**Introduction**

Allergic asthma is a chronic inflammatory respiratory disease associated with eosinophilic infiltration, increased mucus production, airway hyper-responsiveness (AHR), and airway remodeling. Lung airway epithelial cells express a myriad of pattern recognition receptors such as Toll-like receptors (TLRs), enabling them to sense and respond to a variety of external triggers. The epithelial-derived cytokines IL-33, TSLP, and IL-25 are widely recognized as alarmins produced in response to house-dust mites (HDM). These cytokines activate several leukocytes involved in allergic inflammation, such as eosinophils. We have shown that a combination of Pam2CSK4 ("Pam2", TLR2/6 ligand) and a class C oligo-deoxy-nucleotide ODN M362 ("ODN", TLR9 ligand) when delivered together by aerosol ("Pam2ODN") synergistically activate an innate immune response in the lung mucosa. To further study the immunomodulatory effects of Pam2ODN, we have developed allergic models to show that Pam2ODN attenuates an allergic immune response to HDM.

**Methods**

Mice were sensitized with 100 μg HDM extract at day 0, followed by 6 daily challenges of 10 μg HDM from day 7 to 12. Mice were suspended from upper incisors on a board 60 degrees from 0, under isoflurane anesthesia. Mice were challenged with HDM mixed in PBS while suspended from upper incisors at 60 degrees by placing individual droplets onto nose, while under isoflurane anesthesia. Bronchoalveolar lavage was done on mice after challenges were completed. Differential cell quantification with Wright-Giemsa stain was done to evaluate numbers of leukocytes in bronchoalveolar lavage fluid. Mucous metaplasia of lung epithelial cells was assessed by staining lung tissue with fluorescent PAS stain. Mice were given Pam2ODN at intervals before and after sensitization to HDM. To evaluate synergy, Pam2 and ODN were aerosolized individually or in combination with the previous sensitization and challenge paradigm.

**Results**

When mice were treated with a single Pam2ODN treatment 7 days before HDM sensitization, we observed strong reductions in leukocytes collected in lung lavage fluid, compared to HDM/HDM mice (Figure 1). Differential cell quantification with Wright-Giemsa stain revealed an overall decrease in leukocyte recruitment (Figures 1C-1F). Quantitative image analysis of intracellular mucin content revealed that intracellular mucin accumulates moderately in PBS/HDM mice, but HDM sensitization was required for the full phenotype (Fig. 1J). A single Pam2ODN treatment 7 days before HDM sensitization reduced intracellular epithelial mucin content >35%, which was not significantly different from PBS/HDM mice. Pam2ODN treatments similarly showed efficacy when administered 30- and 15-days before sensitization, with a trend toward less efficacy when the treatment-sensitization interval was further increased. When Pam2ODN treatment was administered any day after HDM sensitization, the treatment effect was completely absent (Figure 2A). Neither Pam2 nor ODN exhibited any significant efficacy when administered alone, but the combination Pam2ODN treatment completely blocked allergic inflammation (Fig 3A-D).

**Conclusions**

With the results from this study, we have generated evidence that Pam2ODN induces a powerful immune response that interferes with allergic inflammation in mice. Pam2ODN is only able to attenuate allergic inflammation when administered before HDM sensitization, which suggests that the mechanism of action involves blocking sensitization to HDM. It is possible that Pam2ODN might induce tolerance to Aeroallergens and could be useful as a therapeutic for allergic disease.