Efficacy of PLGA particle vaccine against house dust mite allergen depends on size of PLGA particles and presence of CpG

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ABSTRACT SUMMARY
Poly(lactide-co-glycolide) (PLGA) particles carrying antigen and adjuvant is a promising vaccine system which has been shown to stimulate systemic antigen specific immune responses in various disease models. In this study, we aimed to explore the ability of this vaccine system to prevent airway inflammation caused by house dust mite allergies. C3H/HeBFeJ mice were vaccinated subcutaneously with Der p 2 coated on different sized empty or CpG-loaded PLGA particles followed by week long Der p 2 exposures. We found that PLGA particles showed a size dependent decrease in eosinophilia and airway hyperresponsiveness induced by Der p 2 exposures. Mice vaccinated with the Der p 2 coated on large sized empty PLGA particles showed increased levels of Der P 2-specific IgE and IgG1 antibodies compared to the same sized particles containing CpG. This study shows that the size of the PLGA particles used for vaccination plays a major role in the type and intensity of antigen specific immune responses generated and that the presence of CpG can significantly reduce asthmatic symptoms induced by HDM allergens.

INTRODUCTION
In the United States, 84% of residences have detectable levels of house dust mite (HDM) allergens and a quarter of these houses have higher levels of allergens than the proposed limit for asthma [1]. Absence of a long-term solution to HDM-induced asthma in combination with the dangerous levels of HDM allergens present in households necessitate the requirement for a prophylactic vaccine that would switch the inflammatory immune response induced by HDM allergens to a protective immunity. HDMs produce Der p 2,a highly potent allergen which has shown positive titers in serum samples of 79% of patients suffering from asthma, wheezing and/or rhinitis [2]. To develop prophylactic therapy against allergy-associated lung disorders, induction of high IgG titers and Th1 type immune response is highly desirable. We have previously reported that PLGA particles encapsulating antigen and CpG can stimulate robust immune responses compared to vaccination with antigen and CpG in solution [3]. In this study, we sought to determine the effects of the size of PLGA particle vaccines and the influence of CpG in the overall immune response to Der p 2-coated PLGA particle vaccines.

EXPERIMENTAL METHODS
Different sizes of particles were prepared using a modified double emulsion solvent evaporation method described by Joshi et. al. [3]. Different sizes of suspended particles were collected by sequential centrifugation of particles at 200 rpm (7 × g), 700 rpm (75 × g), 4000 rpm (2880 × g), and 7000 rpm (6790 × g) for 5 minutes. Particles were washed with distilled water and lyophilized.

Table 1: Treatment groups and experimental timeline.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Day 0 &amp; Day 7</th>
<th>Day 14 to Day 23</th>
<th>Day 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. S(Der p2+CpG)</td>
<td>s.c. administratio n of 10μg Der p2</td>
<td>Intranasal instillation of 2.5μg Der p2</td>
<td>Necropsy</td>
</tr>
<tr>
<td>2. S(Der p2)</td>
<td>PBS (s.c.)</td>
<td>PBS</td>
<td></td>
</tr>
<tr>
<td>3. M(Der p2 +CpG)</td>
<td>w/o or w/o 5μg CpG</td>
<td></td>
<td></td>
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<tr>
<td>4. M(Der p2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. L(Der p2 +CpG)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. L(Der p2)</td>
<td></td>
<td></td>
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<tr>
<td>7. Shams</td>
<td></td>
<td></td>
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<tr>
<td>8. Sentinels</td>
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</table>

Male C3H/HeBFeJ mice were vaccinated with small, medium and large sized blank or CpG-loaded PLGA particles. Treatment groups (n=12) are describe in Table 1. For necropsy, 6
mice from each group were sacrificed for lung histopathology, bronchoalveolar lavage fluid analysis and serum antibodies estimation. Six mice were investigated for airway hyper-responsiveness.

RESULTS AND DISCUSSION

Three sizes of blank and CpG-loaded PLGA particles: 9.2 µm, 1.0 µm and 300 nm, were successfully prepared as shown in figure 1.

Figure 1: Scanning electron micrographs of CpG-loaded (i) small, (ii) medium, and (iii) large sized PLGA particles. Particles were spherical with smooth morphology. Scale bar on lower right represents 2 µm.

We found that the ratios of IgG2a:IgG1 antibodies were high and with no increase in IgE antibodies secretion for small and medium sized PLGA particles containing CpG compared to large sized particles suggesting stimulation of a Th1 biased immune response. Analysis of BAL fluids and lung histopathology of vaccinated mice challenged with Der p 2 demonstrated that small sized blank or CpG loaded PLGA particles coated with Der p 2 prevented pulmonary influx of leukocytes. Similarly, no significant increase in airway resistance was observed with small PLGA particles when challenged with increasing doses of methacholine (figure 2).

Mice vaccinated with medium and large sized empty PLGA particles coated with Der p 2 exhibited airway remodeling and increased AHR on Der p 2 exposures when compared to sentinels. On encapsulation of CpG in these particles AHR (figure 2) was significantly reduced compared to medium and large sized empty PLGA particles. These results demonstrate that incorporation of CpG can significantly improve the efficacy of the vaccine.

CONCLUSION

This is the first study that comprehensively evaluates the effect of size of PLGA particles and presence of CpG in generating a vaccine against HDM. We have clearly demonstrated that the size of PLGA particles has a significant impact on the efficacy of the vaccine and that incorporation of CpG into the PLGA particles promotes a Th1-predominant immune response.

REFERENCES

2. Trombone APF, Tobias KRC, Ferriani VPL et al. Use of a chimeric ELISA to investigate immunoglobulin E antibody responses to Der p 1 and Der p 2 in mite-allergic patients with asthma, wheezing and/or rhinitis. Clin Exp Allergy 32(9), 1323-1328 (2002).

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