

Summary

Introduction

This thesis is about the treatment of solid tumors using immunotherapeutic strategies. The immune system recognizes and kills viruses, bacteria and fungi. Furthermore, it can recognize and kill cancerous cells. Unfortunately it is not always successful. Stimulating or aiding the immune system to destroy cancerous cells is called cancer immunotherapy, which is often used as an adjuvant treatment after surgery, chemotherapy or radiotherapy.

The immune system comprises various types of cells and signaling molecules. The main players covered in the thesis are T cells and dendritic cells.

The human body contains millions of slightly different T cells. Each T cell can recognize part of a protein called an epitope with its T cell receptor (TCR). The epitope is placed in a group of molecules called the MHC complex and fits perfectly in a specific TCR. When this happens, the T cell will start to divide and receives an activation signal, readying it to attack cells with the specific epitope. This will happen most efficiently when the epitope is presented by a so called antigen presenting cell. The most capable antigen presenting cell is the dendritic cell.

Not all T cells are good killers. There are several types of T cells. Roughly they can be divided in helper T cells and killer T cells (named cytotoxic T cells). Helper T cells can aid other immune cells to do their job. T cells start off as naïve cells. After they have come in contact with their antigen they develop into either effector T cells or memory T cells. Effector T cells are good at killing their targets, but have a relatively short lifespan. Memory T cells have a longer lifespan, and are responsible for immunological memory. They can be divided into central memory T cells and effector memory T cells. Many immune cells are capable of producing and interpreting chemical messengers called cytokines.

Thesis outline:

- Part one describes various immune therapeutic strategies for the treatment of head and neck cancer.
- Part two describes the use of the cytokine IL-21 in immunotherapeutic strategies.
- Part three describes the outcome of a specific type of immunotherapy for colorectal cancer patients.

Part 1 Immunotherapeutic approaches for head and neck cancer patients

The research described in Chapter 2, is the charting of T cells in the peripheral blood of head and neck cancer patients and comparing that to that healthy donors. Head and neck cancer arises from the oral cavity, larynx or pharynx. The main risk factors for developing head and neck cancer are smoking, excessive alcohol consumption and HPV infection. HPV is a group of viruses that can be divided in low risk and high risk subtypes for developing cancer. When a cell is infected with HPV, virus specific proteins are produced and expressed by the infected cell.

One of the main results of the research described in Chapter 2, is that healthy age-matched donors have more T cells than head and neck cancer patients. The percentage of effector T cells is relatively large in the group of cancer patients, while the percentage of naive T cells is small. Within the group of cancer patients we found that the percentage of effector and effector memory T cells is larger in the patients with HPV positive tumors compared to patients with HPV negative tumors.

The information obtained on the types and numbers of T cells in this patient group has the potential to be used as a prognostic tool.

Chapter 3 and 4 of this thesis describe our efforts to support the immune system in fighting head and neck cancer cells. It is possible to activate T cells of a patient with dendritic cells generated in the laboratory. Monocytes can be used to generate dendritic cells that can subsequently be loaded with antigens specific for (or associated with) tumor cells. After this, the dendritic cells can be injected back into the patient, where corresponding T cells can be activated to kill tumor cells.

Survivin is an antigen that is highly expressed in a large percentage of tumors, including head and neck cancers. Survivin plays an important role in cell mitosis and is an inhibitor of apoptosis (regulated cell death). These characteristics are important for the tumor to maintain a malignant phenotype and would therefore form a good target for immunotherapy. Dendritic cells can be loaded with mRNA encoding survivin. The dendritic cell can translate the mRNA into protein after which the protein is degraded and fragments are presented on their surface so T cells can be activated.

Before testing this setup with survivin on patients, we researched it in the lab. The first step of the investigation was to generate large numbers of survivin specific T cells from the blood of healthy donors. We were able to produce small numbers of survivin specific T cells. We did not succeed in producing large numbers of survivin specific T cells. When the percentage of survivin specific T cells increased, the T cells started to die. While generating large numbers of T cells with other specificities did not form a problem. After additional research we found that activated T cells express survivin themselves, and can therefore be recognised and killed by survivin specific T cells, a phenomenon called fratricide.

The main message of this Chapter is that survivin is not the ideal target for active immune therapy.

Another method to generate large numbers of tumor specific T cells is described in Chapter 4. T cells are made HPV specific by transducing the genetic make-up of an HPV specific T cell receptor into T cells with various specificities. HPV infections not only play an important role in the development of some head and neck cancers but also in (amongst others) cervical cancer.

To obtain the genetic code for an HPV specific T cell receptor, a T cell clone has to be generated. Dendritic cells loaded with fragments of HPV are used to stimulate T cells from healthy donors. After identifying the specific T cells, they can be sorted and cloned. From this clone the genetic material of the T cell receptor can be identified and isolated. We transduced the genetic code of the HPV specific T cell receptor from a cytotoxic T cell into helper T cells. The newly generated T cells were capable of recognising cells expressing HPV and produce the cytokine interferon gamma in response. With this method it is possible to generate large numbers of T cells that are tumor specific.

In the discussion of part 1 (Chapter 5) we reviewed various immunotherapeutic strategies for the treatment of head and neck cancer. Based on our research and that of others we can advise to focus on HPV positive tumors when developing immunotherapy for head and neck cancer patients.

Part 2 : The role of IL-21 in immunotherapy

T cells produce cytokines that effect other T cells. There are cytokines that stimulate growth, a longer lifespan or make them more effective. When developing immunotherapy we strive for the T cells to efficiently recognize and kill target cells. Furthermore, we would like them to persist sufficiently long. In our studies, described in Chapter 6 and 7, we investigated the effects of IL-21 on T cell characteristics that are favourable for anti-tumor immunity.

Normally IL-21 is produced by a subtype of helper T cells. It is, however, also possible to use synthetic IL-21. Large amounts of systemic synthetic IL-21 may lead to auto-immune disease. Therefore, local production is a safer option. In the research, described in Chapter 6, we transfected mRNA encoding IL-21 into dendritic cells, so they will start to produce IL-21. This is combined with a tumor antigen to generate tumor specific antigens. We found that dendritic cells transfected with IL-21 mRNA together with the tumor antigen mRNA, generated more tumor specific T cells than dendritic cells that were only transfected with the tumor antigen mRNA. Furthermore, when IL-21 was used, the T cells were better capable of recognizing and killing target cells.

Culturing large numbers of T cells originating from the tumor environment and injecting them back into the patient is a form of passive immunotherapy. In active immunotherapy T cells are stimulated in the patients, instead of in the lab like in passive immunotherapy. The T cells in the lab are stimulated to grow with the help of antigen presenting cells (APC), such as dendritic cells. In the investigations described in Chapter 7 we made use of APC that were artificially enhanced and are called aAPCs. The aAPC produced either no cytokine, IL-2, IL-15 or IL-21 and were used to stimulate lymphocytes originating from the tumor, called tumor infiltrating lymphocytes (TIL). We found that TIL stimulated with IL-2 producing aAPC gave rise to the most T cells. The IL-21 producing aAPC gave rise to the most cytotoxic T cells. Moreover, the T cells produced with the help of IL-21 had the highest percentage of markers on them that are typical for cells with a long lifespan. The TIL that were stimulated by IL-21 producing aAPC were also best at killing target cells.

The discussion (Chapter 8) of the second part of this thesis discusses the applicability of IL-21 in immunotherapeutic strategies. We advise not to administrate IL-21 systemically but to have it locally produced by APC.

Part 3 : Immunotherapy for Colorectal cancer patients

Active Specific Immunotherapy (ASI therapy) is a form of therapy where autologous irradiated tumor cells are injected back into the patient together with BCG. BCG or Bacillus Calmette Guérin is used to boost the immune response. ASI therapy was used two decades ago to treat colon cancer patients with stage II and III disease. The original research showed that particularly patients with small tumors benefited from this ASI therapy compared to surgery alone (the control group).

Chapter 9 describes the influence of the microsatellite status of the tumors on the effect of ASI. Today we know that about 15 % of the colon cancers are microsatellite instable (MSI). MSI tumors have more genetic alterations than microsatellite stable (MSS) tumors. Our original hypothesis was that patients with MSI tumors would benefit more from ASI therapy than patients with MSS tumors. From the performed retrospective study we learned that patients with MSI tumors already had a very good prognosis, leaving little room for improvement by ASI therapy. Patients with small MSS tumors had the most benefit from ASI therapy. They died less often from colon cancer and had less recurrences of the disease in the 15 years they were followed.

In Chapter 10 we describe our research in the same patient group that was used for the analyses the numbers of T cells and cytotoxic T cells in a colon tumor. Patients with MSI tumors had more T cell infiltration than patients with MSS tumors. Altogether patients with high T cell infiltration had a better prognosis than patients with low T cell infiltration. In addition, we investigated the effect of ASI on MSS tumors with high and low T cell infiltration. We found that patients with high T cell infiltration in the stroma of the tumor benefited the most from ASI therapy.

In the discussion (Chapter 11) of this third part the thesis our findings from Chapter 9 and 10 are analysed. We can conclude that ASI therapy has most clinical benefit for patients with MSS tumors with high T cell infiltration in the stroma.