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Pneumovax®23 directly stimulates B cells *in vivo* generating a predominant IgA response early after vaccination

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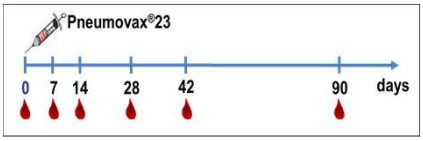
Background

Marginal zone (MZ) and B-1 B cells are the main players during T cell independent (TI) immune responses. In mice, both subsets have been characterized in great detail. In humans, IgM$^+$ memory B cells in peripheral blood have been suggested to represent equivalents of splenic MZ B cells, whereas human B-1 B cells have been recently described as CD19$^+$CD27$^+$CD20$^+$CD43$^+$ B cells. However, both views remain controversial. The aim of this study was to characterize the effect of a TI response using Pneumovax®23 on human B cell subpopulations.

Methods

We analyzed alterations in the composition of the B cell compartment in peripheral blood and pneumococcal polysaccharide (PnPS)-specific antibody production in healthy individuals following vaccination with Pneumovax®23.



Results

1) Increased numbers of CD27^{++} plasma cells and IgA$^+$ but not IgM$^+$ memory B cells

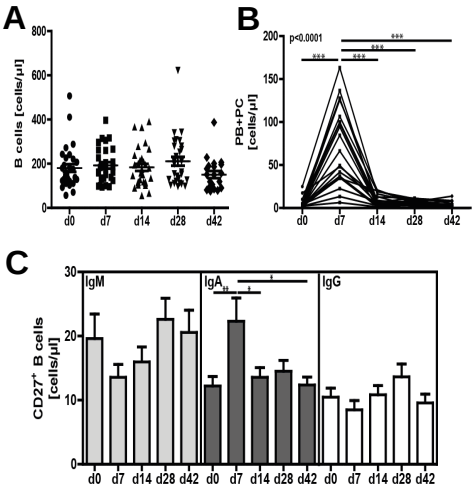


Figure 1: **(A)** Absolute numbers of CD19$^+$ B cells, **(B)** CD27^{++} plasma cells and **(C)** IgM$^+$, IgA$^+$ and IgG$^+$ CD27$^+$ memory B cells after immunization with Pneumovax®23 at indicated time points

2) Pneumovax®23 induces predominantly PnPS-specific IgA mainly produced by CD27^{++} cells

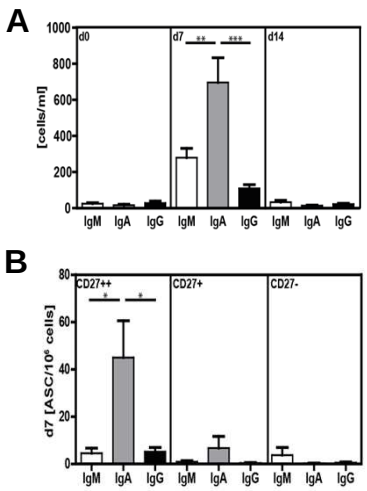


Figure 2: PnPS-specific IgM, IgA and IgG secreted by **(A)** PBMCs on day 0, 7 and 14 and **(B)** sorted B cell subsets on day 7 after vaccination (both measured by ELISpot).

3) Immunization with Pneumovax®23 does not generate a specific memory response

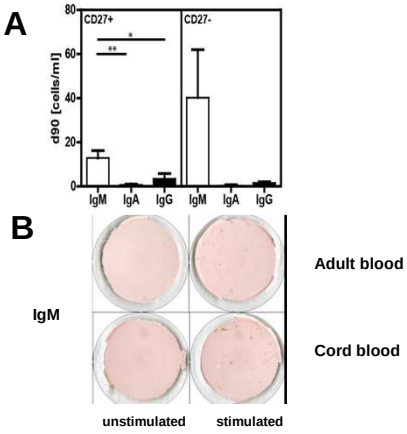


Figure 3: **(A)** Number of PnPS-specific ASC within sorted and restimulated (R848/IL-2) CD27$^+$ and CD27$^-$ B cells 90 days after vaccination **(B)** PnPS-specific IgM production in stimulated (IL-4 / IL-21 / anti-CD40 / CpG / BAFF) and unstimulated cord blood cells and PBMC of healthy adults not recently vaccinated with Pneumovax®23

4) Proposed human B-1 cells are increased in response to Pneumovax®23

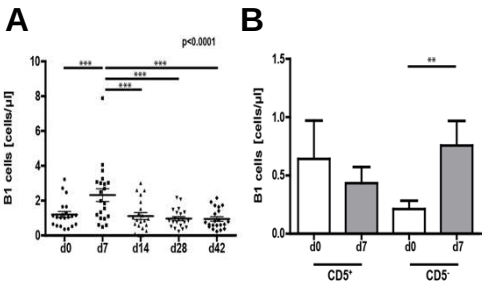


Figure 5: **(A)** Absolute numbers of CD19$^+$CD27$^+$CD20$^+$CD43$^+$ B cells over time after immunization with Pneumovax®23 and **(B)** their expression of CD5 on day 0 and 7.

5) Transient reduction of BAFF serum levels one week after immunization with Pneumovax®23

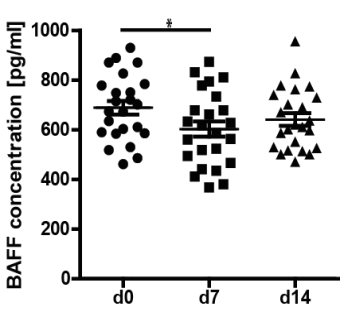


Figure 4: BAFF serum levels on day 0, 7 and 14 after vaccination measured by ELISA.

6) Immunization with Pneumovax®23 leads to transient reduction of CD27$^-$ naïve and transitional B cells indicating an increased turn-over rate

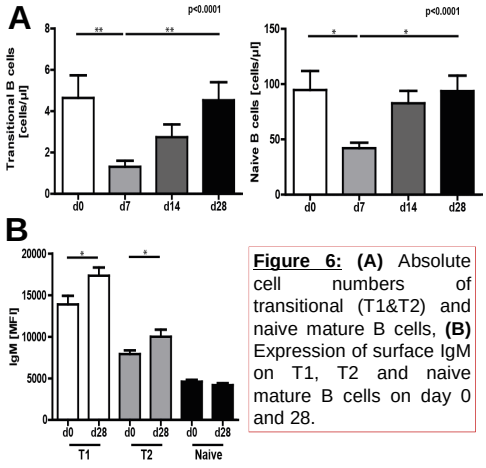


Figure 6: **(A)** Absolute cell numbers of transitional (T1&T2) and naive mature B cells, **(B)** Expression of surface IgM on T1, T2 and naive mature B cells on day 0 and 28.

Summary

Vaccination with Pneumovax®23

- has no effect on IgM$^+$ memory B cells
- results in a significant increase of CD27^{++} plasma cells and IgA$^+$ memory B cells
- leads to an increase of proposed B-1 cells
- does not generate a detectable memory response after 90 days

Abbreviations: PBMC: peripheral blood mononuclear cells, ASC: antibody secreting cells, MFI: mean fluorescence intensity, * p<0.05, ** p<0.01, *** p<0.001

Conclusion

Our data show that Pneumovax®23 primarily induces PnPS-specific IgA-producing plasma cells and IgA$^+$ memory B cells. Whereas we do not see any change in IgM$^+$ memory B cells, we find an increase of proposed human B-1 B cells as previously described. We assume that IgA induction is characteristic for TI and innate immune responses by direct stimulation of B cells.

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