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Immune response in suckling mice fed PBMCs harvested from adult mice and pulsed with Prevnar13: a pilot study

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Abstract

Five-day-old mouse pups were fed either Prevnar 13 alone or peripheral blood mononuclear cells (PBMCs) isolated from adult donors and pulsed *ex vivo* with Prevnar-13. Mice vaccinated with Prevnar-13 or with vaccine-pulsed PBMCs displayed a positive serum IgM response greater than that of mice treated with mock-pulsed PBMCs, though the response of Prevnar-13-treated vs. Prevnar 13-pulsed PMBC-treated groups was not significantly different. However, neither group was protected against lethal infectious challenge. We conclude that it is possible to elicit a neonatal immunological response after vaccine or vaccine-pulsed PBMCs administered via the oral route, but a single dose is insufficient to protect against subsequent infection. Further studies can confirm whether a booster dose may improve protective efficacy and may reveal a difference between vaccine-pulsed PBMC treatment and vaccine alone that is not apparent after a single dose.

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Introduction

PBMC pulsing with vaccine antigens followed by administration by intravenous or oral routes has been shown to induce protective immunity in adult rodents[1]. Neonatal populations are both highly vulnerable to infectious diseases and difficult to protect against infection due to the immaturity of their immune systems. This vulnerability is partially offset by the transfer of maternal immunity during breastfeeding, as the luminal efficacy and permeability of the neonatal gut confer the benefit of both antibodies and immune cells present in breast milk[2][3][4][5][6][7][8][9]. We explored whether this mechanism could be co-opted as part of a strategy to vaccinate neonates using the oral route. The current pilot study was undertaken to determine whether vaccine-pulsed PBMCs administered by the oral route to neonatal mice could induce an immune response

Methods

Ethical approval was in accordance with institutional policies.

Male and female C57Bl/6 mice were housed together as breeding pairs. On day 5 post-birth, neonatal suckling mice were vaccinated orally with Prevnar-13 alone or with Prevnar 13-pulsed or mock-pulsed PBMCs in a volume of 12 µl, the maximum volume that the pups would ingest. PBMCs were obtained by submandibular bleeding from adult mice into tubes containing 50 µl citrate solution.

PBMCs were isolated by mixing blood 1:1 with 2% FBS solution in PBS and centrifugation over Histopaque 1083 and the remaining RBCs were lysed using ACK buffer. Isolated PBMCs were washed and brought to a concentration of $4x10^6$ cells/ml in RPMI, with or without vaccine. PBMC/vaccine suspensions were incubated 3H at 37° C and washed to remove free vaccine.

PBMCs were administered orally to neonatal mice aged 5 days in a volume of 12 µl at a concentration of $4x10^7$ cells/ml for a dose of ~500,000 cells/mouse. A pipette was used to deliver the vaccine drop-wise to the mouth, and pups consumed these droplets instinctively. Mice were subject to lethal infectious challenge 28 days after oral vaccination at an age of 33 days. The lethal challenge consisted of $2x10^6$ CFU of *S. pneumoniae* A66.1 in a volume of 50 µl, a standard challenge dose of A66.1 for our lab based on survival titration.

Following the lethal challenge, mice were observed daily for 21 days to monitor survival.

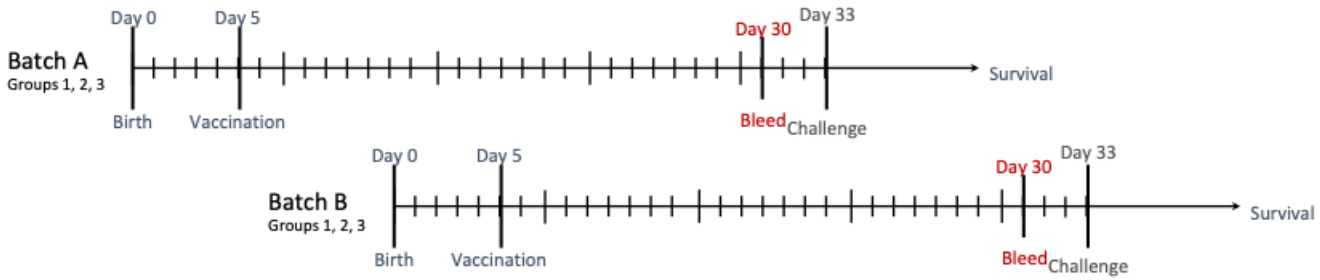
Blood was drawn from vaccinated mice three days before the lethal challenge to assess serum Ab responses by ELISA [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6289435/].

All of the neonates were breastfeeding from immunologically naïve mothers. The neonates all received PBMCs on day 5 (also from immunologically naïve mice), but these PBMCs were pulsed with antigen or not depending on whether they were in the experimental or control group.

This time was chosen to observe the immunological state of the animals as close to the challenge as possible, while giving the animals time to recover before the next stage of the experiment. The positive control was pooled serum from immunized mice from other experiments.

This experiment used mice from two litters, treated identically and at equivalent time points relative to birth. Each litter contained two mice in each treatment group. The schedule of oral vaccination and pathogen challenge is shown in Fig.1.

Fig.1. Oral vaccination using neonatal subjects (Schedule for vaccination and challenge)



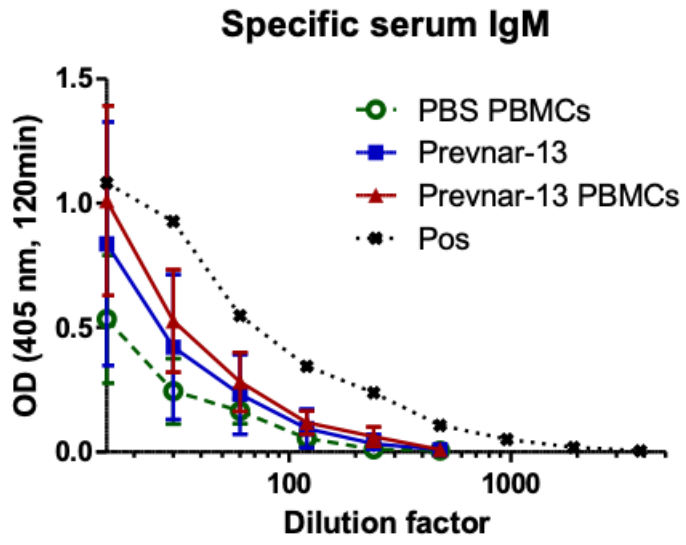
	Treatment	Prevnar-13 (serotype 3 Ag)	PBMCs	Volume	Route
1	Un-pulsed PBMCs	0 µg/dose	$5x10^5$	12 µl	Oral
2	Prevnar-13	0.053 µg/ dose	0	12 µl	Oral
3	Prevnar-13-pulsed PBMCs	0.053 µg/ dose	$5x10^5$	12 µl	Oral

- Experimental requirements made it impossible to conduct entire experiment synchronously
- Experimental design was split into A and B batches
- Each batch represents 1 litter of 6 pups
- Each batch represents 3 groups, with 2 pups/group
- Data to be pooled at end for 4 pups/group

Fig. 1. *Oral vaccination using neonatal subjects (Schedule for vaccination and challenge)*

Results (Fig.2)

Fig.2. Oral vaccination using neonatal subjects (Serum antibody response)



- Vaccination day 5
- Single dose, oral
- Bleed day 30
- Slight IgM response for vaccinated groups
- No difference between vaccinated groups
- No IgG response, consistent with prime-only vaccination

Fig. 2. *Oral vaccination using neonatal subjects (Serum antibody response)*

Mice vaccinated with Prevnar-13 or with Prevnar 13-pulsed PBMCs displayed a positive serum IgM response greater than that of mice treated with mock-pulsed PBMCs. The difference in response between Prevnar-13-treated and vaccine-pulsed PMBC-treated groups was not significant. No serum IgG was detected in any group, consistent with results from other vaccine-pulsed PBMC experiments showing that a boost is required for isotype switching. There was no difference in survival between groups and mortality was total (Fig.3). Mice still alive on day 6 were moribund but we did not record the number of
hours they survived beyond day 6.

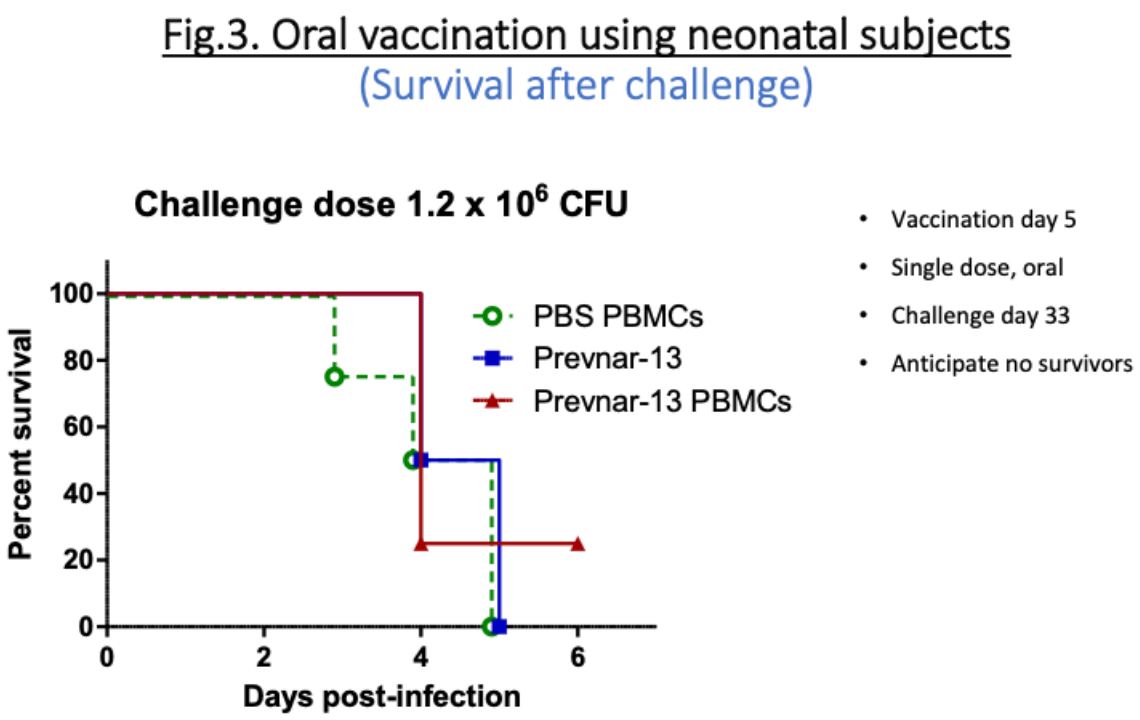


Fig. 3. *Oral vaccination using neonatal subjects (Survival after challenge)*

Discussion

A single oral dose of Prevnar 13 or Prevnar 13-pulsed PBMCs elicits comparable serum IgM Ab responses compared to oral Prevnar-13 in neonatal mice, but neither treatment enhances survival against a lethal challenge. However, as the post-pulse wash step removes free vaccine antigen from PBMC suspensions, mice in the pulsed-PBMC group likely received lower doses of antigen than mice receiving Prevnar-13 directly. The equivalence in serum antibody response suggests the possibility that vaccine-pulsed PBMCs may have elicited enhanced responses compared to Prevnar-13 alone relative to true antigen exposure. Future studies including a booster dose 2-3 weeks following IgM induction are likely to induce a protective effect.

Conclusions

Prevnar 13 alone or Prevnar 13-pulsed adult mouse PBMCs induced an IgM response when given orally to neonatal mice. The mechanism(s), including possible entry of orally administered pulsed PBMCs into Peyers Patches, remains to be determined in future studies.

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