Invariant Natural Killer T cells as Anti-Tumour Immunotherapy

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Aims

- Know in a general way which is the immunologic response when a tumour appears.
- Define what natural killer T cells are and their cellular characteristics.
- Describe why invariant natural killer T cells would be effective as anti-tumour therapy

Materials and Methods

- Scientific literature search in **Pubmed database** using as key words: NKT cells or natural killer T cells, iNKT cells or invariant natural killer T cells, tumour immunology and cancer/ tumour therapy. Papers were selected for: quality, date of publication and journal impact.
- Search in **official websites** of World Health Organization and Clinical Trials.

Introduction

Cancer is a disease that can affect any part of the body. One defining feature of it is the rapid creation of abnormal cells that grow beyond their usual limits, and which can then invade adjoining tissues and spread to other organs.

It is one of the most important diseases nowadays because is a leading cause of death worldwide. The research of **new therapies** is crucial for helping patients that do not respond to classical therapies such as radiotherapy, surgery or chemotherapy.

It is known that our immune system is able to fight against tumours in a process referred to as **cancer immunoediting** which involves three phases: elimination, equilibrium and escape. Because of it many immunotherapies are being set to treat tumours. For example, here we describe **NKT cells** and their demonstrated anti-tumour potential.

Results

Characterization of NKT cells

1. What are NKT cells? NKT cells are a unique subset of lymphocytes that express at the same time NK cell markers (NK1.1 in mice and CD161 in humans) as well as a TCR α/β, with a restricted repertoire. This TCR recognizes glycolipids presented by CD1d, a non-classical antigen presenting molecule that associates with β2-microglobulin. The rapid response of this cells to their antigens is characteristic of an innate immune response, and allows the polarizing cytokines (IFN-γ and/or IL-4) to regulate adaptive immunity.

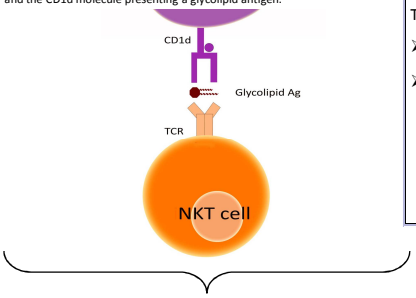
2. Subtypes

	Type I NKT cells (iNKT cells)	Type II NKT cells	NKT-like cells
CD1d dependent	Yes	Yes	No
α-GalCer reactive	Yes	No	No
TCR	Semi-invariant	Variant	Variant
TCR α-chain	Vα14-Jα18 (mice) Vα24-Jα18 (humans)	Diverse	Diverse
TCR β-chain	Diverse	Diverse	Diverse

3. Ligands

Synthetic compounds	α-Galactosylceramide (α-GalCer) OCH (analog of α-GalCer) C20:2 (analog of α-GalCer) C-glycoside (analog of α-GalCer) βGalactosylceramide (βGalCer (C12))
Bacterial molecules	α-gluconosylceramide (GSL-1) α-galacturonosylceramide (GS-1') Phosphatidylinositol mannoside (PIM)
Endogenous molecules	Isoglobotrihexosylceramide (iGb3) Disialoganglioside (GD3) Phosphatidylinositol (PI)

Figure 1. Schematic view of the interaction between the TCR of a NKT cell and the CD1d molecule presenting a glycolipid antigen.



4. Activation

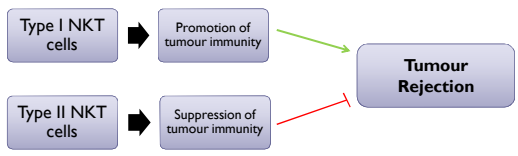
Two pathways:

- **Activation mediated by cytokines**
- **Antigenic activation by TCR signal**
  - Direct: APCs presentation to NKT cells by CD1d-glycolipid antigen complexes.
  - Indirect: APCs are activated through their TLRs that trigger the loading of CD1d molecules with endogenous antigens presented to NKT cells.

5. Functions and Implications in Diseases

Tumours	Transplantation tolerance	Infections	Allergy	Autoimmunity

6. NKT cells in Regulation of Tumour Immunity



iNKT cells for Cancer Treatment

1. Mechanisms to attack tumour cells

Direct

- iNKT cells express high levels of granzyme, perforin and FasL that can produce cytolysis or ligand induced killing of tumour cells.

Indirect

- iNKT cells produce large amounts of IFN-γ, which activates NK cells and CD8⁺ T cells that have cytotoxic activity on tumour cells.

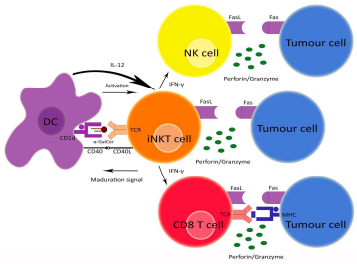


Figure 2. Anti-tumour immune responses induced by activated iNKT cells. After activation, iNKT cells exert direct anti-tumour activity, including perforin, granzyme and Fas ligand, and up-regulate CD40L which stimulates CD40 on DC to produce IL-12. Large amounts of IFN-γ secreted by iNKT cells induce NK cells and CD8⁺ T cells to exert indirect anti-tumour responses.

2. Manipulating iNKT cells for Cancer Immunotherapy

- **Stimulating patient's own iNKT cells by the administration of exogenous activators**
  - **IL-12** → this cytokine exerts multiple anti-tumour effects that include the activation of NK, iNKT and T cells in addition to its immune-cell-independent anti-angiogenic effects to mediate tumour rejection.
  - **α-GalCer** → iNKT cells recognize it in conjunction to CD1d. After activation these cells exert potent direct and indirect anti-tumour activities, inducing the production of large amounts of cytokines (IL-12 and IFN-γ).
  - **α-GalCer-DCs** → the adoptive transfer of α-GalCer-pulsed DCs induce more potent anti-tumour effects than the treatment with soluble α-GalCer.
- **Transferring activated iNKT cells to the patient**
  - ***in vitro* activated iNKT cells** → iNKT cell populations are isolated from a patient with cancer, selected and expanded *in vitro* with cytokines before being infused back into the patient. These expanded iNKT cell populations can be co-infused with CD1d ligand-pulsed DCs to enhance the anti-tumour activity *in vivo*.
  - **iPS-derived iNKT cells** → for patients whose NKT cells are limited, induced pluripotent stem cells can be generated , developed into functional iNKT cells *in vitro* and then injected to the patient. This cells have demonstrated to suppress tumour growth *in vivo*.

3. Clinical trials done with this therapy

According to ClinicalTrials.gov nowadays there are 138 studies working with NKT cells to treat cancer around the world.

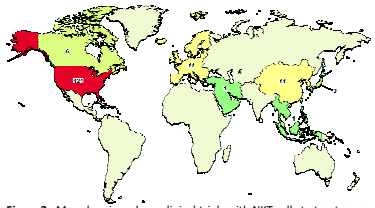


Figure 3. Map showing where clinical trials with NKT cells to treat cancer are being done.
https://clinicaltrials.gov/ct2/results/map?term=%22natural+killer+T+cells%22+AND+%22cancer%22

These are being done with patients with hematological cancers which usually express CD1d and also with solid tumours that do not express CD1d due to the ability of iNKT cells to attack both types of cancers.

Some of the results show that the therapy is safe, generates iNKT cell-specific responses and have immunomodulatory effects in cancer patients.

Conclusions

- NKT cells express NK cell markers and at the same time TCRs α/β with a restricted repertoire that recognizes CD1d. They can be divided then into three subsets: type I NKT cells (or iNKT cells), type II NKT cells and NKT-like cells. Once these cells become activated by endogenous or exogenous ligands, they mediate immunoregulatory functions in different diseases such as cancer. In this case, type I NKT cells potentiate the anti-tumour response, while type II NKT cells suppress this response.

- iNKT cells, characterized by its semi-invariant TCR and by its specific recognition of α-GalCer presented by CD1d, can attack the tumour by indirect mechanisms (releasing cytokines like IFN-γ that activate other effector cells as NK cells or CTLs) or direct mechanisms (by their own cytotoxic activities).

- To use iNKT cells for cancer treatment we can stimulate the patient's own cells with the administration of exogenous activators (IL-12 or α-GalCer) or we can transfer *in vitro* activated iNKT cells to the patient.

- By the moment, there are a lot of clinical trials and research groups working on this promising new therapy with iNKT cells, so it is to hope that their optimal and safe therapeutic use will be as rapidly and efficiently defined as have been defined their various roles and activities and we hope too that iNKT-cell based immunotherapies in combination or not with other standard therapies could be established in the near future.