



2011 Research Grant Program Winning Abstract

Expanded NK Cells for Adoptive Immunotherapy

By Dean Lee

NK cells are well known to provide MHC-independent recognition of transformed, infected, and otherwise stressed cells, leading to cytolysis and local production of pro-inflammatory cytokines, which makes them a very promising tool for tumor immunotherapy. Adoptive transfer of NK cells has been shown to be safe in adult patients with cancer. However, the inability to obtain large numbers of pure NK cells and the low frequency of related KIR-mismatched donors has limited the application of NK cell therapy. Propagation of NK cells in vitro—a major focus of our laboratory research—has been problematic, since there are many activating and inhibitory receptors, cooperative receptor pairs, and overlapping signaling pathways for maturation, activation, and proliferation. Propagation with K562-based aAPC transduced with CD137L and membrane-bound IL-15 (mIL15) achieved a mean NK cell expansion of 277-fold in 21 days, but continued proliferation was limited by senescence from profound telomere shortening (Fujisaki, *Br J Haematol*, 2009). We developed a novel ex vivo expansion method for NK cells that resolves this problem by genetically engineered aAPC to express membrane-bound IL-21 (mIL21), which signals primarily through STAT3, which is known to activate telomerase. We showed that NK cell expansion on mIL21-expressing APCs promotes sustained proliferation without senescence by increasing expression of telomerase—and therefore telomere length—in the expanded cells. Expansion of NK cells from 20 donors with mIL21 resulted in a median 23,413-fold expansion by day 21 (Somanchi, *JoVE* 2011 <http://www.jove.com/details.stp?id=2540>, and manuscript in preparation), sufficient to deliver multiple infusions of NK cells at high cell doses in a clinical trial. MD Anderson Cancer Center has four clinical trials of adoptive NK cell immunotherapy, for ALL, NHL, AML, and neuroblastoma. We are finalizing the production of a master cell bank of these aAPCs to use for future expanded NK cell clinical trials.

We hypothesize that IL-21 plays a pivotal role in NK cell homeostasis through STAT3-dependent telomerase activation, upregulation of CD160 expression, and induction of anti-apoptotic machinery. We propose the following research plan to apply multicolor flow cytometry to study the cell signaling and immune function of NK cells expanded with mIL21 for the immunotherapy of cancer.

Specific Aim 1: To determine the role of IL-21 signaling in regulating telomerase expression. Previously we found that telomeres in NK cells expanded with aAPCmIL21 for 7 days are much longer than those of NK cells cultivated in the presence of aAPCmIL15. It is known that IL-21 induces STAT3, some STAT1, and very little STAT5 phosphorylation, and that the telomerase gene promoter has binding sites for STAT3 and STAT5. We will determine if aAPC with mIL15 or mIL21 induces STAT3 and STAT5 phosphorylation, and determine which is most important in regulating telomerase expression in NK cells, using STAT inhibitors and STAT-specific BD Phosflow™ analysis.



Specific Aim 2: To clarify NK cell subpopulations involved in IL-21–dependent long-term expansion. Using a fluorescence activated cell sorting approach, we will purify NK-cell subpopulations (candidate subsets CD56^{bright}CD16⁻CD62L⁻ and CD56^{dim}CD16⁺CD62L⁺) and determine their proliferative response to aAPCmIL21 using BrdU incorporation.

Specific Aim 3: To determine the role of IL-21 in regulating CD160 expression. CD160 is normally expressed on 80% of peripheral blood NK cells. It has been shown that GPI-specific phospholipase D (GPI-PLD) is responsible for CD160 shedding from the NK-cell surface, and that expression of this metalloprotease is upregulated in response to IL-15 or IL-2. Previously we found that CD160 surface expression by NK cells is upregulated in response to mIL21 even in the presence of IL-2. Using anti-GPI-PLD and anti-CD160 mAb, we will determine if IL-21 affects CD160 expression in NK cells through an indirect mechanism involving GPI-PLD activity.

Specific Aim 4: To explore how IL-21 and CD160 co-regulate survival of NK cells. We compared proliferative responses and apoptosis of NK cells cultivated in the presence of aAPCmIL15 or aAPCmIL21 by following proliferation and Annexin V binding. We found that mIL15-stimulated NK cells proliferated at the same rate as those stimulated with mIL21, but underwent dramatic cell death (up to 80%) during the first four days of activation. We also showed an inverse correlation of apoptosis with CD160 expression. Apoptosis did not occur in NK cells activated with aAPCmIL21 and showed upregulation of CD160 expression. Using mAbs against Bcl family members, we will explore whether engagement of CD160 with its ligands plays a role in expression of pro-survival factors as a secondary mechanism of IL-21 signaling.

These experiments will utilize multiparameter flow cytometry and sorting to determine i) proliferation and cell death using BrdU incorporation, 7-AAD, and Annexin-V, ii) expansion potential of NK cell subsets and phenotypic maturation using BD anti-human CD antibodies applicable to future ASR applications, iii) pathways involved in IL-21 signaling by assessing STAT phosphorylation and expression of apoptosis-regulating Bcl-family members, iv) activation/degranulation responses detected by liberation of LAMP1 and intracellular cytokines, perforin, granzymes, and BD™ Cytometric Bead Array (CBA) assays, and v) surface-receptor regulation of GPI-anchored proteins using anti-human CD and PLD antibodies. This grant will greatly assist in providing the broad array of cytometry reagents necessary for developing this critical new information in NK-cell biology, and the resulting data will inform the development of ex vivo expansion for future clinical trials of adoptive immunotherapy with NK cells.

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