

Modulation of the Adjuvant Activity of DDA/TDB Liposomes with Immunostimulators

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ABSTRACT SUMMARY

Liposomes based on dimethyldioctadecylammonium (DDA) and trehalose 6,6'-dibehenate (TDB) have been evaluated and appears to be promising as vaccine adjuvants for a number of vaccination purposes. By incorporating additional immunostimulatory components into DDA/TDB liposomes, the adjuvant system can be customized to induce the required immunological profile for a given vaccine.

INTRODUCTION

Cationic liposomes are of interest as delivery vehicles for vaccine antigens as they possess positive charge and are able to carry the antigen to and interact with the negatively charged surface of the antigen presenting cells (APCs). Furthermore, studies have demonstrated a superior adjuvant effect of cationic liposomes compared to anionic or neutral liposomes (1). Cationic liposomes based on dimethyldioctadecylammonium (DDA) and trehalose 6,6'-dibehenate (TDB) have been evaluated and show potential as an adjuvant system (designated CAF01) for vaccines against a wide range of diseases (2). It has significant immunostimulatory activity, characterized by the induction of a strong cell mediated immune response as well as a humoral response (3), desirable for a number of vaccine targets. The liposomal adjuvant can be customized further to induce the required immunological profile for a given disease by incorporating additional immunostimulatory components into the liposomes.

Toll-like receptor (TLR) targeting has gained interest in adjuvant research as stimulation of these pattern recognition receptors is important for induction of the immune response. TLRs recognize a wide range of pathogen-associated molecular patterns (PAMPs), e.g. lipopeptides, doublestranded RNA, LPS and its derivatives, bacterial flagellin, and unmethylated CpG motifs in bacterial DNA.

In this study we investigated the versatility of cationic liposomes based on DDA/TDB in combination with different immunostimulatory ligands including several TLR ligands. We evaluated muramyl dipeptide (MDP, NOD2 ligand), polyinosinic-polycytidylic acid (Poly IC, TLR3 ligand), monophosphoryl lipid A (MPL, TLR4 ligand), Gardiquimod (TLR7), Resiquimod (TLR7) and CpG 1826 (TLR9 ligand) in combination with DDA/TDB. Of particular interest is to identify a formulation with the ability to induce cell-mediated responses, characterized by production of CD8⁺ T-cells. Furthermore, it is evaluated how incorporation of immunostimulatory components affects the physico-chemical properties of the liposomes.

EXPERIMENTAL METHODS

The DDA/TDB liposomes were prepared by the thin film method as described previously (3).

The gel-to-liquid phase transition temperature of undiluted vesicles in suspension was determined using differential scanning calorimetry (DSC). Thermograms were obtained using a MicroCal VP-DSC MicroCalorimeter, scanning at a rate of 30 °C/h from 25 °C to 60 °C. VPViewer 2000 and Origin® 7 scientific plotting software were used for data analysis.

Female C57BL/6 mice, 6 to 12 weeks old, were obtained from Harlan Scandinavia. The vaccines were prepared by mixing the liposome formulations with ovalbumin (OVA) as antigen. The final OVA concentration was 25 µg/ml, and the final concentration of DDA and TDB was 1.25 and 0.25 mg/ml, respectively, for all formulations. Mice were immunized intraperitoneally (i.p.) three times with a 2-week interval between the immunizations (0.2 ml/dose). The vaccines were kept isotonic with 9% trehalose. Spleens were harvested 14 days after the last immunization. Splenocyte cultures ($n = 3$) were obtained by passage of spleens through a metal mesh, followed by washing twice in RPMI (Gibco Invitrogen). Isolated splenocytes were stained with PE-labeled H2kb/SIINFEKL pentamer (ProImmune) for 10 minutes at room temperature and washed in PBS with 0.1% BSA. Subsequently, the cells were stained for 30 min at 4°C for surface markers with mAbs using 1:200 dilutions of anti-CD4, anti-CD8, anti-CD62L and anti-CD44 (all BD Pharmingen). After washing, cells were re-suspended in FACS buffer and analyzed on a six-colour BD FACS Canto flow cytometer (BD Biosciences). Finally, responses were analysed using FlowJo software V.7.2.2.

RESULTS AND DISCUSSION

The versatility of DDA/TDB-based liposomes as adjuvant systems is under investigation. Incorporation of certain immunostimulating compounds into DDA/TDB liposomes may affect the immunological properties, as well as the physico-chemical properties, of the adjuvant formulation. Characterization of the latter is important for issues such as formulation with antigen and stability.

Incorporation of immunostimulatory components may affect the particle size, the surface charge and the phase transition temperature. As an example incorporation of MPL resulted in increased average particle size and decreased surface charge (results not shown). A change in phase transition temperature was also observed after incorporation of immunostimulating compounds into the liposomes (Figure 1). Addition of

MPL decreased the phase transition temperature while other immunostimulatory components, such as MDP and Poly IC, gave rise to more peaks, suggesting that the TLR ligands induce an inhomogeneous distribution of lipids resulting in formation of ordered DDA or TDB domains.

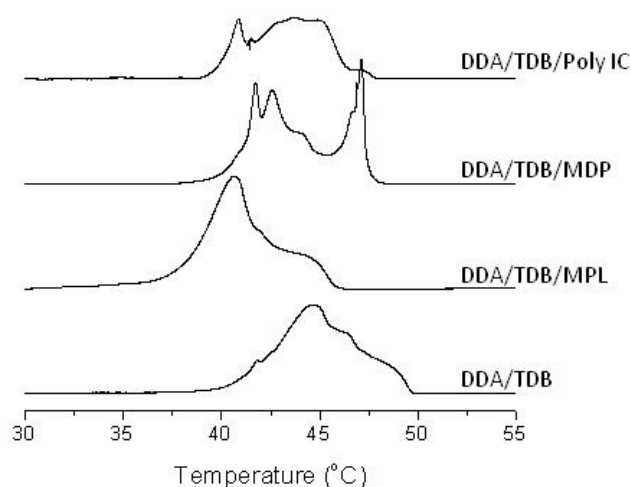


Figure 1 Thermograms of DDA/TDB vesicles containing different immunostimulatory components. Thermograms were obtained by DSC.

The immunogenicity of the liposomal adjuvant formulations with different immunostimulating components was evaluated in mice using OVA as a model antigen. The ability to induce CD8⁺ T-cell responses was evaluated in spleen cells, and OVA-specific (H2kb/SIINFEKL-specific) CD8⁺ T-cells were isolated by FACS. CpG, Poly IC or MPL in combination with DDA/TDB liposomes were able to induce antigen-specific CD8⁺ T-cell responses (Table 1 and Figure 2). DDA/TDB in combination with e.g. gardiquimod or resiquimod gave responses comparable to DDA/TDB alone and naive mice. Stimulation with the TLR3 ligand Poly IC or the TLR9 ligand CpG in combination with cationic liposomes has also previously been shown to induce CD8⁺ T-cell responses in mice (4).

Table 1 TLR ligands divided into categories depending on the percentage of H2kb/SIINFEKL positive CD8⁺ T-cells they induce in combination with DDA/TDB and OVA.

Response	% SIINFEKL positive	TLR ligands
Low	< 0.5%	No TLR ligand Resiquimod Gardiquimod
Medium	0.5 - 5%	CpG MPL
High	> 5%	Poly IC

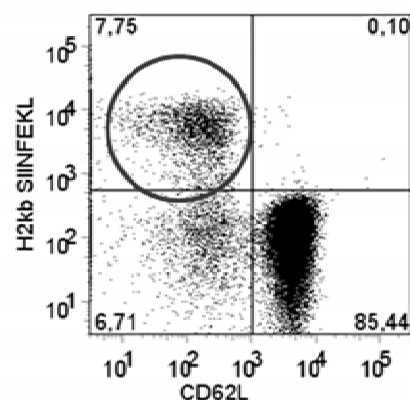


Figure 2 Distribution of CD8⁺ T-cells after immunization with OVA and DDA/TDB in combination with Poly IC. 7.75% of activated CD8⁺ T-cells are H2kb/SIINFEKL positive.

CONCLUSION

Liposomes based on DDA/TDB have previously been demonstrated to show potential as vaccine adjuvants. By incorporating different immunomodulating compounds into these liposomes, it is possible to direct the immune response towards a desired pathway. Incorporation of e.g. Poly IC or CpG in the liposomes directs the immune response towards a cell-mediated response characterized by induction of CD8⁺ T cells.

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