ImmunoTools *special* Award 2014

Elisa Montaldo, PhD

Department of Experimental Medicine, University of Genova, c/o U.O.C Immunologia, Torre A1 Pad 90, IRCCS Azienda Ospedaliera Universitaria San Martino - IST Largo R. Benzi 10, 16132 Genova, Italy

Analysis of human innate lymphoid cell development

Innate lymphoid cells (ILCs) represent an extended family of developmentally related hematopoietic cells, which contribute to host immune defence. ILCs derive from a common precursor expressing the transcriptional repressor inhibitor of DNA binding 2 (ID2) and they require the common cytokine receptor γ-chain for their development. Accordingly to the newly proposed nomenclature, ILCs are classified in three main groups of cells on the basis of their transcription factor profile and functional properties. Group 1 ILCs includes NK cells and other Tbet$^+$IFN-γ$^+$ cells; group 2 ILCs express *GATA3* and produce type-2 cytokines such as IL-5 and IL-13; and group 3 ILCs characterized by *RORC* expression and IL-22/IL-17 production. Natural Killer (NK) cells represent the best characterized cell population belonging to ILC1 group. NK cells play a key role in tumor immune-surveillance and in host defence against virus and other pathogens thanks to their ability to mediate cytolytic activity and to release cytokines. ILC2 are involved in responses against helminthic infections and in allergic lung inflammation. ILC3 play a key role in lymphoid organogenesis in the foetus and after birth are involved in lymphoid tissue homeostasis and immunity to extracellular bacteria.

Constantinides *et al*. have recently identified in murine foetal liver a common progenitor committed to all ILC subsets, but not towards cytotoxic NK cells. Although an *ID2*$^+$ common precursor has been postulated also for human ILC, a common precursors has not been identified yet. In humans, it was shown that umbilical cord blood (UCB)-CD34$^+$ cells differentiate towards ILC3 and NK cells. However, no information exists on the sites of ILC3 differentiation after birth.

The aim of the proposed project is to clarify whether in humans distinct precursors of group ILC1, ILC2, and ILC3 exist, which is their tissue localization, and which cytokines and environmental stimuli might influence their development.

CD34$^+$ hematopoietic precursor cells (HPC) from different tissues, including (peripheral blood (PB), bone marrow (BM), umbilical cord blood (UCB), tonsils, lymph

nodes, thymus, liver, intestinal lamina propria and decidua) have been shown to differentiate towards NK cells. $CD34^+$ cells isolated from these tissues will be tested for the ability to differentiate also towards the other ILC lineages. HPCs, identified for the ability to differentiate towards one or more ILCs lineage, will be analysed by multiparametric flow cytometry for surface markers, transcription factors and cytokine profile in order to identify HPC subsets displaying features suggestive of specific commitment towards different ILC populations. By cell sorting we will isolate the eventually identified ILC committed precursors, and their differentiation ability will be compared to the respective HPC bulk cultures.

We expect that $CD34^+$ cell isolated from PB, BM and UCB can give rise to all ILCs since they contain HPC capable of generating all leuco- and eritro-lineages. Furthermore we will analyse thymic and tonsil $CD34^+$ cells as examples of peripheral HPCs, which might be committed to different ILCs fate upon interaction with specific microenvironment.