POSTER 25

RT-PCR ON MAGNETICALLY SORTED B-CELL SUBSETS FROM PEDIATRIC BONE MARROW

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Acute lymphoblastic leukemia (ALL) is characterized by the uncontrolled proliferation of lymphoblasts which have been arrested in an early stage of B-cell differentiation. A key obstacle in identifying biomarkers of malignant B-cells in ALL patients has been constructing a cDNA library of normal B-lineage committed lymphoblasts from pediatric bone marrow for comparison. In order to identify unique biomarkers, a relatively quick method for isolating B cell subsets from pediatric bone marrow for RNA expression profiling is in development. B cells were magnetically sorted from bone marrow aspirate by immunophenotype into pro-B, pre-B, and immature B-cell subsets using antibodies covalently linked to paramagnetic microbeads. RNA was isolated and amplified from the subsets then probed using RT-PCR for expression of B-lineage marker (*PAX5*), myeloid-lineage marker (*MPO*), terminal deoxynucleotidyltransferase (*DNTT*), and immunoglobulin μ-heavy chain (*IGHM*) to determine the efficacy of the separation. Both *PAX5* and *IGHM* expression were directly proportional to B-cell maturity with *PAX5* expression residing between the positive (RL) and negative (U266) controls. Expression of *DNTT* was inversely related to B-cell maturity. Expression of *MPO* in all B cell subsets was higher than in RL, but parallel to the AML cell line, KG-1. The expression patterns of *PAX5*, *IGHM*, and *DNTT* were consistent with successful separation of the B-cells into pro-B, pre-B, and immature B subsets, but further validation of this method with flow cytometry is necessary.