

IMMOBILIZED DELTA4 INDUCES DIFFERENTIATION AND EXPANSION OF FUNCTIONAL HUMAN NK CELLS FROM CD34+ HPCs, INDEPENDENT OF STROMAL CELLS

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Introduction: We have previously shown that the Notch ligands Jagged2, Delta1, and Delta4 cause both differentiation and expansion of functional human NK cells from CD34+ hematopoietic progenitor cells (HPCs) derived from umbilical cord blood. In the presence of murine OP-9 stromal cells expressing Notch ligand, the Notch-induced NK (N-NK cells) expand over 500-fold and reach up to 90% purity. N-NK cells have both cytokine-secreting and cytotoxic functions and express activating NK cell receptors important for tumor cell recognition and killing, while at the same time they lack a majority of NK cell inhibitory receptors. Thus, the phenotype and functionality of N-NK cells suggests a potential clinical use as cell therapy for malignancy. For the generation of N-NK cells for clinical use, we have designed a culture system which can be easily adapted to large-scale production and which avoids the use of xenogeneic stromal cells. This culture system uses recombinant, immobilized Delta 4 attached to microbeads for the differentiation and expansion of NK cells from HPCs, independent of stromal cells.

Methods: HEK 293T cells were transfected with a vector encoding a chimeric molecule consisting of the outer membrane component of murine Delta4 fused with human IgG1 Fc, termed Delta4:Fc. The presence of the Delta4:Fc chimera in the cell supernatant was verified by Western Blot and quantified by ELISA using anti-human Fc antibody. Concentrated supernatant was combined with Protein A microbeads for a target concentration of 40-60 ng Delta4:Fc per μ l of beads. The amount of Delta4:Fc bound to the beads was confirmed by ELISA performed on the post-bind supernatant. Delta4-beads were incubated with CD34+ HPCs isolated from cord blood, in the presence of cytokines including IL-7 and IL-15 to promote NK cell development. A commercially available chimera, CD14:Fc, was also bound to beads and served as a negative control. After 14 days, cultures were monitored weekly by flow cytometry for the development of NK (CD56+CD3-) cells. Receptor phenotype of NK cells was determined by flow cytometry, and the ability of NK cells to lyse hematopoietic tumor cell lines was examined by a non-radioactive cytotoxicity assay (Promega CytoTox-Glo™).

Results: Incubation of CD34+ HPCs with 0.33 μ g/well Delta4:Fc in the presence of IL-7, SCF, FltL, IL-3, and IL-15 resulted in an average of 73.4% NK cells after 21 days in culture, with the percentage reaching up to 90% in some experiments after 28 days. The non-NK cells in culture consisted of CD33+ granulocytes; no T cells or B cells were seen. Expansion of NK cells reached an average of 248-fold after 28 days in culture. The addition of IL-6 to the above 5-cytokine mix increased the expansion of NK cells to 334-fold, although the percentage of NK cells was only marginally increased. NK cell development and expansion was specific to the presence of Delta4, as cultures containing CD14:Fc-beads routinely resulted in less than 5% NK cells and less than 10-fold NK cell expansion. The receptor phenotype of NK cells derived from the Delta4-bead system included expression of the natural cytotoxicity receptors (NKp30,44,46) and CD244 (2B4), absent/low expression of CD16 and NKG2D, and absence of the inhibitory receptors KIR and NKG2A. This phenotype is similar to that previously reported for NK cells cultured with Delta4-expressing OP-9 stromal cells. NK cells from the bead system were capable of lysing various human hematopoietic tumor cell lines (K562, RPMI-8226, and OCI-AML3).

Conclusion: Recombinant Delta4 immobilized onto microbeads is capable of inducing expansion and differentiation of functional, inhibitory receptor-negative NK cells from CD34+ HPCs, independent of the presence of stromal cells. The Delta4-bead culture may be a practical strategy for the generation and expansion of NK cells for cell therapy purposes.