

**[3756.3] Single Cell Sorting To Decipher the Checkpoints for B Cell Selection in Neonatal Cord Blood**

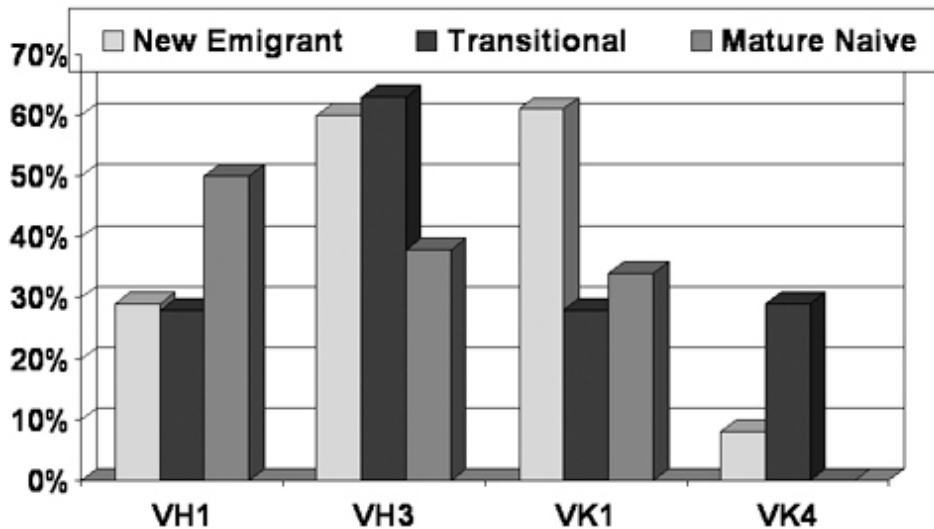
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BACKGROUND: Autoimmune diseases are caused by dysfunction in the immune system leading to the production of self-reactive antibodies. In healthy adults, polyreactive and self reactive antibodies are removed at two checkpoints. In the neonate, studies have shown that polyreactive B cells are a major constituent of cord blood B cells, and the neonate is reactive to more antigens than adults. Our initial data on B cell phenotyping shows that the predominant B cells in neonatal cord blood include transitional cells, new emigrant cells, and mature naive cells. The checkpoints at which polyreactive cells are removed from the neonatal repertoire have not been evaluated. The role of polyreactive antibodies in the neonatal immune defense is unclear.

OBJECTIVE: To determine the checkpoints for B cell selection in the neonate.

DESIGN/METHODS: Single cell sorting of cord blood B cell subsets was performed using flow cytometry. RT-PCR was done on all cells, followed by two rounds of PCR to amplify the immunoglobulin heavy chain and light chain. DNA sequencing was used to identify gene usage by NCBI Blast analysis.

RESULTS: Single cell sorting was done on 3 cord blood samples, followed by RT-PCR and PCR on 1440 cells. Seven percent contained PCR product. Results of DNA sequencing of selected genes is shown below:



CONCLUSIONS: Cord blood immature B cells, such as transitional and new emigrant cells, more frequently express  $V_{H3}$ ,  $V_{K1}$ ,  $V_{K4}$ , and longer CDR3s, when compared to mature naive cells. These immunoglobulin gene segments have been associated with self reactivity in previous studies. Thus, these immature cells may be responsible for the polyreactivity seen in neonates. The checkpoints for B cell selection in neonates could exist at the new emigrant and transitional B cell stage. The predominance of polyreactive, low affinity B cells in neonatal cord blood, with fewer specific antibody secreting cells, partially accounts for the poor initial response to infection leading to impaired adaptive immunity in the neonate.

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