Structural Characterization of Lipid-based Adjuvants for Subunit Vaccines

Structure-Activity Relationship for Analogues of the Mycobacterial Cell-Wall Lipid Monomycoloyl Glycerol

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May 2017

Abstract

Subunit vaccines based on *e.g.* highly purified recombinant antigens are promising, safer and efficient alternatives as compared to traditional vaccines based on live attenuated or whole inactivated pathogens. One of the challenges in developing subunit vaccines is the poor immunogenicity of most antigens and therefore co-administration of adjuvants is often needed to potentiate the immune response.

Identification of new adjuvants usually involves *in vitro* evaluations of candidate compounds testing their ability to stimulate cells of the immune system. Both a specific molecular structure and an optimal presentation of specific moieties of the compounds are required for successful stimulation of the cells. Nevertheless, the supramolecular structure of the adjuvants is rarely evaluated although it may have a drastic impact on the presentation of the compounds to the cells.

The purpose of the present project was to investigate a promising adjuvant family consisting of synthetic double-tailed surfactant-like lipids based on the mycobacterial cell wall lipid monomycoloyl glycerol (MMG). An array of synthetic MMG analogues, differing in the alkyl chain length or the headgroup and lipid tail stereochemistry, was studied and the supramolecular structure was related to the immunopotentiating properties *in vitro*. The results indicated that the self-assembly of these synthetic MMG analogues into supramolecular structures in excess buffer or excess biologically relevant medium has a major impact on their immunopotentiating properties *in vitro*, since the presentation of the compounds to the cells is affected. Thus, fully hydrated MMG analogues adopting an inverse hexagonal (H_2) structure were less prone to activate the cells, as compared to analogues adopting a lamellar structure.

Furthermore, the thermal phase behavior of the synthetic MMG analogues in excess buffer was investigated and revealed that all analogues exhibit a direct phase transition from a lamellar to an inverse hexagonal phase. The lamellar phase was of an interdigitated, subgel type for a majority of the investigated MMG analogues. The headgroups

of these analogues were also in a crystalline state, which was evident from the formation of a two-dimensional superlattice. This might be the first example of a lipid system showing both interdigitation and superlattice formation. Thus, the MMG analogues are not only interesting from a vaccine perspective, but also from a physico-chemical perspective, as the lipids might serve as an interesting model system for future studies.

Additionally, the presented MMG analogues were incorporated into dimethyldioctadecylammonium (DDA) bromide dispersions, and the effect of the lipid composition on the structural and morphological characteristics of these dispersions was studied. The structural and morphological analysis revealed the formation of both polydisperse unilamellar and multilamellar vesicles and/or self-assemblies with an internal inverse hexagonal structure (hexosomes). Selected DDA:MMG dispersions were subsequently tested in mice. The *in vivo* study revealed that the tested DDA:MMG unilamellar vesicles were immunogenic and induced Th1 and Th17 responses when tested with a chlamydia antigen, regardless of the immunoactivity of the neat MMG analogues *in vitro*.

In conclusion, the study demonstrated the impact of the specific molecular features of these MMG analogues on i) the self-assembled nanostructures in excess buffer, ii) the *in vitro* immunoactivity of the self-assembled nanostructures, iii) the structural characteristics of DDA:MMG dispersions, and iv) the adjuvant efficacy of DDA:MMG dispersions. Thus, the study demonstrated the importance of the structural characteristics of the adjuvants and emphasized the need for relevant biophysical studies in adjuvant research with an attempt of introducing a rational approach for efficient vaccine design.