ImmunoTools *special* Award 2014

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Study of Natural Killer cells in double-unit cord blood transplantation

Double umbilical cord blood transplantation (dUCBT) is an efficient alternative when fully HLA-matched related or unrelated donors are unavailable. Interestingly, when full chimerism is obtained after dUCBT, it is usually derived from only one of the two UCB units injected. To date, neither genetic nor biological data allows predicting which UCB unit will engraft. By contrast to T lymphocytes, there is a rapid recovery of Natural Killer (NK) cells after UCBT. The effector functions of NK cells are tuned by inhibitory and activating receptors such as the Killer cell Immunoglobulin-like Receptors (KIR), which are specific to allotypic determinants shared by different HLA-class I molecules. Thus, HLA incompatibilities can trigger NK cell alloreactivity which is involved in Graft-versus-Leukaemia effect and can also contribute to engraftment.

The overall objective of my research project is to determine the impact of KIR and HLA incompatibilities 1) in engraftment of one UCB unit and 2) in anti-leukemic effect.

To develop the project, we combine a clinical study from samples of recipients and cord blood units to perform a KIR/HLA genetic analysis and a cellular study on recipient cells, to determine the phenotypic and functional profile of NK cells. We focus on NK cells (**CD56**), T lymphocytes (**CD3, CD4, CD8**), monocytes (**CD14**), B lymphocytes, (**CD19**) evaluating different NK cell markers.

In parallel, we investigate the biology of cord blood NK cells which are commonly characterized as “immature cells”. We realise a large phenotypic study of cord blood NK cells in collaboration with the CHU of Nantes. NK cell cord blood phenotype is compared to the study of healthy adult donors. This study is realized by flow cytometry, focusing on **$CD3^-$ $CD56^+$** NK cells and evaluating the expression of KIR,

NCR, HLA class I molecules (**HLA-A,B,C)** and different markers. We also evaluate the effect of cytokine stimulation (**IL-12, IL-15**) on these immature cells and evaluate their activation (**CD38, CD69**).

To investigate which cord blood NK cell subset are potentially alloreactive against leukemic cells, we develop an in vitro model. We evaluate the functional potential of cord blood NK cells using a panel of leukemic cell lines which must also be phenotypically characterized using **CD33**, **CD34**, **CD20**, **HLA-A,B,C** antibodies. We perform cytotoxic assay based on the evaluation of **IFN-gamma** production and CD107a expression of NK subsets against leukemic targets.

Taken together, our investigation aims to increase our knowledge on cord blood NK cell biology and it also brings useful tools for clinicians to better select cord blood unit based on the KIR/HLA profile to improve both engraftment and anti-leukemic effect.