Membrane-bound IL-15 stimulation on peripheral blood natural kiler progenitors leads to the generation of an adherent subset co-expressing dendritic cells and natural kiler functional markers

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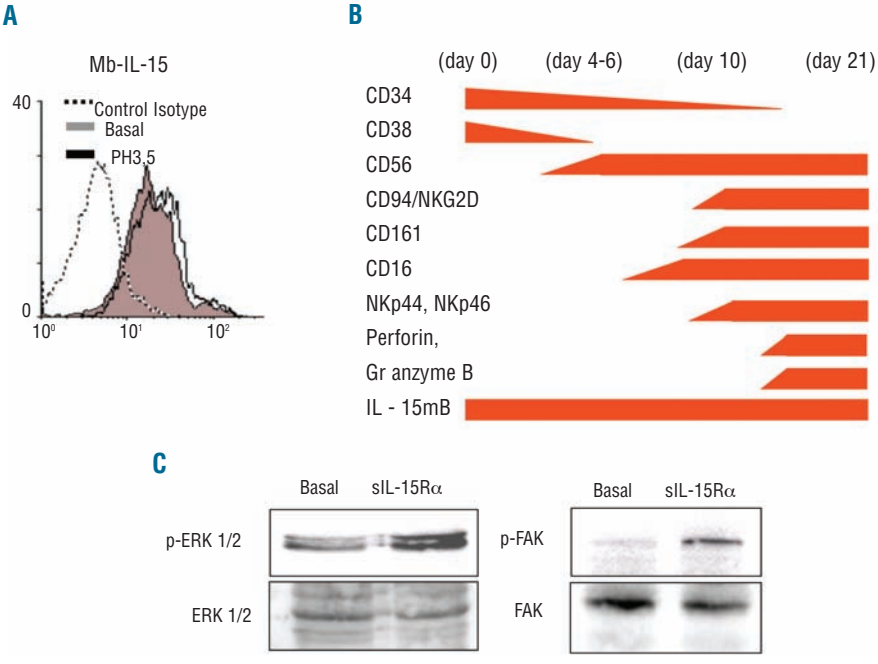
Citation: Negrini S, Giuliani M, Durali D, Chouaib S, and Azzarone B. Membrane-bound IL-15 stimulation on peripheral blood natural kiler progenitors leads to the generation of an adherent subset co-expressing dendritic cells and natural kiler functional markers. Haematologica 2011;96(05):762-766. doi:10.3324/haematol.2010.033738

Supplementary Appendix

mRNA extraction and cDNA synthesis

PB-NKPs were amplified with Stemα A medium supplemented with 100 ng/ml of SCF and Flt3-L (NK pathway). These progenitors were incubated with 1 μg/ml of human soluble recombinant IL-15Rα chain or with control murine IgG1.

At different times of culture (1-6 days) RNA was extracted using the Chomoczinsky method, as modified in the RNAble kit (Eurobio, les Ulis, France). RNA was resuspended in 10 μL diethyl pyrocarbonate (DEPC)-treated water and stored at -80°C until use. cDNAs were obtained by using random hexamers and avian myeloblastosis virus reverse transcriptase (First Strand cDNA Synthesis Kit, Roche Diagnostics, Meylan, France). Reverse transcription-polymerase chain reaction (RT-PCR) controls containing no RNA, and others containing RNA but no reverse transcriptase, were always used as negative control.



Online Supplementary Figure S1. Human PB-NK progenitors express a membrane bound IL-15 competent for delivering a bi-directional signalling: effects on the differentiation potential. (A) Detection of membrane-bound IL-15 (mb-IL-15) expression by flow cytometry (mAb 247-PE, R&D Systems) in PB-NKp (black open peak) and analysis of the sensitivity to acid buffer (shadowed peak). Isotype-matched Abs were used as control (dotted peak). These data are representative of 3 independent experiments. (B) Kinetic evolution of early hematopoietic markers and NK markers in human PB-HP expanded at high cell density in STEMα−A medium (Stem Alpha, Saint Clement les places, France) supplemented with 100 ng/mL of SCF and FLT-3L (Immunotools, Friesoythe, Germany). (C) Western blot analysis of MAPK ERK1/2, and FAK phosphorylation in 18-day old PB-NKp stimulated or not with 10 ng/mL of s-IL-15Rα chain. Membranes were reblotted with ERK1/2, and FAK Abs used as loading controls. One experiment representative of 3 is shown.