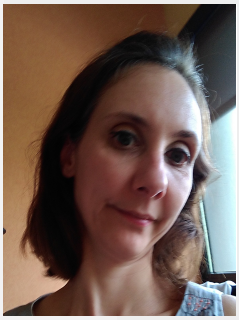
ImmunoTools *special* Award 2018



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NK Cell immunotherapy for medulloblastoma brain tumor

Description of the project:
Medulloblastoma is the most common brain tumor in children. While Standard treatment have improves survival, patients frequently relapsed due to tumor escape to treatment. One of tumor escape mechanism is the induction of immune cell exhaustion through the programm cell death pathway (PD1) leading to the development of immunotherapies targeting this immune check point. As natural killer cell anti-tumoral effect has been observed in some brain tumors and as these cells express PD-1, it would be interesting to determine if an anti-PD1 antibody could enhance their cytotoxicity against medulloblastoma.

• Objectives of the project and description of the methods used
- to develop a mouse model of human medulloblastoma to evaluate the anti-tumor effect of NK cells and the effect of blocking the PD1 / PDL1 pathway on their cytotoxicity. We wish :
1. to Analyze the fate of NK cells after administration, with and without pre-treatment with antiPD1
2. to Evaluate response to NK cell therapy on:
a. Reduction of tumor mass
b. Improving survival

Our experimental design will be as follow:
- Selection of the human medulloblastoma cell line the most sensitive to the cytotoxicity of NK cells in vitro
- Heterotopic implantation of the tumor on the back of nude mice and treatment with IL-15 activated NK cells incubated or not with anti-PD1 antibodies. Distribution and persistence of NK cells, will be determined at D2 and D10 at the optimal time of tumor development by confocal microscopy on tumor slides and flow cytometry after tumor dissociation.
- Same experiments carried out on the orthotopic tumor (cell infiltration, with evaluation of the tumor mass by petscan, carried out on animals treated or not with NK cells incubated or not with the anti-PD1 at 5 and 10 days after injection.

-Expected results
These results will make it possible to determine the impact of blocking the PD1 / PDL1 pathway on the anti-tumor activity of NK cells in vivo.

ImmunoTools reagents would be very useful to:
-phenotype NK cells after immunomagnetic selection using CD3 PerCP, CD16-FITC, CD56-APC.
After selection, NK cells would be expand using rh IL15. After expansion, NK cell activation state would be determine using CD69 antibody, and NK cell exhaustion would be determined using anti CD279 antibody. The different subpopulations of NK cells obtain after expansion would be characterized by the expression of CD56 (bright and dim sub-populations), CD62 L and CD57 (maturation marker of NK cells).

-NK cell functionality against medulloblastoma cell lines would be evaluated using anti-TNFa and anti-IFNg ELISA. The induction of medulloblastoma cell line apoptosis after co-culture with NK cells would be determined by the use of Annexin V FITC.

-The modulation of PDL1 expression by medulloblastoma cell lines would be studied with or without priming them with rh IFNg.

- CD3 PerCP, CD16-FITC, CD56-APC would also be used after dissociation of the tumor developed into the animal model treated with NK cells, to determine if human NK cells have reached the tumor. Anti-murine NK cell antibody would be use to analyse wether mouse NK cells are attracted into the tumor since nude mice still have NK cells but no T or B cells.

Expected results and impact:
Children with medulloblastoma suffer from neuro-intellectual and significant physical effects related to the impact of toxic treatments for nervous system. It is therefore essential to consider new less toxic therapeutic approaches to healthy tissues and specific cancer cells. The development of relevant animal models is essential to evaluate these new treatments, and especially if immunotherapy based on NK cells can be a therapeutic option.

The expected results will observe a reduction in tumor mass and improved survival in animals treated by adoptive immunotherapy and an adding value of combining them with an anti-PD1 antibody. Following the establishment of this animal model and the achievement of expected results, we then wish to graft mice with tumor cells obtained by resection from patients and determine whether they develop in vivo and respond similarly to adoptive immunotherapy alone or combined.

ImmunoTools *special* AWARD for **Veronique Decot** includes 21 reagents

FITC - conjugated anti-human: CD3, CD16, CD56, CD57, CD69, Annexin V

PE - conjugated anti-human: CD57, CD62L, CD279

PerCP - conjugated anti-human: CD3

APC - conjugated anti-human: CD56

recombinant human cytokines: rh IFN-g, rh IL-15

human ELISA-set (for one 96 plate): human TNF-a, human IFN-g

DETAILS more AWARDS