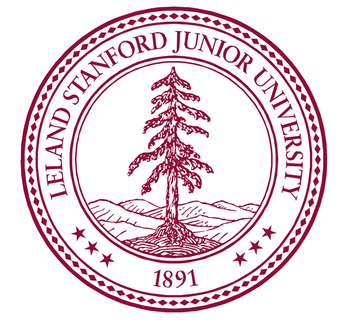

Plasmacytoid Dendritic Cells Are Dispensable For Antiviral Immune Responses Following Cationic-Lipid-DNA-Complex (CLDC) Adjuvanted Influenza Vaccination

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INTRODUCTION

Cationic-Lipid DNA complex (CLDC) is a novel adjuvant consisting of cationic liposomes combined with a non-coding DNA plasmid. Inactivated influenza A virus adjuvanted with CLDC elicits high levels of influenza-specific antibodies and T cells. The DNA component of this vaccine is a plasmid that lacks a cDNA segment and which stimulates leukocytes by both unmethylated CpG DNA-dependent and -independent mechanisms. CLDC administration *in vivo* induces high levels of cytokines that promote Th1 immunity (e.g., IL-12p70), type I interferons (IFN-αs and IFN-β), and IFN-γ. At least part of this stimulation is likely to be dependent on triggering of Toll-like receptor (TLR)-9. Plasmacytoid dendritic cells (pDCs), which express particularly high levels of TLR-9, and conventional dendritic cells (cDCs) have been shown to interact after unmethylated CpG DNA administration to provide an optimal innate immune response, e.g., IL-12p70 production. pDCs, which are a major source of circulating type I IFNs after unmethylated CpG administration *in vivo*, have also been shown in some viral infection models to promote responses mediated by NK cells, cytotoxic T lymphocytes (CTL), Th1 cells, and B cells. Here we investigate the impact of pDC depletion on CLDC/influenza vaccine-induced adaptive immune responses.

EXPERIMENTAL DESIGN

Plasmacytoid Dendritic cell depletion: C57BL/6 mice received 2 intraperitoneal (i.p.) injections of 100 μg of rat anti-mouse PDCA-1 antibody (Miltenyi Biotec) or control rat IgG 24 hours apart.
Flow cytometric detection of pDCs: Whole blood, spleen and lymph nodes (pooled axillary, brachial and inguinal node tissue) were harvested from the 2 groups of mice. Single cells suspensions were prepared and stained in PBA (PBS, 1% BSA, and 0.1% sodium azide) with antibodies to PDCA-1 (Miltenyi Biotec), CD11c and CD45R/B220 (BD). Samples were read on a FACSCalibur (BD) and analyzed using FlowJo software (Tree Star, Inc.). Dead cells were excluded from the analysis with size scatter and 7-amino actinomycin D (AAD) exclusion.
Functional test of pDC depletion: We injected anesthetized mice with 200 μl containing 5.0 μg of PS-CpG ODN1826 (5'-TCCATGA**CG**TTCCTGA**CG**TT-3', Sigma-Genosys) complexed with the cationic lipid DOTIM (octadecenoyloxy[ethyl-2-heptadecenyl-3-hydroxyethyl] imidazolinium chloride, Avanti Polar Lipids Inc.). 30 μl DOTIM were mixed with 5.0 μg ODN in 170 μl 10% sucrose solution, incubated 20 min then injected intravenously in the tail vein. Blood samples were collected by tail clippings at 6 hours. The IFNα was quantified using an ELISA assay (PBL).
Immunization: Twenty four hours after the last injection of control or depleting antibodies, we injected i.p. 5.0 μg of purified HKx31 influenza virus (Charles River) adjuvanted with CLDC. Tail vein blood samples were obtained at the indicated time points.
Detection of HKx31-specific antibodies: Briefly, plates were coated overnight with 1.0 μg/ml purified inactivated HKx31 virus in PBS. After blocking in diluent assay (10% FCS in PBS), ten-fold serial dilutions of sera were incubated for 2 hours, washed, and specific isotypes were detected with anti-mouse-IgG1 and -IgG2c HRP-conjugated antibodies (Southern Biotech). TMB substrate was use for the final detection step. Plates were read at 450 and 570 nm. HAI titers were performed using standard methods.
IFN-γ T-cell secretion assay: Single-cell suspensions were prepared from spleens harvested 4 weeks post- immunization. Splenocytes were cultured at $2x10^6$ cells/ml, and stimulated with 1.0 μg/ml purified inactivated HKx31 virus or 20 HA units live HKx31 virus. Culture supernatants were harvested at 24, 48, 72 and 96 hours post-stimulation, and the levels of IFN-γ were quantified using an ELISA assay (BD Biosciences) following the manufacturer's conditions.

RESULTS

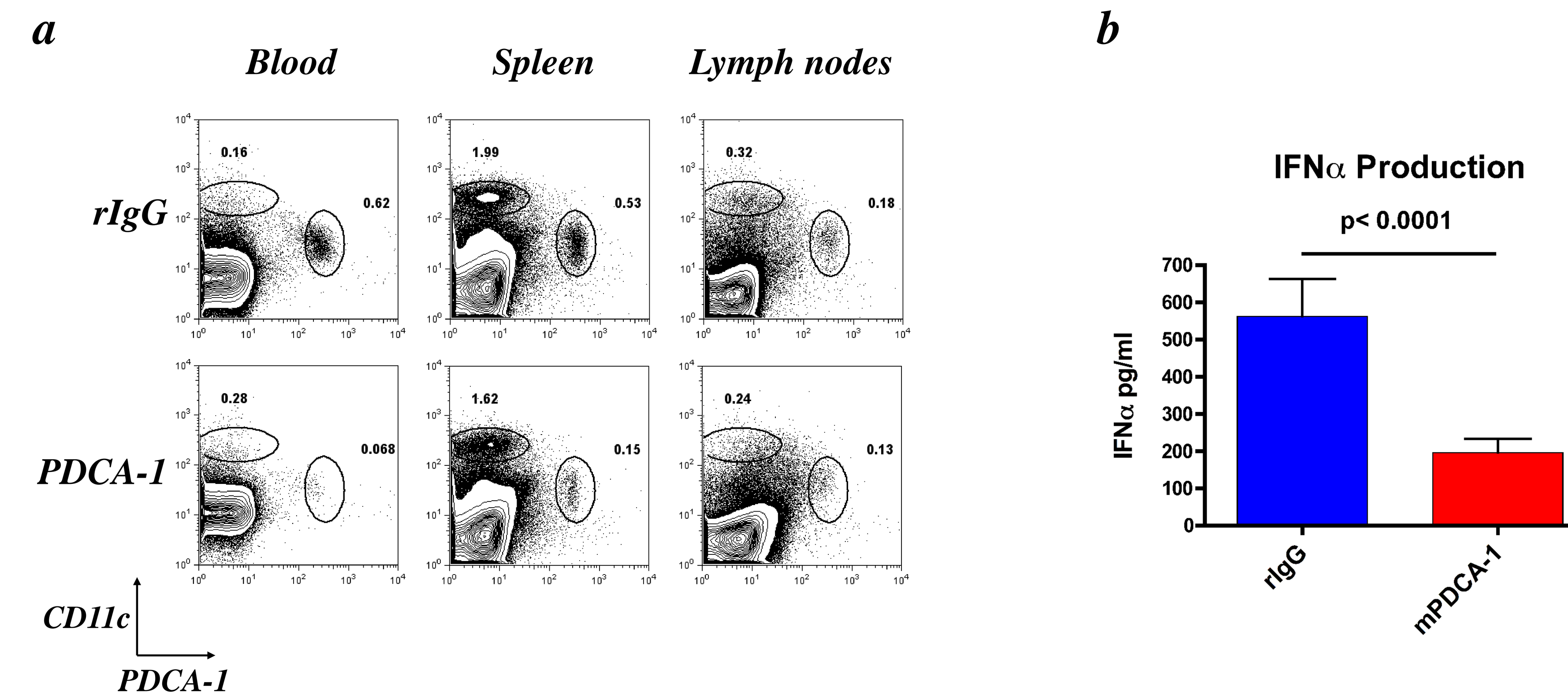

Figure1: ***In vivo*** **pDC depletion**. **a-** Flow cytometric analysis analysis of mice injected with anti-mouse PDCA-1 or rat IgG antibodies. The depletion resulted in a 75 to 90% decrease in pDCs. **b-** IV injection of a CpG ODN resulted in a 60-70% decrease in IFN-α in the group injected with the anti-mPDCA-1 antibody.

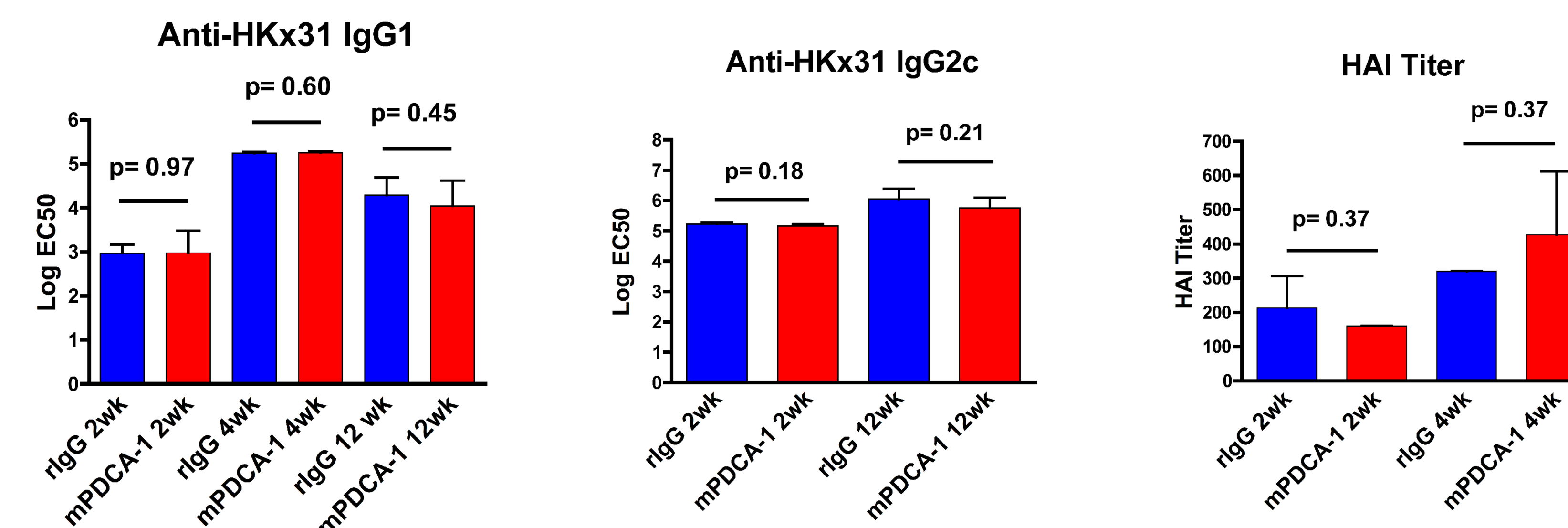

Figure2: Post-immunization antibody responses. There were no statistical differences in anti-HKx31 IgG1 or IgG2c isotype levels at any time point tested after one immunization. The level of protective antibodies (HAI titers) were similar at 2 and 4 weeks post-immunization for the pDC-depleted and non-depleted groups.

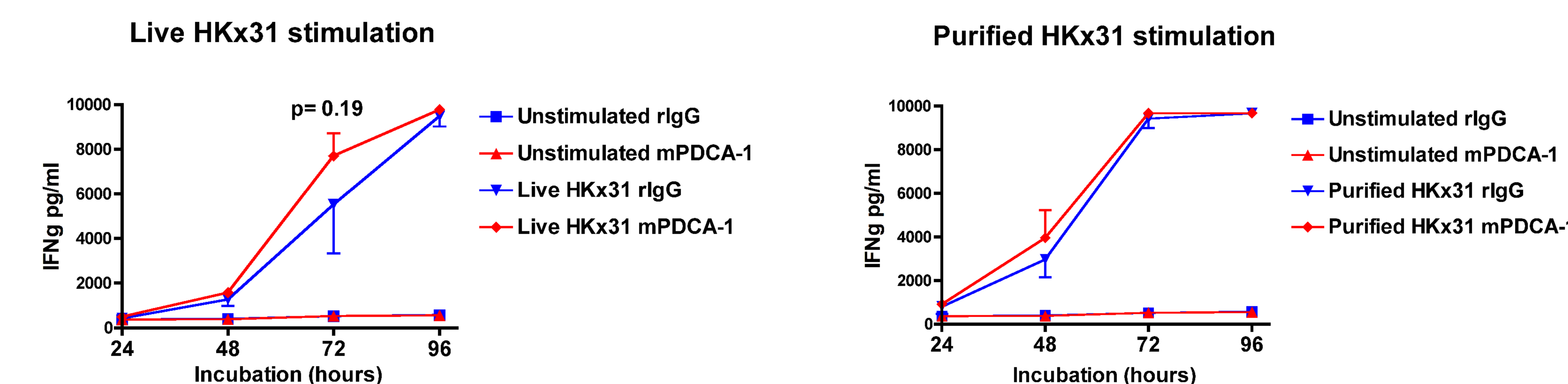

Figure3: ***In vitro*** **splenocyte stimulation for IFN-γ.** There were no statistical differences in the amounts of IFN-γ secreted after activation with either live or purified HKx31 influenza virus in cultures of splenocytes harvested from pDC-depleted or non-depleted mice. The spleens were harvested at 4 weeks post-immunization.

SUMMARY

•Our *in vivo* pDC depletion protocol results in a 75 to 90% decrease in pDCs numbers.
•Despite this depletion, there were no significant differences in antibody responses in the 2 groups of mice, as seen by equivalent influenza-specific IgG1 and IgG2c titers as well as comparable levels of protective antibodies, as assessed by HAI.
•Upon stimulation of splenocytes with live or purified inactivated influenza virus, there is no statistical differences in the level of IFN-γsecreted between the two groups of mice. Our previous work has found that this secretion is mediated mainly by CD4 T cells.

CONCLUSIONS

Despite a substantial *in vivo* decrease in pDCs following antibody administration as evaluated by flow cytometry, we did not see an impact of this depletion on influenza-specific B-cell or T-cell adaptive immune responses following immunization with CLDC/HKx31 influenza A. This suggests that pDC-derived IFN-α is not a limiting factor for a robust immune response induced by CLDC/antigen vaccination. However, we cannot exclude the possibility that the remaining pDCs are still secreting enough type I interferons, as seen in our IFN-α assay, to shape the adaptive immune response. It is also possible that CLDC might be able to induce IFN-α by a pDC-independent mechanism.

FUTURE DIRECTIONS

•Determine the impact of the pDC depletion on the generation of the memory T-cell and B-cell response
•Determine the importance of type I IFNs in CLDC immunization by evaluating immune responses in type IFN receptor deficient mice

ACKNOWLEDGEMENTS

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