Neonatal B-Cell Receptor Induces a STAT-Dependent Negative Regulator

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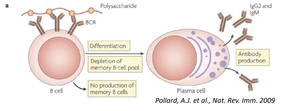
Abstract

Introduction: Neonates and infants have suboptimal immune responses to immunization compared to adults, resulting in high susceptibility to microbial infection. B lymphocytes have a central role in humoral immunity to immunization and infection, and signals via B cell receptors (BCRs) are essential for the development and maintenance of B lymphocytes. Neonatal B cells, however, do not respond to BCR activation as robustly as adult cells do.
Purpose: Our hypothesis is that unique features of neonatal BCR signaling may be contributing to suboptimal humoral responses. Thus, the aim of this project is to decipher the neonatal BCR signaling pathways and identify those that may be responsible for suboptimal B cell functions.
Methodology: Splenic B cells purified from neonatal and adult mice were stimulated ex vivo with anti-mouse IgM F(ab')2 to crosslink BCRs. We sought to identify uniquely activated or inactivated signaling pathways downstream of BCRs through RNA sequencing analysis. Subsequently, we performed western blot assay, quantitative polymerase chain reaction, and flow cytometry to investigate protein targets of the signaling pathways.
Results: We have identified that signal transducer and activator of transcription (STAT)-related signaling pathways were uniquely activated in neonatal B cells following BCR cross-linking. Among the STAT proteins, we found that STAT3 and STAT5 were highly phosphorylated in neonatal B cells compared to adult B cells in response to BCR activation. Using small molecule inhibitors and small interfering RNA, we identified that STAT5-induced IL-6 activates STAT3 in an autocrine or paracrine manner. Moreover, IL-6-induced STAT3 activation leads to the production of anti-inflammatory cytokine IL-10.
Conclusion: Neonatal spleen is known to contain higher numbers of IL-10 producing Breg cells than adult spleen and IL-10 is known to act as a negative regulator of T cell functions including survival and cytokine production. Our studies unveiled the pathways involved in the production of IL-10 in BCR-activated neonatal cells. We showed that BCR-activated neonatal cells secrete IL-6 in a STAT5-dependent manner. Subsequently, IL-6 signaling through STAT3 leads to the production of IL-10 from B cells.

Introduction

Despite advances in medicine and technology, approximately 4 million children under the age of 6 months die globally every year[1-3]. The major causes of neonatal death are infectious diseases including pneumonia, tetanus, and meningitis[4,5]. The leading causes of these serious bacterial infections are the polysaccharide-encapsulated bacteria[6]. In host immunity, B cells play a central role in humoral immune responses to infection. The polysaccharide-encapsulated bacteria are recognized by B cells via B cell antigen receptors (BCRs) in a T cell independent (TI) manner[7]. BCRs detect the capsular component polysaccharide and initiates B cell differentiation to antibody secreting cells. However, in humans, TI response are absent in neonates and do not reach adult levels until around 2 years[8], suggesting that BCR signaling is defective in neonate. Thus, the aim of this project is to decipher the neonatal BCR signaling pathways and identify those that may be responsible for suboptimal B cell functions.



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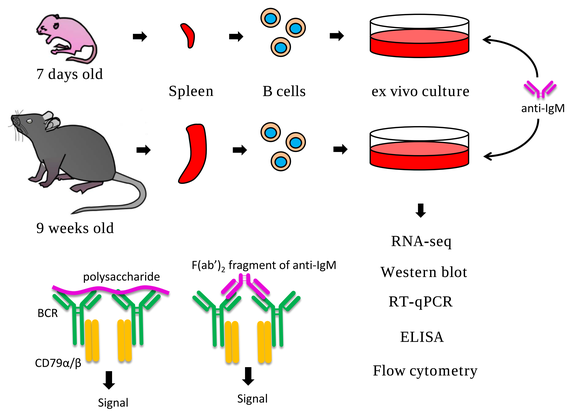
Materials and Methods



Results and Discussion

Signal transducer and activator of transcription (STAT)-related signaling pathways were uniquely activated in neonatal B cells following BCR cross-linking.

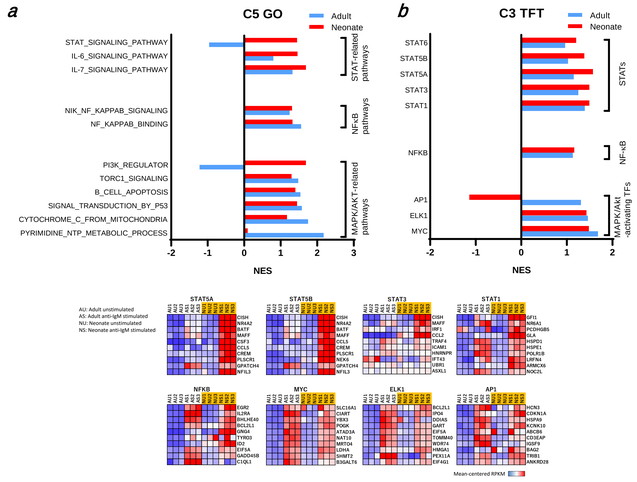


Figure 1 *Isolated CD19+ B cells were stimulated with 10 μg/mL F(ab')$_2$ fragments of anti-IgM for 7 h, and total RNA was isolated for RNA sequencing. **a**, Pathway analysis of signaling pathways upregulated following BCR activation. Gene set enrichment analysis (GSEA) was performed using the C5 gene ontology (GO) gene sets in MSigDB. NES for the changes in the same pathways were compared between Adult and Neonatal B cells. **b**, GSEA using the hallmark gene sets in C3 transcription factor targets (TFT). NES for each TF were compared between Adult and Neonate (upper) and heat maps of top 10 genes in selected TFs (lower).*

Results and Discussion

Among the STAT proteins, STAT3 and STAT5 were highly phosphorylated in neonatal B cells compared to adult B cells in response to BCR activation.

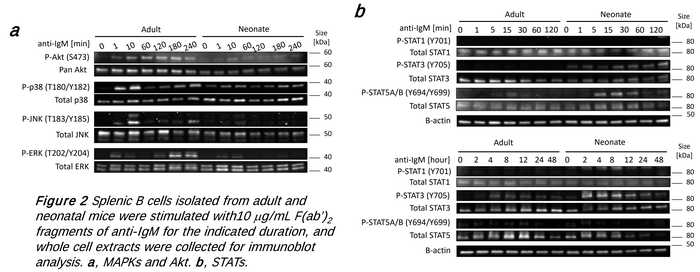


Figure 2 *Splenic B cells isolated from adult and neonatal mice were stimulated with10 μg/mL F(ab')$_2$ fragments of anti-IgM for the indicated duration, and whole cell extracts were collected for immunoblot analysis. **a**, MAPKs and Akt. **b**, STATs.*

BCR-activated neonatal cells secrete IL-6 in a STAT5-dependent manner.

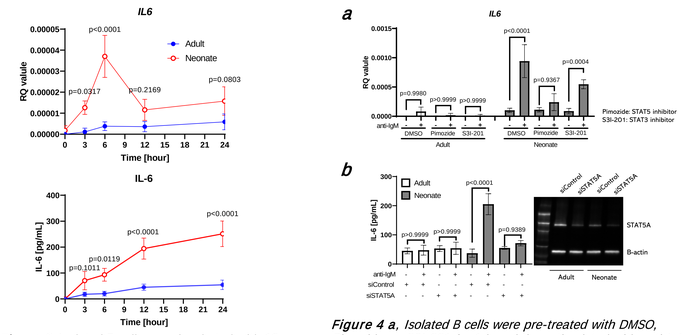


Figure 3 *Isolated B cells were incubated with 10 μg/mL F(ab')$_2$ fragments of anti-IgM for the indicated duration. Il6 mRNA expression in B cells was determined by RT-qPCR (upper) and the amount of IL-6 secreted from B cells was determined by ELISA (lower).*

Autocrine IL-6-induced STAT3 activation leads to the production of anti-inflammatory cytokine IL-10.

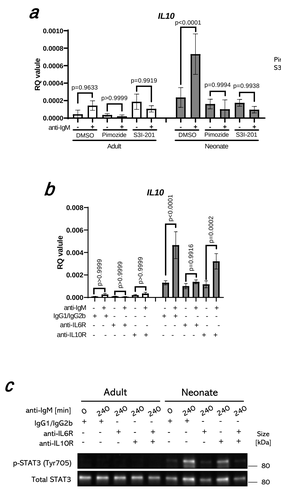


Figure 4 *Isolated B cells were pre-treated with DMSO, Pimozide, or S3I-201 for 1 h and then stimulated with anti-IgM for 4 h. Il6 mRNA expression in B cells were determined by RT-qPCR. **b**, Isolated B cells were transfected with 1 μM siRNA targeting STAT5A (siSTAT5A) or non-targeting control siRNA (siControl) for 48 h, and then then stimulated with anti-IgM for 4 h. The amount of STAT5A was examined by immunoblot analysis. The amount of IL-6 secreted from B cells was determined by ELISA.*

STAT5-induced IL-6 activates STAT3 in an autocrine or paracrine manner.

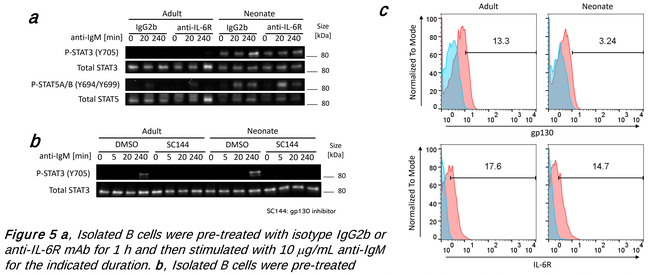


Figure 5 ***a**, Isolated B cells were pre-treated with isotype IgG2b or anti-IL-6R mAb for 1 h and then stimulated with 10 μg/mL anti-IgM for the indicated duration. **b**, Isolated B cells were pre-treated with DMSO or SC144 for 1 h and then stimulated with 10 μg/mL anti-IgM for the indicated duration. Whole cell extracts were collected for immunoblot analysis. **c**, Isolated B cells were analyzed for surface levels of gp130 and IL-6R by FACS.*

Conclusion

IL-10 inhibits the activity of immune cells including T cells during infection[9]. IL-10 can be secreted by several different types of cells. IL-10-producing regulatory B cells (Bregs) have been identified[10], and splenic B cells in neonate produce a significant amount of IL-10[11]. A previous study using B cell-specific IL-10-deficient mice showed the dominant effect of B cell-derived on suppression in inflammatory T cell development[12]. Our studies unveiled the pathways involved in the production of this anti-inflammatory cytokine in BCR-activated neonatal cells (Fig. 7).

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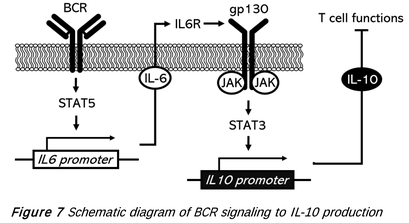

Figure 7 *Schematic diagram of BCR signaling to IL-10 production*