New strategies for simplifying influenza vaccination
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Chapter 1

General Introduction
Introduction

Influenza is an infectious disease caused by influenza A viruses. Influenza occurs as seasonal epidemic and pandemic. Almost every human is susceptible to contract this virus. In seasonal outbreaks, the influenza virus causes illness ranging from mild fever to death. In future pandemics, there is a serious risk for high mortality rates because humans are naïve to new influenza virus strains especially the bird flu strains H5N1 and H7N9 [1]. Therefore, it is very important to control the spread of influenza virus during both epidemic and pandemic outbreaks.

The spread of influenza during outbreaks can be controlled either by the administration of antivirals (neuraminidase inhibitors) or by vaccination [2,3]. The neuraminidase inhibitors like oseltamivir and zanamivir are used to control the disease in seasonal and pandemic outbreaks. During the 2009 swine flu pandemic, oseltamivir was the drug of choice for the treatment and prophylaxis of swine flu (H1N1) [4]. However, it does not prevent one's ability to acquire the virus from an infected person. In principle, influenza vaccines are capable of inducing an immune response that can immediately neutralize the virus. Therefore, vaccination is considered to be more effective than the administration of antivirals. Influenza vaccines are available as inactivated vaccines i.e. subunit, split, virosome and whole inactivated virus (WIV) and live attenuated virus (e.g. Flumist®). WIV, subunit and split vaccines are widely used because virosomal and live attenuated virus vaccines are only approved in a few countries [5–7].

Although influenza vaccines are advantageous over antivirals there are several shortcomings related to the route of administration and the production and properties of the vaccine formulation. Except for the live attenuated virus vaccine, which is administered intranasally, all the other types of influenza vaccines are administered either through the intra muscular or subcutaneous route. These routes of vaccination require resources like syringes, sterile needles and the help of trained healthcare workers. These prerequisites further add up to the costs of vaccination. Moreover, due to needle phobia compliance can be compromised. Furthermore, the possibilities of needle stick injuries also add up to limitations of conventional vaccination routes. In terms of vaccine production capacities, both egg and cell culture based influenza vaccine production is limited. As a result during a pandemic, mass vaccination involving high and multiple vaccine doses may not be feasible at the present situation. Current influenza vaccines also have several shortcomings with respect to the immune response they elicit. Despite vaccination, people still can get sick and this could be overcome by inducing stronger immune response in the respiratory tract, i.e. the port of entry of virus. Finally, current influenza vaccines including the live attenuated virus vaccine are unstable and therefore require refrigerated storage and transport, the so-called cold chain. In particular for stockpiling pandemic and seasonal influenza vaccines, storage at ambient temperature would be highly advantageous, as it would significantly reduce the costs. Summarized, there is a need for an influenza vaccine that can be self-administered via a non-parenteral route,
induces a potent immune response at relatively low dose, and is stable. In this thesis, we explored two different strategies to develop a vaccine/vaccination routine that could possibly fulfill these requirements.

In the first strategy, we envisage to develop a dry and stable powder vaccine that can be administered through the pulmonary route. The main advantage of this route of administration is its ease of administration. Therefore, it requires a minimal involvement of trained health care workers [8]. A dry powder formulation is preferred over a liquid formulation because it has been shown before that when dried in the presence of stabilizing excipients, the antigenicity of the vaccine is not affected during drying and storage for extended period of times at ambient conditions. [9]. Moreover, with the application of proper drying techniques, powder particles can be produced that are suitable for inhalation [10–12]. Obviously, it is of utmost importance that during storage not only the antigenicity of the vaccine is maintained but also the physical powder properties. Changes in physical powder properties e.g. irreversible agglomeration, would severely compromise the suitability of the powder for pulmonary administration. As the physical characteristics of vaccine powders during storage have not been studied before, this issue will be addressed in this thesis. The feasibility of pulmonary administration of influenza vaccine has been studied before. In several studies it has been shown that pulmonary vaccination can induce not only potent systemic immune responses but can also elicit immune responses in the respiratory tract, the port of entry of virus [9,12]. However, it fails to induce a balanced Th1/Th2 immune response [9]. In addition, pulmonary vaccination induces poor IgA antibody responses in the nose. We hypothesize that the quality of immune response can be improved by the co-administration of adjuvants. Moreover, application of adjuvants may facilitate dose sparing. Therefore, in this thesis various adjuvants were incorporated in the dry powder formulations and evaluated in a mouse model.

In the second strategy, which might be in particular suitable during a pandemic outbreak, we envisage to develop a sublingual tablet containing a stabilized vaccine of a previous strain, which can be used as primer for a booster vaccine prepared from the current drifted strain. As described above, the influenza pandemic outbreaks create a big risk of vaccine shortage. It has been described that the immune response against newly drifted influenza strains can be primed with old vaccine strains [13]. The major advantage of this strategy is that the old vaccine can be produced in large quantities because time constraints lack. However, the priming doses used in that study were unstable liquid formulations, which will hamper stockpiling of these vaccines. Moreover, the liquid formulations were administered by injection, which has the shortcomings as described above. To overcome both the disadvantage of the antigen instability and the parenteral administration, we propose to develop a stable tablet formulation that can be administered sublingually. As described above, using stabilizing excipients and proper drying techniques, the vaccine can be brought in a dry and stable state. In this thesis, it is investigated whether or not this powder can be formulated into a tablet suitable for sublingual applications while the antigenicity
of the vaccine is maintained. We propose to apply a sublingual tablet because it is in particular suitable for mass vaccination campaigns during a pandemic. Furthermore, we have chosen for this route of administration because it has been shown in previous studies that the sublingual administration of influenza vaccines can successfully induce immune responses [14–16]. However, these studies were performed with unstable liquid influenza vaccines. Moreover, to our knowledge the sublingual prime and heterologous booster strategy has never been explored before.

**Highlights of this thesis**
The highlights of this thesis are,

- The storage stability for stockpiling influenza vaccines
- Improve the immune response by co-administering adjuvants with stabilized pulmonary influenza vaccines
- The prime-boost strategy for vaccination during pandemics with sublingual tablet and heterologous i.m. booster vaccine.

**Outline of the thesis**

**In chapter 2**, we reviewed the importance of particulate influenza vaccines. The current status of liposomes, virosomes, nanoparticles and virus like particles in influenza vaccination was discussed in a broader context. The advantages and limitations of these vaccine systems were described. This review also includes a discussion on regulatory challenges, compliance issues and perspectives of particulate influenza vaccines.

**In chapter 3**, the physical powder properties and the immunogenic stability of pulmonary influenza vaccine powders during storage was evaluated. The influenza vaccine was spray freeze dried in the presence of inulin, dextran and a mixture of dextran and trehalose. The dry powder formulations were stored at different temperatures for 3 months. The physical powder properties such as particle size and specific surface area before and after storage were evaluated. The immunogenic stability of the stored influenza vaccines was evaluated in mice.

**In chapter 4**, δ-inulin, a carbohydrate based adjuvant was evaluated for its adjuvant effect when co-administered with pulmonary influenza vaccines. The immune responses induced by adjuvanted and pulmonary influenza vaccines alone in mice were compared.

**In chapter 5**, monophosphoryl lipid A, a lipid based vaccine adjuvant was tested with pulmonary influenza vaccine powder in mice. The receptor binding capacity and immunostimulatory properties of the adjuvanted influenza vaccine was evaluated. The antibody levels and memory B cell levels induced by adjuvanted vaccine were compared to that of pulmonary vaccine alone. The advantages of
adding monophosphoryl lipid A to influenza vaccine are discussed in comparison
to vaccine alone.

In chapter 6, a wide range of vaccine adjuvants were evaluated with pulmonary
influenza vaccine powders in mice. The adjuvants tested were Pam3CSK4,
monophosphoryl lipid A, CpG-ODN-1826 and GPI-0100. The immune responses
induced by these adjuvanted influenza vaccines were compared. The choice of
optimal adjuvant for pulmonary influenza vaccine is also discussed in this chapter.

In chapter 7, a prime-boost strategy of influenza vaccination was evaluated in an in-vivo
study using mice. In this study, the immune responses were primed with a single
dose of sublingual influenza vaccine. Later, the immune response was boosted with a
heterologous i.m. influenza vaccine. Furthermore, the possibilities to formulate the
influenza vaccine into stable sublingual tablet were explored.

In chapter 8, the results of the research described in this thesis are summarized and
the perspectives are discussed.

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