Chapter 8

Summary and concluding remarks

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Summary

Vaccination against influenza is the most important strategy to control the virus spread during seasonal epidemics and pandemics. However, there are several shortcomings related to current vaccines, i.e. they have to be administered parenterally (except for the live attenuated virus vaccine Flumist®), their production capacities are limited, the immune response they elicit is sub-optimal, and they are unstable.

In this thesis, we explored two different strategies to avoid these shortcomings. In the first strategy, a dry and stable adjuvanted vaccine powder has been developed that can be administered through the pulmonary route. In the second strategy a sublingual (s.l.) tablet containing a stabilized vaccine of a previous strain was developed that can be used to prime the immune response for a booster of a vaccine prepared from the current drifted strain.

Hence, in this thesis we evaluated,

1. The storage stability of powdered pulmonary vaccines.
2. The preservation of immunogenicity of adjuvanted pulmonary influenza vaccines during spray-freeze drying process, we also evaluated the adjuvant effect of adjuvanted vaccines.
3. The sublingual priming (with stabilized vaccine) for subsequent intra muscular (i.m.) boosting of immune response with heterologous influenza vaccine

In chapter 2, a literature review is given on particulate influenza vaccines. The particulate influenza vaccines resemble the intact influenza virus. The particulate nature of the vaccines holds great promise to induce better immune response than subunit or split vaccine. Furthermore, the review discusses about the regulatory challenges and perspectives of particulate influenza vaccines.

In chapter 3, we evaluated the physical and immunogenic stability of powdered WIV for pulmonary vaccination. WIV was spray freeze-dried in the presence of inulin, dextran and mixture of dextran and trehalose. The properties of spray freeze-dried powders were found to be suitable for pulmonary immunization. Furthermore, during spray freeze drying, WIV retained its particulate nature and its antigenicity was maintained. The powdered vaccines were stored at -20, 2-8, 30 and 40 °C for 3 months. The physical properties of all three vaccine powders, i.e. the particle size and specific surface area were maintained when stored at temperatures up to 30°C. The powdered vaccines stored at 30 °C for 3 months were used to evaluate their receptor binding capacity and immunogenicity. It was found that the receptor binding capacity of dry powder vaccines was preserved which was confirmed by the maintenance of the hemagglutination titers. The immunogenic stability of the formulated powders was evaluated in mice. The results revealed that the immunopotentiating effects of dry powder vaccines were maintained as the immune responses elicited by the stored vaccines were comparable to freshly prepared spray freeze-dried vaccines. During
storage, however, most likely some particle-particle interaction occurred which interfered with a proper vaccine delivery to the lungs when using the dry powder insufflator. In contrast, this problem was not observed when the RODOS powder disperser was used. Hence, it should be noted that the stored vaccines require an efficient disperser for proper delivering the powder to lungs.

In chapter 4, the adjuvant effect of δ-inulin with liquid pulmonary vaccine was evaluated. δ-inulin was mixed with influenza vaccine and then administered to mice either via i.m. injection or the pulmonary route. The immunological readouts including serum IgG, IgG subtypes, nose IgA and serum hemagglutination titers were evaluated. In terms of serum IgG and nasal IgA antibody levels, pulmonary vaccination induced a better immune response than i.m. vaccination. However, pulmonary vaccination of vaccine alone induced a Th2 dominant immune response. The addition of δ-inulin to vaccine induced a more balanced Th1/Th2 immune response without compromising the Th2 immune response. Furthermore, the δ-inulin adjuvanted vaccine enhanced the nasal IgA antibody levels compared to pulmonary vaccine alone. The adjuvanted pulmonary vaccine induced hemagglutination titers of > 40 which is generally considered to be protective. Thus, the addition of δ-inulin to the pulmonary influenza vaccine has an advantage over both the non-adjuvanted pulmonary vaccine and the adjuvanted i.m. vaccine.

In chapter 5, we incorporated the adjuvant monophosphoryl lipid A (MPLA) together with WIV in sugar glass matrix of inulin by spray freeze-drying technique. The physical and immunological properties of the vaccine powder for pulmonary immunization were evaluated. Maintenance of the adjuvant activity of MPLA during spray freeze drying was confirmed by NFκB activation with RAW-Blue™ cells. The particle size of the vaccine powder appeared to be within the acceptable range for inhalation. The maintenance of the receptor binding efficacy of WIV during spray freeze drying was confirmed by the hemagglutination titer. The immunogenicity of WIV in mice was confirmed by antibody immune responses. The MPLA adjuvanted pulmonary vaccine induced high IgA antibody levels compared to vaccine alone. The incorporation of MPLA in influenza vaccines induced a more balanced Th1/Th2 immune responses than pulmonary vaccine alone. Moreover, the adjuvanted pulmonary influenza vaccine equally neutralized the influenza virus as seen in the control (i.m.). Overall, the MPLA adjuvanted pulmonary influenza vaccine induced better mucosal and systemic immune responses than pulmonary vaccine alone.

In chapter 6, various adjuvants were evaluated for pulmonary vaccine powders. The adjuvants include palmitoyl-3-cysteine-serine-lysine-4 (Pam3CSK4, TLR 1 and TLR 2 ligand), MPLA (TLR 4 ligand), CpG oligodeoxynucleotide (CpG-ODN-1826, TLR 9 ligand) and GPI-0100 (a saponin based, non-pattern recognizing receptor binding compound). The adjuvants were incorporated in the vaccine powders by spray freeze-drying a mixture of the adjuvant, WIV and inulin as lyoprotectant. The NFκB reporter cell line studies confirmed the preservation of immunostimulating properties of these vaccine powders. All the TLR ligands were capable of inducing
systemic immune responses in mice, but they failed to induce potent mucosal immune responses. However, GPI-0100 induced both potent mucosal and systemic immune responses compared to the vaccine alone. The GPI-0100 also protected the mice from a lethal challenge of a heterologous influenza strain. Thus, GPI-0100 holds great promise to act as a potent adjuvant for pulmonary influenza vaccines.

In chapter 7, it was investigated whether s.l. administration of influenza vaccine can prime the immune system for a later i.m. boost with a heterologous influenza vaccine, the priming efficacy of a stabilized influenza vaccine through s.l. route for a heterologous i.m. booster vaccine. Ideally, the vaccine for s.l. applications should be administered as a tablet in which the vaccine is stable. Therefore, WIV was freeze-dried with inulin and then formulated as a s.l. tablet. During processing, the vaccine stabilized with inulin in the sugar glass matrix remained intact, which was confirmed by SDS-PAGE and hemagglutination titers. The sugar-glass stabilized vaccine was successfully formulated into a s.l. tablet. For animal studies, however, we reconstituted the freeze-dried vaccine to test the priming efficacy of vaccine because of practical difficulties related to s.l. administration of tablets to mice. The i.m. boosting efficacy of heterologous vaccine was tested. The i.m. booster vaccine of a heterologous strain resulted in the induction of serum IgG and nasal IgA antibody immune responses against both new and old strains. Therefore, it was concluded that the s.l. vaccination can indeed prime the immune response for a subsequent i.m. booster vaccine.

Concluding remarks and perspectives

In this thesis, two different strategies we explored to overcome / minimize the shortcomings of conventional influenza vaccines. First, a stable influenza vaccine powder for pulmonary administration was developed and the possibilities of adding adjuvants to pulmonary vaccines were evaluated. Second, as a pre-pandemic measure, we tested the priming efficacy of stable s.l. vaccine for a subsequent heterologous i.m. booster vaccine, especially with H5N1 strains, which pose a threat for future pandemic.

To prepare a dry powder vaccine formulation for pulmonary administration, WIV was spray freeze-dried in the presence of inulin, dextran or a mixture of dextran and trehalose (dex/tre). Spray freeze-drying was selected as a technique to dry the vaccine as it can yield powder particles with proper characteristics for pulmonary administration. It has been shown before that WIV can be spray freeze-dried in the presence of inulin without loss of its antigenicity [1]. In this thesis, it has been shown that both dextran and dex/tre can be used as stabilizing excipients. Furthermore, it was found that the antigenicity of WIV incorporated in these sugars was maintained during storage for at least three months at temperatures up to 40 °C. Also the powder characteristics during storage were evaluated, as a change of these characteristics can be highly detrimental for pulmonary deposition. It was found that the particle size distribution and specific surface area of the inulin and dex/tre based formulations
did not change during storage for three months at a temperature up to 30 °C. Interestingly, the powder characteristics of the dextran based formulation was even stable at 40 °C. Hence, the inulin and dext/tre stabilized influenza vaccines have a potential for an excellent long term storage stability at ambient temperatures while the dextran based formulation even has a superior stability profile (Chapter 3). Therefore, stockpiling of these dry powder vaccine formulations are not or less dependent on refrigerated conditions. Inulin, dextran and trehalose are approved for parenteral and oral use in humans, but not for pulmonary administration. In studies of Zijlstra et al. and Audouy et al. it was shown that pulmonary administration of inulin to the lungs of rats and mice respectively, only induced a mild inflammatory response [1,2]. However, further research towards the safety of administering inulin and the other two sugars to the lungs have to be tested in more detail in appropriate animal models as well as in humans.

Previous studies indicate that pulmonary administration of WIV elicits comparable or higher immune responses than conventional i.m. vaccines [1,3]. However, it induces a Th2 skewed immune response while a balanced Th1/Th2 immune response is preferred [4]. In addition, the induction of nasal IgA antibodies is poor. To improve the quality of immune response, a range of potential vaccine adjuvants was incorporated by spray freeze-drying in inulin glasses together with WIV. It was found that the co-incorporation of adjuvants PAM3CSK, CpG ODN, monophosphoryl lipid A and GPI-0100 in inulin glasses did not affect their adjuvant activity. Pulmonary administration of these powders to mice indicated that in particular monophosphoryl lipid A and GPI-0100 elicit improved immune responses (Chapter 5 and 6). Both the cellular immune response and the local immune response in nose were improved when compared with the vaccine alone. After a challenge with a heterologous influenza strain, the formulation with GPI-0100 even reduced the lung virus titer 10 times more than the vaccine alone. Furthermore, in a preliminary study δ-inulin was evaluated as adjuvant for pulmonary immunization. In this study, the adjuvant was ad-mixed with WIV dispersion and pulmonary administered to mice. This study showed that also δ-inulin improved the immune response with respect to cellular and local immune responses when compared to the vaccine alone (Chapter 4). Therefore, these studies clearly indicate that various adjuvants can be used to potentiate pulmonary influenza vaccines. The studies described in this thesis were performed at a fixed adjuvant dose. Thus, to further improve the immune response the adjuvant dose should be optimized. In these studies, also the level of protection by these immunizations against a virus challenge should be investigated more extensively, not only in mice but also in more appropriate animal models such as cotton rats and ferrets. More interestingly, the possibilities of dose sparing for the vaccine should be investigated. The δ-inulin adjuvant was evaluated as a liquid formulation. From previous studies it is well known that the pulmonary administration of influenza vaccine in dry powder state induces a more potent immune response than in liquid state [1]. Thus, it would be interesting to investigate the pulmonary administration of dry powder formulation of a spray freeze-dried formulation containing δ-inulin
and WIV. Furthermore, similar to the safety aspects described above for the applied sugars, also the toxicity of the adjuvants in the lungs should be studied in detail before application in human.

The priming efficacy of s.l. vaccine was tested for a subsequent heterologous i.m. booster vaccine in an animal study using mice. The immune response was primed by s.l. administration of WIV prepared from NIBRG-14 and booster by i.m. injection of WIV prepared from and NIBRG-23 to test the prime-heterologous boost strategy for H5N1 bird flu vaccines. The results showed that indeed s.l. immunization with NIBRG-14 primed for a subsequent heterologous i.m. booster immunization with NIBRG-23 vaccine as the serum HI titers and nasal IgA antibody titers against NIBRG-23 were much higher than after i,m. immunization with NIBRG-23 alone. In this study, WIV was also freeze-dried together with inulin as lyoprotectant and then formulated using appropriate excipients into a stable tablet suitable for s.l. administration (Chapter 7). Such tablets holds great promise for a future pandemic as a number of advantages: 1) They can be prepared from a previous influenza strain, thus reducing the risk for vaccine shortage of vaccine prepared from the virus spreading at that moment. 2) They are stable and can thus be stockpiled under no or less stringent refrigerated conditions. 3) They are easy to distribute and administered. Therefore, priming of the immune response with a s.l. tablet for a booster vaccine prepared from heterologous drifted strain can be considered as a step forward in pandemic preparedness. In this thesis, the s.l. prime was administered as a liquid formulation due to technical problems associated with the s.l. administration of tablets to mice. Therefore, the s.l. tablet primer and heterologous i.m. booster strategy should be investigated in bigger animals e.g. cotton rats or ferrets. Furthermore, the protective immunity should be evaluated in challenge models. To improve the priming efficacy, potent adjuvants could be incorporated into the s.l. vaccine tablets. The mucosal adjuvants discussed in this thesis like MPLA, GPI-0100 or other potent adjuvants may serve as future adjuvant candidates for s.l. vaccines. Moreover, adjuvation of s.l. vaccines might be a step forward to further facilitate vaccination e.g. by replacing the s.l. prime and i.m. boost strategy with non-invasive s.l. prime and s.l. boost strategy. By eliminating the need for invasive (i.m.) booster vaccination, the reach of vaccine could be improved.

References


