ImmunoTools *special* Award 2021

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Exploring the link between PTEN Hamartoma Tumour Syndrome and tumour immunology

PTEN Hamartoma Tumour Syndrome (PHTS) is a rare and heterogeneous disease affecting at least 1 in 200.000 people. The underlying cause of this heterogeneous syndrome is a PTEN loss of function.

PTEN stands for Phosphatase and Tensin Homologue. It was first discovered as a tumour suppressor gene: somatic loss of function is found in many malignancies. PTEN is an enzyme with pleiotropic function, but its most prominent role is the dephosphorylation of PIP_3 into PIP_2. Thereby PTEN negatively regulates the PI3K/Akt/mTOR pathway. PTEN inactivation leads to exacerbated activity of the PI3K pathway , which promotes cell survival, division, proliferation and glucose metabolism. In PHTS, PTEN loss of function is congenital and thus is present in all tissues and all stages of development.

PHTS patients suffer from neurodevelopmental problems, benign neoplasms from early adulthood, and an increased risk of developing malignancies. In addition, many PHTS patients show signs of a defective immune response. Some present with recurrent upper respiratory infections, while others present with autoimmune disease. There is a need to characterise the cellular processes underlying these symptoms. Currently, the only available information on the implications of PTEN malfunction in immunity comes from *in vitro* studies or studies in mice.

As mentioned, PHTS patients are at high risk of developing malignancies. Immunity plays a pivotal role in the elimination of cancerous cells. Immunotherapy, which restores and/or boosts the capacity of the host's immune system to eliminate cancer, greatly improved modern cancer treatment. However, it is crucial to identify the right treatment for each patient. Underlying immune dysregulation is very likely to determine the treatment success of PHTS patients with cancer. An accurate picture of their immune landscape is therefore warranted.

Our first goal thus, is to map the PHTS immune landscape directly in humans. The Radboud University Centre Nijmegen is a national centre of expertise for PHTS. We are in the position to follow PHTS patients who are managed by our clinicians.

Not all PHTS patients develop malignancies, and if they do, the malignancies are different from one patient to another. To generate hypotheses, we want to start by characterising the immune system at baseline, and the immune response to a well-defined stimulus. We chose the seasonal influenza vaccine, which is routinely administered both to patients and to healthy controls.

Blood and serum samples are drawn from study subjects just before vaccination, and two weeks after. Serum is used for vaccine-specific antibody titration. With a small fraction of the blood we measure the proportion of several immune cell populations. From the rest we isolate Peripheral Blood Mononuclear Cells (PBMCs). With the PBMCs we can study the response to the vaccine *in vitro*. High cell proliferation upon incubation with the vaccine antigens indicates the presence of vaccine-specific clones in the PBMC population. Cytokine production in the supernatants of the stimulated PBMCs provides information about which cells are getting activated, and what their phenotype is.

Furthermore, we freeze PBMCs for follow-up research. The results of the exploratory phase will bring us to new hypotheses. With the stored PBMCs we can zoom in further. The **ImmunoTools** **ELISA kits** will allow quantification of certain cytokines and chemokines, when the PBMCs are stimulated with the hemagglutinins present in the vaccine or with other ligands of interest.

Our lab is especially interested in Dendritic Cell biology. $CD14^+$ PBMCs can be differentiated into monocyte-derived Dendritic Cells (moDCs) by incubating them with **rh IL-4** and **rh GM-CSF**. We could investigate whether moDCs of PHTS patients behave differently from moDCs from age-matched healthy controls.

In conclusion, we are in the process of collecting very valuable, unique samples. PHTS is a rare disease, but the investigation of the PHTS immune response extends further than PHTS patients and their families. Our investigations will indirectly teach us more on the implications of PHTS function in health and disease.

ImmunoTools *special* AWARD for **Estel Collado Camps** includes 10 reagents

recombinant human rh GM-CSF, rh IL-4

human ELISA-set: IFN-gamma, IL-10

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