexpression and aberrant glycosylation of MUC1 are proven to be linked with tumor formation in several cancer types. Thus, a growing interest to target aberrantly glycosylated mucines is shown. One way to selectively inhibit the tumor growth is to generate tumor cell specific T-cell response. Since MUC1 is overexpressed in tumor cells, a vaccine against this glycosylated peptide might enable such a response. Hence, a number of groups are working on this research topic, including Richard Payne´s lab.[5-7]

In their earlier publications, Payne group described the synthesis of MUC1 vaccine candidates decorated with several T and T_N antigens. However, despite proven humoral response, no cytotoxic T-cell (CTL or CD8+) response was obtained. To solve this problem, they designed a new peptide carrying less glycans (two T_N antigens) and decorated with Pam_2Cys TLR-2 ligand as an adjuvant (Figure 2b). Previous attempts to obtain peptide vaccines with TLR-2 ligands involved NCL and were successful but elaborate, e.g. utilization of liposomes[6] or use of detergents[7]. Payne and coworkers envisioned a one-pot, detergent-free ligation strategy using their recently published diselenide selenoester ligation (DSL)[8]. In this approach, the researchers generate a selenoester on C-terminus of peptides, whereas the N-terminal selenocysteine dimer of a second peptide enables a yet not fully explored ligation mechanism resulting in formation of a nascent peptide bond (Figure 2b). One major advantage of this method is the fast reaction rate without the need of additives. Thus, lipidated peptides, which are usually hard to ligate due to their hydrophobic nature (low yields) and therefore require longer reaction times (>24h), might be ligated to deprotected peptide segments bearing N-terminal diselenide-peptides faster and in better yields. The researchers demonstrated that under optimized conditions the conversion was fast (2 mins) and gave rise to quantitative conversion yields. Moreover, upon hexane extraction to remove diphenylselenol, one-pot deselenization at acidic pH with TCEP and DTT was performed (62% overall yield after HPLC purification).

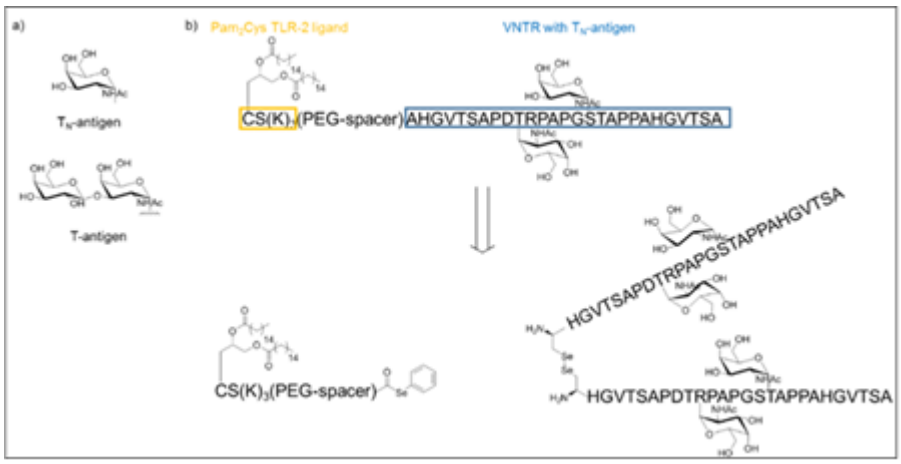

Fig. 2. Retrosynthesis of self-adjuvating MUC1 vaccine

With that in hand, Payne and coworkers evaluated the immunogenicity of their self-adjuvating vaccine candidate. *In vivo* experiments were done injecting the vaccine candidate to C57BL/6 mice subcutaneously. Adoptive transfer of a mixture of splenocytes and MUC1 specific T-cells to both control and vaccinated mice resulted in significant increase in cytotoxicity (cell lysis) of MUC1 labelled cells in vaccinated mice but not in control mice injected with PBS. Next, Ig titers in mice sera were measured demonstrating that vaccination led to high IgM but not to IgG titers. To improve the IgG titer, researchers added a pan helper T-cell epitope (PADRE) of an irrelevant protein, a common method used in vaccination to increase immunogenicity. Interestingly, the IgG titer did not increase, whereas PADRE specific T-cells secreted pro-inflammatory cytokines upon exposure to the self-adjuvating vaccine and external PADRE.

To summarize, Payne and coworkers reported the generation of a self-adjuvating MUC1 vaccine leading to CTL stimulation for the first time. Furthermore, they demonstrated that their recently reported DSL chemistry is fast, quantitative and does not require any additives during the ligation process, even in the case of hard-to-ligate peptide fragments such as lipidated peptides.

SELF-ADJUVATING PEPTOID VACCINES

Very recently, Taillefumier and colleagues described a similar approach for the synthesis of a self-adjuvating (TLR-7 ligand, a 8-oxoadenine derivative) β-alanine-glycopeptoid vaccine, which was oligomerized and decorated with T_N antigen on N-heteroatoms (Figure 3) of the peptoid backbone.[4] The vaccine was further conjugated to the helper CD4+ T-cell epitope of ovalbumin (ISQAVHAAHAEINEAGR) to boost immune activation and IgG production. Besides the elegant synthetic route combining carbohydrate chemistry, peptide coupling and copper(I)-catalyzed-azide-alkyne-cycloaddition (CuAAC), the authors delivered convincing immunological data highlighting activation of dendritic cells (DCs, cytokine secretion upon maturation), B-cells (antibody response) and T-cells (against T helper epitope, cytokine secretion).

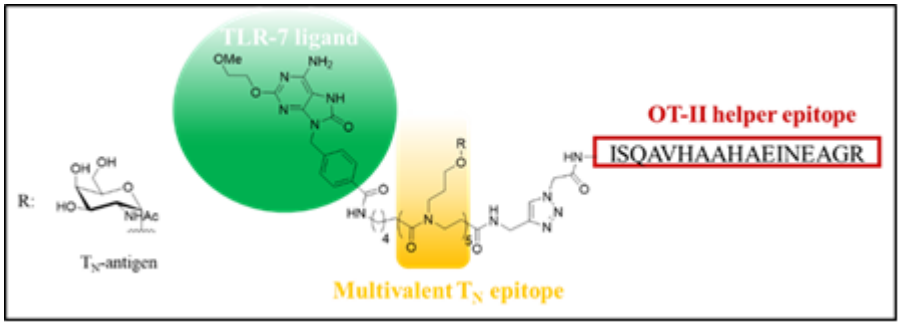

Fig. 3. Schematic representation of self-adjuvating peptoid T_N vaccine

The self-adjuvating effect of TLR-7 ligand was demonstrated via OT-II T-cell activation experiments, in which the researchers isolated T-cells from spleen and the lymph node (LN) of mice injected with the vaccine and the control mice vaccinated with single ovalbumin OT-II epitope without the adjuvant. They found 30-fold more OT-II T-cells in the spleen and LN of mice injected with the self-adjuvating vaccine compared to those of control mice. Interestingly, further evaluation of B-cell activation showed that IgG response was not raised against T_N antigen but against the TLR-7 ligand. On one hand, it was a drawback, since this unexpected response did no accompanied with T_N-specific immunization. On the other hand, the researchers demonstrated a TLR-7 vaccine with potent IgG antibody titers for the first time. TLR-7 is

linked to emergence of autoimmune diseases such as systemic lupus erythematosus (SLE) and ligands of TLR-7 (and TLR-9) have been into involved with activation of B-cells and inhibition of IFN-I.[9] Hence, a vaccine helping to produce circulating antibodies against TLR-7 might lead to inhibition of TLR-7 associated pathway of SLE.

To summarize, here I commented on two recent publications describing the synthesis and immunological evaluation of self-adjuvating pept(o)ide vaccines. Payne and coworkers reported the first self-adjuvating MUC1-T_N specific vaccine leading to CTL activation in the absence of external adjuvants, whereas Taillefumier and colleagues demonstrated that TLR-7 ligands can act as self-adjuvants and even be the agent against which specific antibody response could be raised. While both articles present very elegant synthetic methods (DSL chemistry and combination of solution chemistry with solid-phase synthesis involving chemoselective conjugation techniques) to generate the vaccines, the immunological outcome should be explored further. Nevertheless, I believe that these advances might draw the attention of chemical biologists to the emerging field of peptide vaccine research. Our knowledge on the impact of immune system in cancer is still limited and yet peptide vaccines used in fundamental research projects may represent the first steps to develop tailor-made therapeutic peptide drugs to fight cancer.

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