Interleukin-7-dependent B lymphocytes are required for the anti-pneumococcal polysaccharide response and protective immunity to *Streptococcus pneumoniae*

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Abstract: Unlike human adults or adult mice, young children or young mice respond poorly to pneumococcal polysaccharides (PPS). In mice, B1b lymphocytes are the major responders to a variety of bacterial polysaccharides including PPS. Despite having B1b cells, young mice are severely impaired in responding to PPS, suggesting that B cells in the young are distinct from those in adults. Since B lymphopoeisis early in life is largely Interleukin-7 (IL-7)-independent, while in adults it is IL-7-dependent, we hypothesize that B cells developed in the presence of IL-7 are required for generating anti-PPS antibody responses. In support of this, we found that despite having B1b cells, young wildtype and adult mice deficient either in IL-7 or IL-7Rα are severely impaired in responding to Pneumovax®23 vaccine, and do not survive pneumococcal challenge. Furthermore, we found that transgenic expression of IL-7 promotes the anti-PPS response in young and confers protective immunity to young mice. To translate these findings to human infants we have utilized neonatal NOD/SCID/γc^{null} mice engrafted with human umbilical cord blood CD34$^+$ hematopoietic stem cells to create a "Human Immune System" mouse (HISmouse) model. We have found that these HISmice generate several B cell subsets including B1 (CD19$^+$CD20$^+$CD27$^+$CD43$^+$CD70$^-$CD69$^-$) and the majority of them exhibit an immature phenotype. Moreover, just as young children, HISmice responded poorly to PPS. IL-7 is produced mainly by non-hematopoietic stromal cells, and unlike the human IL-7, the murine IL-7 is poor stimulator of human B lymphocyte development. Although our data indicate that IL-7-dependent B cells are crucial for generating anti-polysaccharide response, we also found that enforced expression of a polysaccharide (α1,3, dextran)-specific B cell antigen receptor heavy chain (V_H J558) in mice can overcome the lack of anti-polysaccharide antibody responses in young mice even in the absence of an IL-7-dependent B lymphopoiesis.

Results

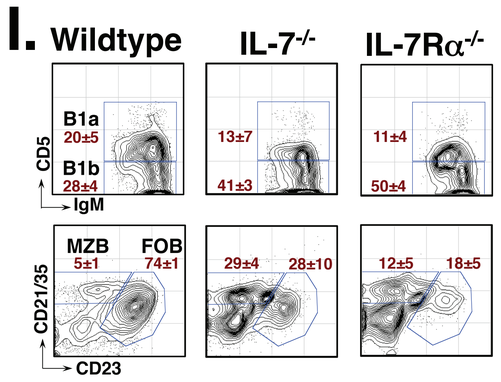

Figure 1. Mice deficient in IL-7 signaling are sufficient in generating B1b, B1a and MZ B cell subsets. The frequencies of B1a and B1b cells in the peritoneal cavity, or marginal zone (MZ) and follicular (FO) B cells in the spleens of mice were determined by flow cytometry. The data are representative of at least 5 mice from each indicated genotype.

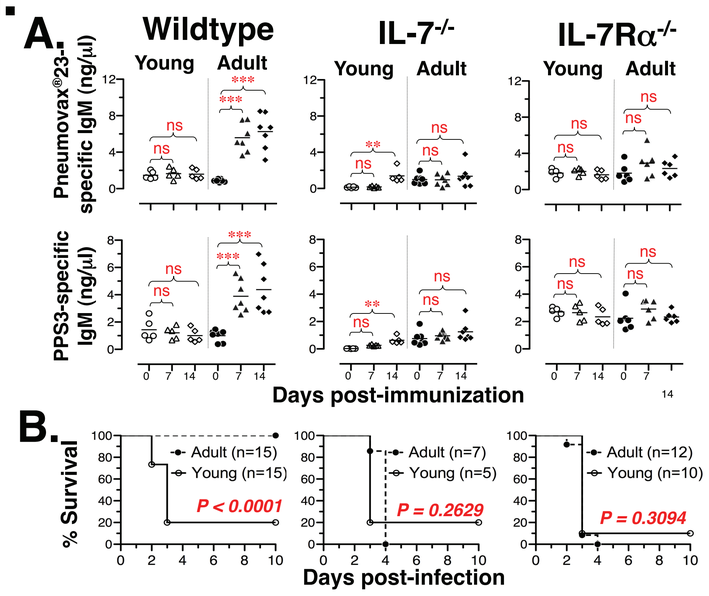

Figure 2. Pneumovax®23 immunization neither elicits an antigen-specific antibody response nor confers protection against *Streptococcus pneumoniae* in mice deficient in IL-7 or IL-7Rα. (A). Young (3 week-old) or adult (8-14 weeks old) wildtype, IL-7$^{-/-}$ and IL-7Rα$^{-/-}$ mice were immunized i.p.with 10 μg of Pneumovax®23. Blood samples were obtained on 0, 7 and 14 days post-immunization and Pneumovax®23- or PPS3-specific IgM levels were determined by ELISA. Each dot represents a mouse and the mean antibody levels are indicated with a solid line. Statistically significant differences between preimmune and immune mice are indicated with p values < 0.05 (*), < 0.01 (**) or <0.001 (***).“ns” denotes not statistically significant. **(B)**. Four weeks following Pneumovax®23 immunization mice were challenged with 5000 cfu of *S. pneumoniae* WU2 (serotype 3) and survival was monitored. Survival statistics were performed using log-rank test and p values are given.

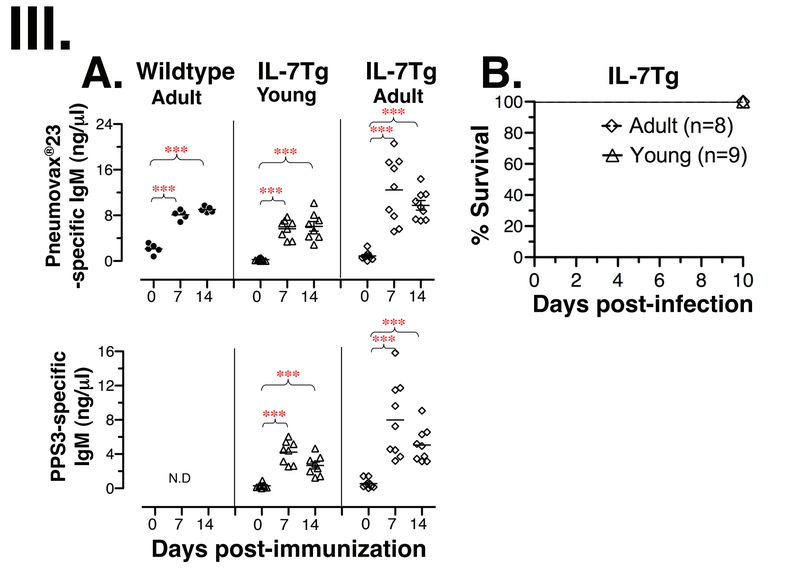

Figure 3. Transgenic expression of IL-7 restores anti-pneumococcal polysaccharide antibody response and protective immunity in young mice. (A). Wildtype adult mice and young or adult IL-7 transgenic (IL-7Tg) mice were immunized i.p.with 10 μg of Pneumovax®23. Blood samples were obtained on 0, 7 and 14 days post-immunization and Pneumovax®23- or PPS3-specific IgM levels were determined by ELISA. Each dot represents a mouse and the mean antibody levels are indicated with a solid line. Statistically significant differences between preimmune and immune mice are indicated with p values <0.001 (***).“N.D” denotes not determined in this experiment but see Fig 2A. **(B)**. Four weeks following Pneumovax®23 immunization mice were challenged with 5000 cfu of *S. pneumoniae* WU2 (serotype 3) and survival was monitored. Survival statistics were performed using log-rank test and p values are given.

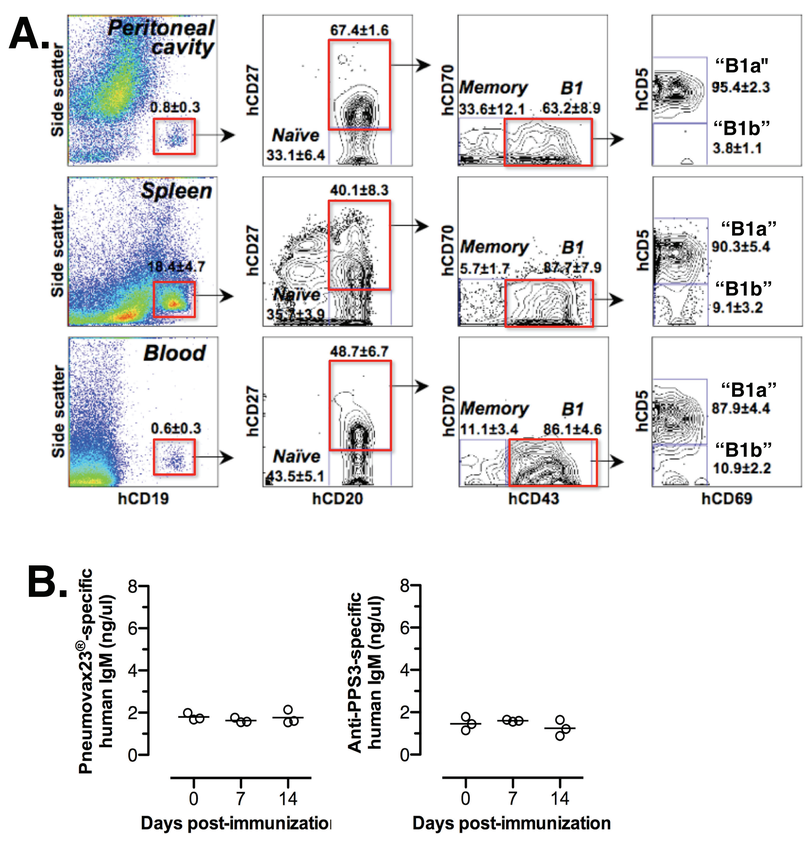

Figure 4. Human Immune System mice generate several human B cell including B1 cell subsets in the apparent absence of human IL-7 and are completely impaired in responding to pneumococcal polysaccharide. (A) Peritoneal cavity, spleen or peripheral blood lymphocytes of HISmice were stained with antibodies specific for human CD19, CD20, CD27, CD43, CD70, CD69 and CD5 and analyzed by flow cytometry. All B cells were first identified by CD19-positivity and were further resolved (indicated by arrows) as naïve (CD20+ CD27-), memory B cells (CD20+ CD27+ CD43- CD70-) and B1 cells (CD20+ CD27+ CD43+ CD70-). The frequency values of the indicated B cell populations were shown within the plots. The data were representative of 3-5 mice. **(B)** HISmice were immunized i.p. with 10 μg of Pneumovax®23. Blood samples were obtained on 0, 7 and 14 days post-immunization and Pneumovax®23- or PPS3-specific IgM levels were determined by ELISA.

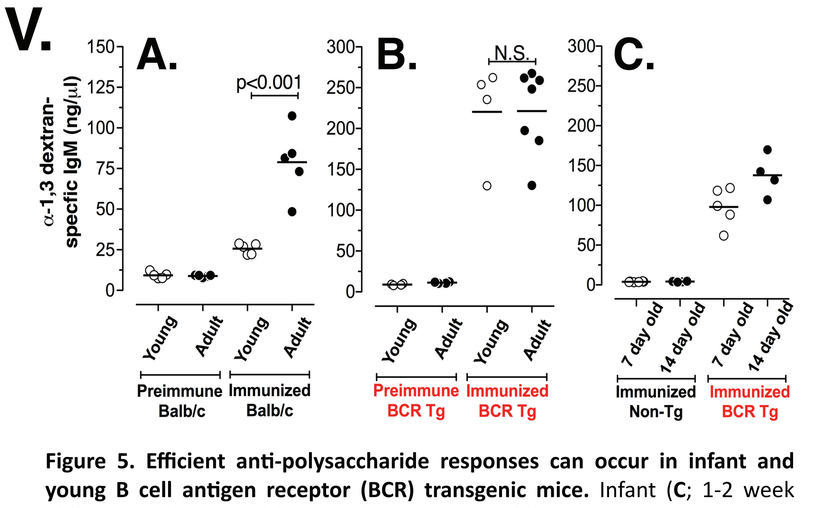

Figure 5. Efficient anti-polysaccharide responses can occur in infant and young B cell antigen receptor (BCR) transgenic mice. Infant (**C**; 1-2 week old), Young (3 week old) or adult (8-14 week old) mice on non-BCR or BCR transgenic (**This is an immunoglobulin heavy chain V_HJ558 transgene product and its random pairing with λ1 light chain results in α1,3 dextran recognition**) mice background were immunized i.p. with 10^8 heat-killed *Enterobacter cloacae* bacterium expressing a capsular polysaccharide (α1,3 dextran). Blood samples were obtained on 0 and 7 days post-immunization and α1,3 dextran-specific IgM levels were determined by ELISA. Each dot represents a mouse and the mean antibody levels are indicated with a solid line. Statistically significant differences between preimmune and immune mice are indicated. N.S. denotes not significant.

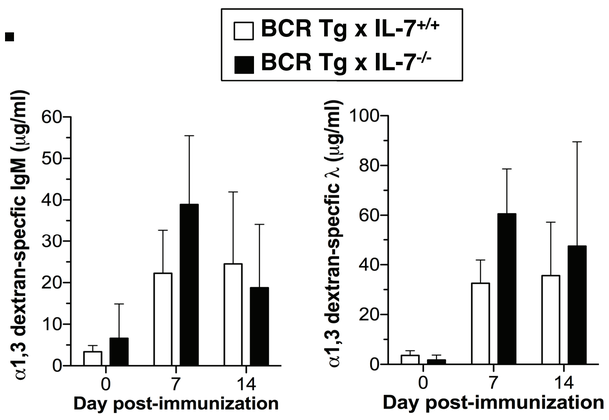

Figure 6. Anti-polysaccharide response can occur in B cell antigen receptor (BCR)-heavy chain V_HJ558 transgenic mice even in the complete absence of IL-7-dependent B lymphopoiesis. BCR transgenic mice sufficient (n=3) or deficient (n=6) in IL-7 were immunized i.p. with 50 μg of phenol-purified α1,3 dextran. Blood samples were obtained on 0, 7 and 14 days post-immunization and the levels of α1,3 dextran-specific antibodies of IgM isotype or λ light chain were determined by ELISA.

Conclusions:

- **Mice deficient in IL-7 signaling are sufficient in generating B1b, B1a and MZ B cell subsets**
- **Pneumovax®23 immunization neither elicits an antigen-specific antibody response nor confers protection against *Streptococcus pneumoniae* in mice deficient in IL-7 or IL-7Rα**
- **Transgenic expression of IL-7 permits anti-pneumococcal polysaccharide response and protective immunity in young mice**
- **immune system mice generate several human B cell compartments including human B1 cell subsets in the apparent absence of human IL-7, but are completely impaired in responding to pneumococcal polysaccharide**
- **Enforced expression of polysaccharide-specific B cell antigen receptor permits anti- polysaccharide responses in infant and young mice even in the complete absence of IL-7**

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