Delivery of the latent tuberculosis antigen Hybrid56 in a liposome delivery system: does liposome surface charge have an effect on immune response?

Alexander Wilkinson1, Randip Kaur1, Dennis Christensen2, Afzal R. Mohammed1 and Yvonne Perrie1

1School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET, England 2Statens Serum Institut, Copenhagen, Denmark

wilkina1@aston.ac.uk

ABSTRACT SUMMARY

To investigate the role of vesicle surface charge in the development of adjuvants, liposomes were formulated from either the cationic lipid dimethylldiodotadecylammonium (DDA) bromide or the anionic lipid surfactant, 1,2-distearyl-sn-glycero-3-phospho-L-serine (DSPS) in combination with the immunomodulating glycolipid, trehalose 6,6′-dibehenate (TDB). The anionic latent tuberculosis subunit vaccine, Ag85B-ESAT6-Rv2660c (Hybrid56) was combined within these liposome adjuvant formulations.

Initial findings from this study showed that there is a liposome charge-dependent trend in the immunogenicity of these vaccine formulations, with cationic DDA/TDB:H56 displaying a Th1-biased immune response with enhanced Th1 cytokine production (both at the injection site and spleen) and antibody titres. However complete replacement of cationic DDA with anionic DSPS within the formulation changes the immune response to a Th2-biased response with slightly increased production of IL5 and IL10 at the spleen.

INTRODUCTION

Liposomes are a flexible and versatile adjuvant delivery system. The ability of these vesicles to protect antigens from degradation, increase antigen uptake by dendritic cells (DCs) as well as increase the controlled release of antigen over time has led to these systems being an ideal formulation for the delivery of subunit vaccines. Sub-unit protein antigens in vaccine formulations are becoming more common due to their increased safety profile, however sub-unit antigens lack immunogenicity when administered alone but when formulated with adjuvants such as liposome delivery systems they are able to deliver a powerful immune response. The aim of the present study was to investigate the role of liposome surface charge of adjuvants in effective delivery of subunit protein antigens and also the subsequent immune response generated.

EXPERIMENTAL METHODS

Liposomes were formulated from dimethylldiodotadecylammonium (DDA) bromide or 1,2-distearyl-sn-glycero-3-phospho-L-serine (DSPS) in combination with the immunostimulatory glycolipid trehalose 6,6′-dibehenate. Liposomes were prepared by the lipid film hydration method with a final lipid concentration of 1.98 mM and TDB concentration of 0.25 mM (8:1 M/M) with the liposomes adsorbing Ag85B-ESAT6-Rv2660c (H56) antigen to a final concentration of 0.1 mg/ml (5 µg/vaccine dose). As negative control formulations, mice were immunised with H56 antigen alone and PBS buffer respectively.

All mice were immunised intramuscularly (i.m.) into the left quadricep with the proposed vaccine (50 µl/dose) three times, with two week intervals between each immunisation. Blood (50 µl) was collected from tail at various timepoints throughout the study (Day 0, 14, 28, 35 and 49). On day 49 of the vaccine study mice were terminated with the spleens and muscle from the site of injection collected. Duoset sandwich ELISAs were carried out on supernatant fractions in order to determine the presence of various cytokines (IFN-γ, IL2, IL5, IL6, IL10, IL1β, IL18 and IL33). Antibody ELISAs were carried out to determine titres of IgG1 and IgG2b in the blood sera.

RESULTS AND DISCUSSION

Upon the final timepoint of the study, mice immunised with DDA/TDB:H56 gave rise to higher production of Th1 cytokines IFN-γ and IL2 thus representing a cell-mediated immune (CMI) response which has been shown to be required against diseases such as TB. Whereas the DSPS/TDB:H56 formulation gave rise to slightly higher production of Th2 cytokines IL5 and IL10 thus representing a shift in bias towards a humoral immune response (Figure 1).

Figure 1 - Cytokine production from splenocytes restimulated in vitro with 5 µg/ml H56 antigen. Results denote mean ± SD of duplicate wells of 5 mice per formulation.
During this investigation there was a need to monitor and study ‘leg immune responses’ at the site of injection (SOI). This was in order to monitor the immune response in regards to cytokines associated with the inflammasome complex such as interleukin-1-beta (IL1β), interleukin-18 (IL18) and interleukin-33 (IL33).

Results from this study demonstrate a liposome charge-dependent relationship in the cytokine response at the vaccination site. IL1β and IL-18 levels from mice immunised with DDA/TDB:H56 were in the region of 16,000 pg/g tissue and 7,000 pg/g tissue respectively (Figure 2). DSPS/TDB:H56 immunised mice were able to produce slightly higher levels of IL33 (12,000 pg/g tissue) at the vaccination site in comparison to DDA/TDB:H56, free H56 and PBS-control immunised mice respectively which gave levels of 10,000, 1800 and 500 pg/g tissue respectively (Figure 2).

This data suggests a role for the inflammasome in the enhanced production of these cytokines, as upon activation this complex contains caspase-1, which is able to convert the inactive pro-form to the active cytokine\(^4\). These cytokines subsequently lead to increased immune responses and cytokine production at the vaccination site (Figure 1).

Figure 2 – Cytokine production from muscle supernatants (IL1β, IL18 and IL33) derived from immunised mice. Results denote mean ± SD of duplicate wells of 5 mice per formulation.

In terms of antibody response in the blood sera, these results showed there was a liposome charge-dependent trend present in which the cationic DDA/TDB:H56 formulation gave rise to higher antibody titres than anionic DSPS/TDB:H56, free H56 and PBS-immunised mice respectively (Figure 3) with the antibody response measured peaking at either day 36 or day 49 p.i of formulations (Figure 3).

These results also demonstrate the importance of a liposome delivery system in the delivery of subunit protein antigen, as the delivery of subunit vaccine alone leads to a significant reduction in immune responses in terms of cytokine production and antibody response (Figures 1-3).

**CONCLUSION**

These studies have demonstrated that upon i.m injection of H56 antigen in combination with DDA/TDB, this leads to enhanced immune responses both at the SOI and the spleen, as indicated by increased cytokine responses. The presence of pro-inflammatory cytokines (IL1β, IL18 and IL33) at the vaccination site suggests a role for the inflammasome in the ensuing immune response which correlates with cytokine production in the spleen. Current studies are ongoing to see if the antigen depot can be improved using various entrapment processes and to correlate these findings with the generated immune response.

**ACKNOWLEDGMENTS**

This work was partly funded by NEWTBVAC (Contract No. HEALTH-F3-2009-241745). NEWTBVAC has been made possible by contributions from the European Commission.

**REFERENCES**