

# Probability binning in immunophenotypic analysis of immunodeficiencies: HIV & CVID

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Probability Binning of Dendritic Cells in HIV patients:

Dendritic cells (DC) are considered an important reservoir for the initial spreading of HIV to regional lymph nodes following viral infection and subsequently to CD4+ T-cells encountering infected DC. We investigated the phenotypical changes of circulating dendritic cells of HIV infected patients by flow cytometry. Dendritic cells were defined as CD4+ mononuclear cells lacking CD3 and CD14 expression, and the expression of CD11c, CD40, CD86, HLA-DR, and CXCR4 was measured after logarithmic amplification of 4 colour fluorescence signals and software compensation for spectral overlap of listmode data.

Bright CD86 staining was confined to CD11c+ DC in cord blood, peripheral blood from healthy adults as well as adult HIV infected patients. CD11c is generally considered a marker of myeloid DC. CD40 and CD86 expression was slightly upregulated, whereas HLA-DR expression was 2 to 8fold upregulated in DC from HIV patients, similar to the increase in HLA-DR expression of CD4- T cells as compared to cells from healthy adults.

CXCR4 expression was at or below detection limit in peripheral blood DC in cord blood and peripheral blood from controls or HIV patients, whereas CXCR4 expression in CD4+ T-cells was significantly upregulated in HIV patients, and exhibiting positive correlation to HLA-DR expression of CD4- T cells.

In conclusion, phenotypical changes in DC of HIV patients are modest, but activation as deduced from increased expression of HLA-DR is reflected in modulations of CD40 and CD86 expression, whereas CXCR4 expression is unaffected.

Probability Binning of B1 lymphocytes in CVID patients:

The pathogenesis of common variable immunodeficiency is incompletely characterised at the molecular level though recent findings have drawn the attention to defective co-stimulation. Adult-onset panhypogammaglobulinemia is a clinical hallmark and is associated with decreased somatic hypermutation of the variable region of the B-cell immunoglobulin encoding gene.

CD5+ B-lymphocytes have been argued to secrete germ-line, non-hypermutated immunoglobulins under little or no regulation by T-cell factors. The routine immunophenotypic screening of immunodeficient patients at our institution includes stainings for CD5 expression in CD20+ lymphocytes. CD2+ events are neglected to rule out "contamination" with T-/B-lymphocyte doublets.

We decided to evaluate retrospectively the CD5 expression pattern in B-cells from known CVID patients and their children, and to compare the findings to those of patients admitted under the suspicion of other immunodeficiencies or controls. Probability binning was used for the comparison to simultaneously include differences in CD5 and CD20 staining intensities.

A marked and statistically significant association of  $T(\chi)$  to age of patients or controls was demonstrated, but it was not possible to discriminate offspring of CVID patients from other children suffering from repeated infections.