# Unique Response to IL-6 Signaling for T cell-mediated antibody response in neonatal stage

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## Introduction

- Neonates mount adult like protective immune responses to vaccines only after multiple immunizations.
- Impaired follicular helper T cell ($T_{FH}$) and germinal center (GC) B cell development likely responsible for weak vaccine responses in neonates.
- The underlying mechanisms in ablated $T_{FH}$ and GC B cell response are not known.

## Results

### 1. High $T_{FR}$ : $T_{FH}$ Ratio Persists Through Out the Immune Response in Neonatal Mice

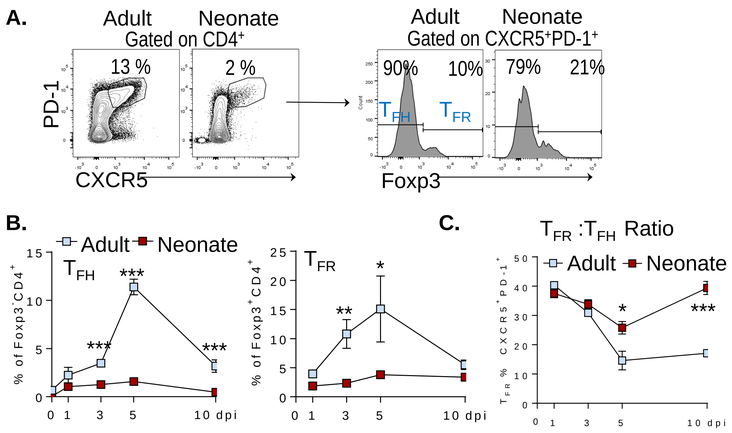


**Figure. 1.** Adult (10-wks) and newborn (5-days) mice were immunized with Sheep red blood cells (SRBC). **(A)** Percent of $T_{FH}$ ($CD4^+CXCR5^+PD\text{-}1^+Foxp3^-$) and $T_{FR}$ ($CD4^+CXCR5^+PD\text{-}1^+Foxp3^+$) cells in $CD4^+$ cells at 5 dpi is shown. **(B)** Percent of $T_{FH}$ cells among $Foxp3^-CD4^+$ and $T_{FR}$ cells among $Foxp3^+CD4^+$ cells are plotted. **(C)** The ratio of $T_{FR}$ to $T_{FH}$ cells ($T_{FR}$ : $T_{FH}$) are plotted.

### 2. Co-administration of IL-6 with PPS14-TT Vaccine Suppresses $T_{FH}$ Cell Generation and Antibody Responses in Neonatal Mouse

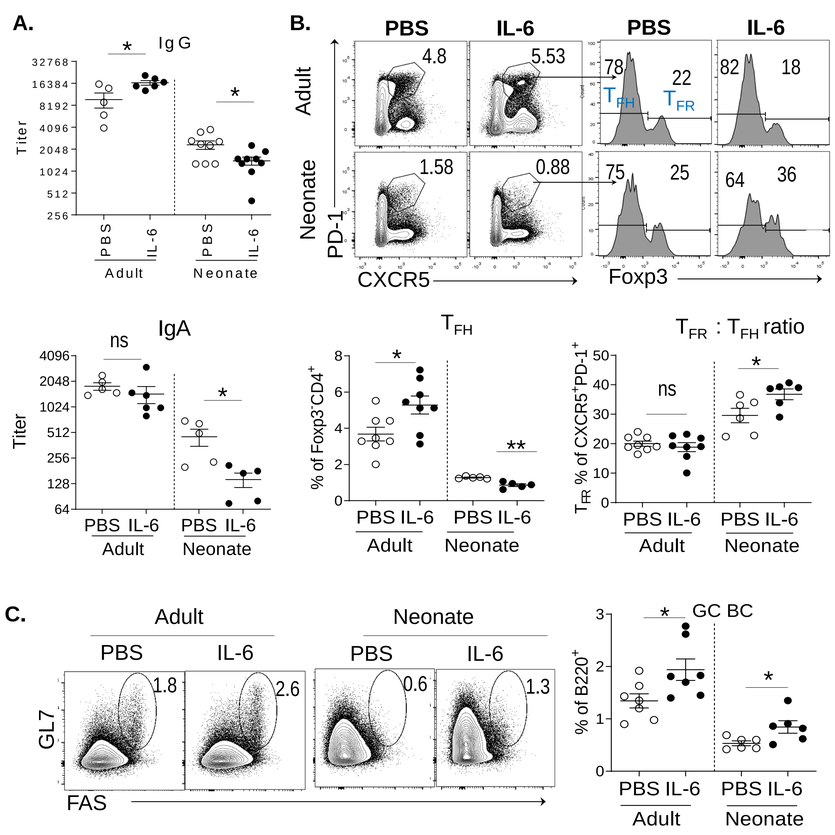


**Figure. 2.** Adult and neonatal mice were immunized with pneumococcal type 14 polysaccharide-tetanus toxoid (PPS14-TT) alone (0.2 µg/mouse) or with IL-6 (500 ng/adult, 100 ng/neonate) and splenocytes were analyzed in FACS at 7 dpi. **(A)** Serum anti-PPS14 IgG and IgA titers were determined in ELISA 6 weeks after immunization. **(B)** Percent of $Foxp3^-$ $T_{FH}$ cells among $CD4^+CXC5^+PD\text{-}1^+$cells and $Foxp3^+$ $T_{FR}$ cells among $CD4^+CXC5^+PD\text{-}1^+$ cells are shown. The ratio of $T_{FR}$ to $T_{FH}$ cells ($T_{FR}$: $T_{FH}$) are plotted. **(C)** Percent of GC B ($GL\text{-}7^+FAS^+$) cells among $B220^+$ cells are shown.

## Results

### 3. IL-6 Signaling Is Impaired in Neonatal $T_{FH}$ Cells

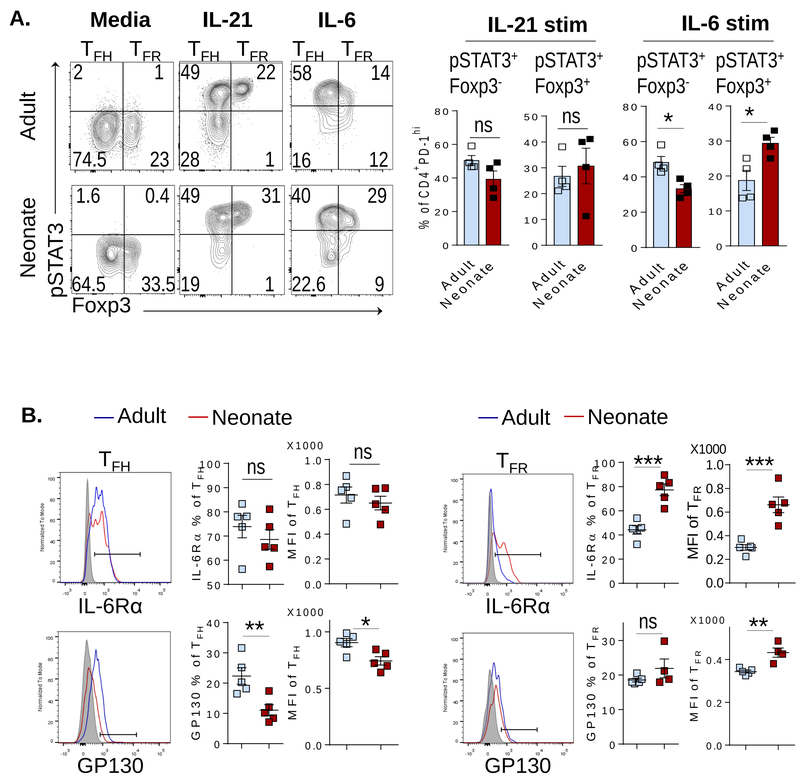


**Figure 3.** Adult (10-wks) and neonatal (5-days) mice were immunized with SRBC, and STAT3 activity in splenic $CD4^+$ cells was analyzed in flow cytometry at 5 dpi. **(A)** Splenocytes were stimulated with IL-21 (50 ng/ml) or IL-6 (100 ng/ml) for 15 min, followed by phospho-flow staining. Plots showing the percent of $pSTAT3^+$ cells among $Foxp3^+$ or $Foxp3^-$ populations were pre-gated on $CD4^+PD\text{-}1^{hi}$ cells. **(B)** Cells were pre-gated on $CD4^+CXC5^+PD\text{-}1^+Foxp3^-$ $T_{FH}$ and $CD4^+CXC5^+PD\text{-}1^+Foxp3^+$ $T_{FR}$ cells.

### 4. Inhibitory IL-2 signaling is promoted by IL-6 in neonatal vaccine

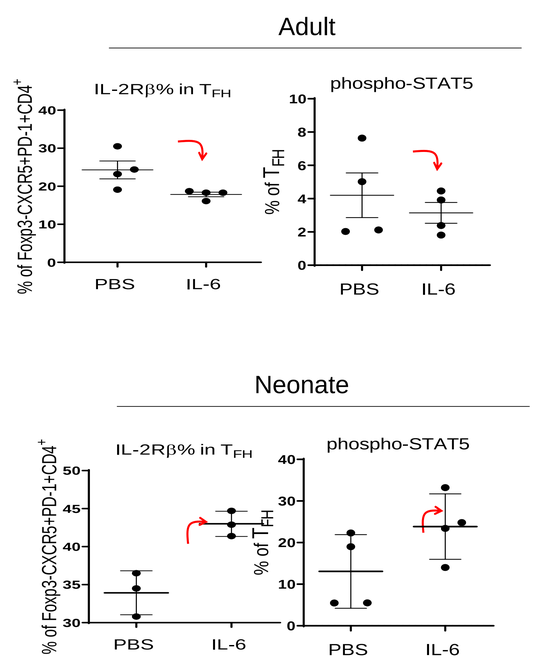


**Figure 4.** Adult (10-wks) and neonatal (5-days) mice were immunized with pneumococcal type 14 polysaccharide-tetanus toxoid (PPS14-TT) alone (0.2 µg/mouse) or with IL-6 (500 ng/adult, 100 ng/neonate) and splenocytes were analyzed in FACS at 7 dpi. Percent of IL-2R beta on TFH cells among Foxp3-CXCR5+PD-1+CD4+ cells are plotted.

### 5. TLR9 and detoxified TLR4 ligand improve anti-bacterial Th 1 response

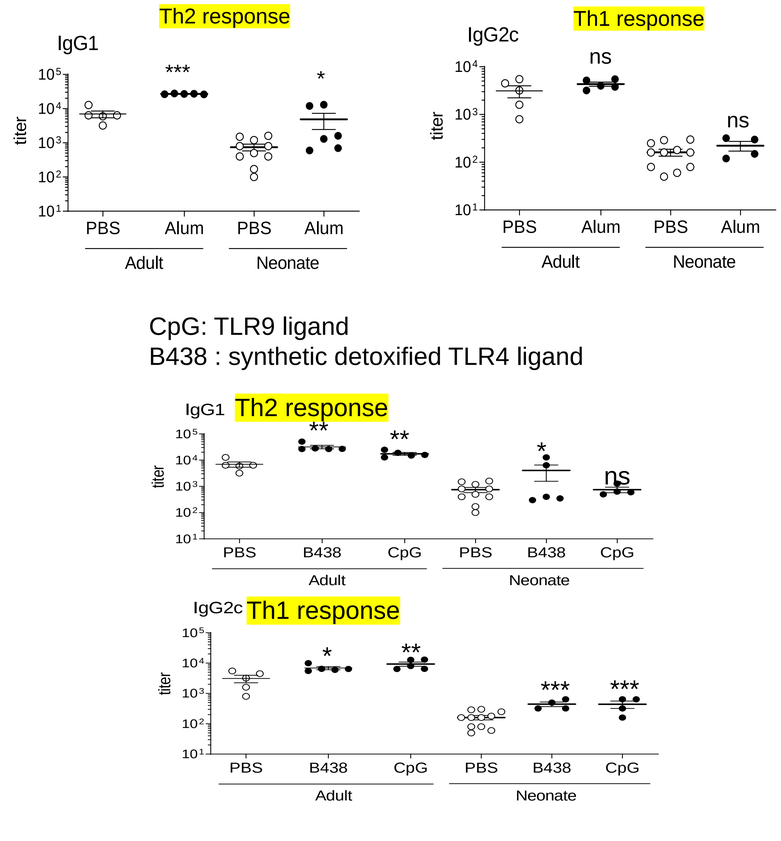


**Figure 5.** Adult (10-wks) and neonatal (5-days) mice were immunized with pneumococcal type 14 polysaccharide-tetanus toxoid (PPS14-TT) alone (0.2 µg/mouse) or with Alum (200mg), CpG or B438 and antibody response was assessed at 4 weeks.

### 6. Mechanism of TLR ligand-based adjuvant in neonatal immune system

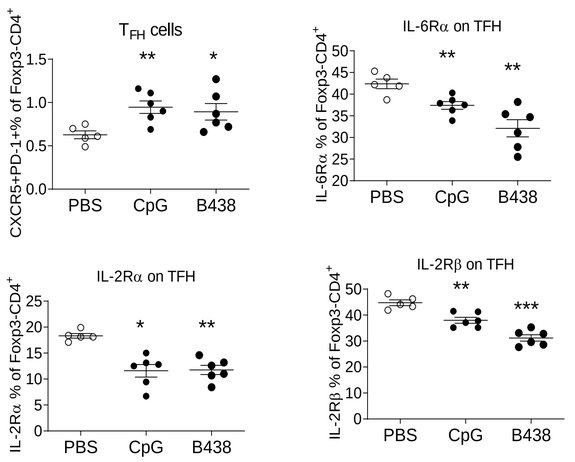


**Figure 5.** Adult (10-wks) and neonatal (5-days) mice were immunized with pneumococcal type 14 polysaccharide-tetanus toxoid (PPS14-TT) alone (0.2 µg/mouse) or with Alum (200mg), CpG or B438 and antibody response was assessed at 4 weeks.

## Summary and Conclusion

- IL-6 improves TFH generation by suppressing IL-2R in adult mice. In contrast to our surprise, IL-6 is detrimental for TFH generation in neonatal mice because IL-6 stimulates IL-2R expression on TFH cells, thereby rendering them susceptible to IL-2 mediated suppression. Our adjuvant study further highlight this mechanism because CpG and LPS both reduced IL-6R and IL-2R expression on TFH cells.