Conjugation free oral microparticulate meningitis vaccine formulation and evaluation.

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ABSTRACT SUMMARY

*N. meningitidis* is a leading cause of bacterial meningitis and sepsis in young children and young adults in the U.S. with a high mortality rate. Capsular polysaccharides (CPS) are a major virulence factor in meningococcal infections and form the basis for serogroup designation and protective vaccines. In the present work, we have developed an oral microparticulate vaccine using CPS for meningitis.

INTRODUCTION

The current meningococcal vaccines are available but are very expensive and require chemical conjugation¹. Therefore, the search for novel vaccine formulations that overcome the limitations of the current conjugate vaccines is important. To address this challenge, we designed a novel meningococcal vaccine formulation consisting of meningococcal CPS polymers encapsulated in albumin-based biodegradable microparticles (MP) that slowly release antigen and induce robust innate immune responses. Vaccines that elicit innate immunity and upregulate co-stimulatory molecules are reported to have enhanced adaptive immune responses² (Fig 1). However, activation of the death receptor CD95 also called Fas receptor, in antigen presenting cells lead to cell death which impact antigen presentation resulting in suboptimal adaptive immune responses.

In order to determine the optimal dose of vaccine-loaded MP that can elicit robust immune responses without apparent cell death, we investigated CD95 expression in macrophages and dendritic cells pulsed with meningococcal CPS-NP³. Further, we examined the expression of co-stimulatory molecules that impact antigen presentation in human macrophages pulsed with meningococcal CPS polymers by qRT-PCR. To determine the correlates of protection by the vaccine, the total IgG and its subtypes IgG₂a and IgG₂b were measured after immunizing mice.

EXPERIMENTAL METHODS

Antigen was formulated in an albumin matrix to prepare microparticles using Buchi 290 spray dryer. Macrophages expressing GFP-LC3 were exposed to microparticles and observed by fluorescence microscopy for autophagosome formation to investigate uptake and autophagy that is important for a strong adaptive immune response. We also evaluated antigen presentation (CD80) and death signal (CD 95) in an in vitro setup where APCs primed by the antigen were used to stimulate splenocytes that had never been exposed to the antigen. The upregulation of other costimulatory
molecules was also checked. The vaccine was administered to Swiss Webster mice to test its in-vivo efficacy. Serum samples were collect at 2, 4, 6, 8 weeks past immunization and checked for antibodies. Animals were used as per protocol approved by Institutional Animal Care and Use Committee and Mercer University.

RESULTS AND DISCUSSION
Antigen loaded microparticles strongly induced autophagic vacuoles indicating induction of uptake and immunostimulatory activity that leads to functional presentation. TNFα release from human macrophages was induced by CPS-loaded nanoparticles, but not by empty nanoparticles. There was significant high levels of CD 95 and antigen presentation i.e. CD 80 in micro-particulate group as compared to antigen solution or blank particles group.

Fig 2: Meningococcal CPS nanoparticles induce CD95 expression in murine dendritic cells.

There was an increased in the expression of co-stimulatory molecule in human macrophages, 2184 times up regulation of CD80 was observed which indicates much better antigen presentation. Other co-stimulatory molecules such as CD95, 95L and 86 also showed significantly high levels (Table 1).

High levels of antibody (total IgG) were observed in the group receiving vaccine microparticles at week 4 and 6. This level was significant high as compared to the antigen solution group and the blank particles group.

Similar results were reported for the IgG subgroups, IgG2a and IgG2b.

Table 1: Expression of co-stimulatory molecules in human macrophages.

<table>
<thead>
<tr>
<th>Co-stimulatory molecule</th>
<th>Fold up-regulation</th>
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<tbody>
<tr>
<td>CD 80</td>
<td>2184</td>
</tr>
<tr>
<td>CD 86</td>
<td>30</td>
</tr>
<tr>
<td>CD 95</td>
<td>18</td>
</tr>
<tr>
<td>CD 95L</td>
<td>64</td>
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<tr>
<td>CD 40</td>
<td>17</td>
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<td>CD 45L</td>
<td>9</td>
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Further we are testing the effects of various commercially available adjuvants along with the antigen to test its immunogenicity. Thus we have reported a microparticulate vaccine for meningitis.

CONCLUSION
The novel meningococcal vaccine nanoparticles are robustly taken up by macrophages and upregulate co-stimulatory molecules that enhance antigen presentation which is a pre-requisite for inducing adaptive immunity.

REFERENCES

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