Combining an engineered costimulatory vaccine with NK cells induces an anti-tumor effect against murine neuroblastoma in vitro and after bone marrow transplant in vivo

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Abstract
High risk neuroblastoma remains a challenge to cure with only 50% survival, despite multi-modality treatments. Nektar Therapeutics (NKT) cells have been previously shown to induce immune responses against murine neuroblastoma with vaccine in engineered CSU5, CSU6, CSU5, and CSU5 cells, resulting in AgN-specific cytotoxic activity in vivo and in vitro. NK cells and engineered AgN.2 cells were co-cultured in ratios of 1:1, 3:1, and 5:1 and AgN.2 cells were then treated with IL-15, IL-2, and IL-15 cytokine cocktail (IL-15:IL-2:IL-15:IL-20) and then assayed by flow cytometry, magnetic cell sorting, and cytotoxic assays in vitro and in vivo after 1, 2, 3, 4, and 5 days. To study the efficacy of an in vitro vaccine with AgN.2 cells after bone marrow transplant (BMT), C57BL/6 at 9B recipients were lethally irradiated, followed by transplantation of 10^7 T cell depleted (TCD) C57BL/6 bone marrow cells, with or without adoptive transfer of NK cells, then subcutaneous injection of 4 x 10^6 neuroblastoma cells, and cytotoxicity and cytokine analysis. No differences were seen between the 1:1, 3:1, and 5:1 co-cultures in vitro. However, IL-15:IL-2:IL-15:IL-20 cytokine cocktail, significantly increases NK cell killing in vitro and in vivo. In vivo, NK cell adoptive transfer is critical for immune responses against neuroblastoma. Cytotoxicity of NK cells against neuroblastoma cells was higher in vivo than in vitro.

Methods

- Groups 1: NK cells were adoptively transferred and treated with IL-15, IL-2, and IL-15 cytokine cocktail (IL-15:IL-2:IL-15:IL-20) before adoptive transfer into mice
- Groups 2: NK cells were adoptively transferred and treated with IL-15 before adoptive transfer into mice
- Groups 3: NK cells were adoptively transferred and treated with IL-15:IL-2:IL-15:IL-20 cytokine cocktail before adoptive transfer into mice
- Groups 4: NK cells were adoptively transferred and treated with IL-15 before adoptive transfer into mice

Results

- Image 1: AgN.2 4P vaccine
- Image 2: Experimental design for in vitro co-culture groups with NK cells
- Image 3: Experimental design for in vitro co-culture with NK cells
- Image 4: Experimental design for in vivo co-culture with NK cells
- Image 5: Cytotoxic activity of NK cells against neuroblastoma cells in vitro and in vivo
- Image 6: Cytotoxic activity of NK cells against neuroblastoma cells in vitro and in vivo
- Image 7: Cytotoxic activity of NK cells against neuroblastoma cells in vitro and in vivo
- Image 8: Cytotoxic activity of NK cells against neuroblastoma cells in vitro and in vivo

Conclusion

- Co-culture of murine NK cells and AgN.2 4P cells in IL-15 increases the percentage of Ly6KD NK cells in vitro as well as secretion of higher concentrations of IFN-gamma, IL-6, and CXCL1, as compared to IL-15 activated NK cells.
- Murine NK cells co-cultured with AgN.2 4P and IL-15 induce higher levels of cytotoxicity to two separate murine neuroblastoma tumors, compared to IL-15 activated NK cells. The 1:1 ratio of NK:AgN.2 4P is the most effective cytotoxic ratio tested.
- After T cell replete allogeneic BMT, combining the AgN.2 4P vaccine with NK cells significantly reduces tumor growth as compared to BMT + vaccination or BMT alone, implying that NK cells and T cell are needed for vaccine effectiveness.
- AgN.2 4P is a safe and potentially effective vaccine for stimulating NK cells in vitro and enhancing a T cell and NK cell-mediated graft-versus-tumor effect in vivo.

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Introduction

- There are no current vaccines in the clinic that prevent recurrence of neuroblastoma.
- Currently, neuroblastoma treatment involves the use of only autologous bone marrow transplant.