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CROSS-SPECIES CHARACTERIZATION OF SPLENIC NATURAL KILLER (NK) CELLS REVEALS ORGAN-SPECIFIC HETEROGENEITY WITH IMPLICATIONS FOR CANCER IMMUNOTHERAPY

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Background Natural killer (NK) cell immunotherapy is a promising modality for cancer immunotherapy, but questions of optimal donor characteristics for in vivo persistence, engraftment, and effector function remain. Splenic NK cells have been poorly characterized in humans and dogs. Although they are not readily available as a source for adoptive immunotherapy, we hypothesized that splenic NK harbor unique phenotype and function which can inform optimal NK immunotherapy.

Methods Matched NK cells were isolated from spleen and blood of 4 human and 3 dog patients undergoing surgery. Immune phenotype, proliferation, viability, and apoptosis were assessed pre and post 14 days of co-culture with irradiated clone9 K562 feeders. Differential gene expression was assessed using 3'-Tag-RNA-sequencing.

Results NK cell frequencies in resting human spleen were significantly lower compared to PBMCs per live CD45+ cells ($9.4 \pm 0.3\%$ vs. $16.7 \pm 8.8\%$, $P=0.05$) whereas the frequencies of NKT, CD3+, and CD8+ were not significantly different ($P>0.05$ all). Phenotypically, TIGIT expression was higher in resting human spleen NK compared to PBMCs ($39.8 \pm 2.4\%$ vs. $19.0 \pm 8.6\%$), as was CD69 ($23.8 \pm 8.7\%$ spleen vs. $14.4 \pm 7.6\%$ PBMCs). Across both dog and human, spleen cells expanded more significantly than PBMCs with human showing 266-fold versus 105-fold expansion. In human, maximal expansion was also greater for spleen NK cells ($690 \pm 1080 \times 10^6$) compared to PBMC NKs ($111 \pm 165 \times 10^6$), although not statistically significant. Similar results were obtained with dog NK expansions. Purity of human NK cells at day 14 was similar for spleen and PBMC-expanded NK cells at $>90\%$, and both groups showed similar Ki67 (70-90%) and Granzyme B (97-100%). In killing assays against human sarcoma targets (SAOS2, A673), there was greater cytotoxicity with spleen-expanded NK cells compared to PBMC-expanded at 10:1 E:T (40-50% vs. 30-35%). Day 14 spleen-expanded NK cells from dog showed greater killing compared to PBMC-expanded against osteosarcoma and melanoma targets at 10:1 E:T (45% vs. 25%). At day 14, there was no difference in apoptosis between human spleen and PBMC-expanded NK cells. Sequencing results showed an upregulation of genetic pathways associated with persistence and metabolic fitness for both human and dog spleen-expanded NK cells at day 14.

Conclusions NK cells derived from spleen appear to show greater activation and expansion compared to PBMC-derived in human and dog subjects with no difference in apoptosis. Further characterization of NK cells from the spleen may provide novel insights into mechanisms to overcome barriers to successful NK immunotherapy for solid tumors in both human and canine models.

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