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How CD4+ T-cell help shapes functional and dysfunctional CD8+ T-cell differentiation

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English Summary

How $CD4^+$ T-cell help shapes functional and dysfunctional $CD8^+$ T-cell differentiation

Our immune system protects us against infections by bacteria, viruses and parasites. This happens by attacking these pathogens themselves, but also by recognizing and killing infected body cells. The immune system can also recognize and kill cancer cells, because cancer cells look different than healthy cells.

The immune system is a complex interplay between different types of immune cells, each with their own function. It can be divided in two arms, the so-called 'innate immunity' and 'adaptive immunity'. The innate immune system forms the first barrier against pathogens and elicits a fast, broad response that also leads to the activation of the adaptive immune system. The adaptive immune system starts up a bit later but works very specifically against a certain pathogen. It also forms so-called 'memory', which enables it to attack the same pathogen even faster and more efficiently the next time. This principle of immunological memory is used in vaccination. During vaccination our immune system is exposed to a small amount of pathogen, such as virus particles, which drives the formation of memory cells. These memory cells can quickly induce an effective immune response upon a second exposure to the same virus, which protects you from infection. You are then immune.

Two important cell types of the adaptive immune system are B cells and T cells. B cells make antibodies, these are molecules that can bind specifically to a target, such as a pathogen. Binding of antibodies can disarm pathogens, or makes them more easy to be seen and killed by immune cells. In my thesis I focus on T cells. T cells can specifically recognize cells that look different from normal, for example because they have been infected by a virus, or because they have turned into tumor cells. Thus, T cells play an important role in the immune response against cancer.

T cells have a sort of antenna, called the T-cell receptor, which they use to recognize small pieces of protein, called antigens. The so-called cytotoxic T cells, or $CD8^+$ T cells, can kill infected body cells or tumor cells. In addition there are the helper T cells, or $CD4^+$ T cells, which can help $CD8^+$ T cells or B cells. In this thesis I investigated the role of $CD4^+$ T-cell help to $CD8^+$ T cells during their activation.

Chapter 1

In this chapter I introduce the subject and explain how $CD4^+$ T-cell help during $CD8^+$ T-cell activation works.

Before a T cell can do its job it needs to be activated. This is true for both $CD8^+$ and $CD4^+$ T cells. Our body has a diverse repertoire of T cells, that all recognize something different. Only the T cells that recognize a specific antigen will be activated when this antigen is present in the body.

This is called an antigen-specific T-cell response. T cells that have never seen 'their' antigen are called naïve T cells.

Naïve T cells are activated by other immune cells, called antigen-presenting cells (APCs). Dendritic cells (DCs), a subset of APCs, can take up parts of dead cells in tissues. They process the debris, present the antigens that are produced on their surface, and subsequently migrate to the lymph nodes to show the antigens to T cells. Besides presenting antigens, DCs also pass on other activating signals to T cells. This happens via direct cell-cell contact (called costimulation) and via secreted molecules (called cytokines). This process where T cells are activated by DCs is also called priming. If a T cell is primed, it starts to divide and develop specific traits that are necessary for its function. This development process is called T-cell differentiation. Next, the T cell leaves the lymph node and travels via the blood to the tissues where it can do its work.

The priming of $CD8^+$ T cells happens in two steps. During the first step the T cell becomes activated by a DC, but it does not yet receive help. During the second step, a $CD4^+$ T cell can deliver help to a DC, specifically to a subtype called cDC1, which subsequently can deliver this help to the $CD8^+$ T cell. In this scenario, 'help' consists of multiple activation signals (costimulation and cytokines) that are passed on between cells. Previous studies have shown that $CD4^+$ T-cell help is crucial to generate properly functional effector $CD8^+$ T cells during virus infections and cancer.

Chapter 2

In this chapter we investigated the role of $CD4^+$ T-cell help for the formation of $CD8^+$ memory T cells.

As mentioned previously, cells of the adaptive immune system, such as $CD8^+$ T cells, can also form memory cells. In contrast to cytotoxic effector cells, which are short-lived, memory cells are maintained long-term, even after an infection is cleared. Upon a second encounter with the same antigen, memory T cells respond extra quickly. Central memory (T_{CM}) cells start dividing a lot, while effector memory (T_{EM}) cells rapidly exert their cytotoxic effector function.

To investigate the role of $CD4^+$ T-cell help for the formation and function of $CD8^+$ memory T cells, we used laboratory animals, mice, which were vaccinated with two types of vaccines. One vaccine (Help) activated both $CD8^+$ and $CD4^+$ T cells, whereby $CD4^+$ T cells could provide help to the $CD8^+$ T cells. The other vaccine (No Help) only activated $CD8^+$ T cells, which could therefore not be helped by the $CD4^+$ T cells. In mice that were vaccinated with the Help vaccine, we saw that helped T_{CM} cells were maintained longer, and many more T_{EM} cells were formed. The profile of helped T_{EM} cells was very similar to helped effector $CD8^+$ T cells. When we vaccinated the mice a second time, the helped $CD8^+$ T cells did not need to receive $CD4^+$ T-cell help a second time, but

could already mount a robust response. Thus, $CD8^+$ T cells 'remember' that they have received $CD4^+$ T cells during priming, and can respond help-independently during a secondary challenge.

Chapter 3

In this chapter I show that helpless $CD8^+$ T cells resemble so-called 'stem-like' cells in chronic infection and cancer, and I propose that these may be the same cells.

In chronic infection and cancer, $CD8^+$ T cells often don't work very well. They divide less, they look different and they are unable to kill their target cells. This dysfunctional state is called exhaustion. Previously, researchers believed that exhausted $CD8^+$ T cells were generated from effector cells that were exposed to their target for too long. However, in the past years, new studies have shown that exhausted cells do not originate from effector cells, but from a precursor population called 'stem-like' cells. In this chapter I summarize the scientific literature on this subject. Next, I investigate to which extent stem-like cells from cancer or chronic infection resemble un-helped, or 'helpless' cells from our vaccination model. This is done based on the gene expression profiles of these cells, showing which genes (parts of DNA) are actively used by a cell to make proteins. The gene expression profile says something about identity, differentiation state and function of a cell, and can be compared between different cells. By comparing the profiles of our helpless cells to those of stem-like and exhausted cells, obtained from published datasets by other research groups, I concluded that stem-like cells strongly resemble helpless cells based on their gene expression profile.

Based on the gene expression data and literature review, in this chapter I propose a new theory: The precursors of exhausted $CD8^+$ T cells in chronic infection and cancer, which are the stem-like cells, are formed as a result of priming in absence of $CD4^+$ T-cell help. In this model, which I call the 'helpless dysfunction model', stem-like cells and helpless cells are the same.

Chapter 4

In this chapter we supported the 'helpless dysfunction model' from Chapter 3 with experimental evidence.

Using the same vaccination model as in Chapter 2, we showed that when naïve $CD8^+$ T cells are primed, they adopt a whole spectrum of differentiation states. From least to most developed, this spectrum consists of T_{CM} cells, stem-like cells, T_{EM} cells and lastly the cytotoxic effector cells. $CD4^+$ T-cell help shifts this spectrum, in the direction of more effector-like. We show that in mice that are vaccinated without $CD4^+$ T-cell help, $CD8^+$ T cells get stuck in the stem-like state. However, these helpless cells can still develop further into effector cells upon receiving $CD4^+$ T-cell help.

Next, we looked at $CD8^+$ T-cell differentiation in tumors. In an immunogenic mouse model of colon cancer, we saw formation of tumor-specific stem-like $CD8^+$ T cells, but not helped effector cells. Even when we manipulated the tumor cells to express $CD4^+$ T-cell activating antigens, and $CD4^+$ T cells were actually activated, still helped effector cells were not generated. These results suggest that in tumors, $CD4^+$ T-cell help delivery to $CD8^+$ T cells by DCs is probably impaired. This could be an important explanation why $CD8^+$ T cells do not exert their functions properly in cancer, and eventually become exhausted.

Chapter 5

In this chapter we investigated the differentiation of $CD4^+$ T cells, and found that precursor $CD4^+$ T cells can develop into T helper 1 (Th1) cells or follicular helper (Tfh) cells, depending on their interactions with other immune cells.

As mentioned previously, $CD4^+$ T cells can offer help to $CD8^+$ T cells or to B cells. This is done by different subtypes of $CD4^+$ T cells: Th1 cells help $CD8^+$ T cells differentiate into effector cells, and Tfh cells help B cells make effective antibodies. In this chapter we showed that Th1 cells and Tfh cells originate from the same Th1/Tfh precursor. These precursors are formed when naïve $CD4^+$ T cells undergo their first step of priming. Whether they become a Th1 or Tfh after this depends on the second step of priming: If this happens through interaction with a cDC1 the $CD4^+$ T cell becomes a Th1 cell, but if this happens through interaction with a B cells the CD4+ T cell becomes a Tfh cell. Also the costimulation signals that are passed on differ between these scenarios.

Chapter 6

In this chapter we reviewed the scientific literature about the importance of Type I interferon (IFN-I) for orchestrating $CD4^+$ T-cell help to $CD8^+$ T cells in cancer.

IFN-I is a cytokine (messenger molecule) which is secreted by immune cells or other cells when they encounter pathogens or damage. This signal works like an alarm bell: something is going on here, the immune system needs to be activated! IFN-I induces better (tumor) antigen presentation by DCs, as well as better migration to the lymph nodes and upregulation of costimulatory molecules. Due to these effects, DCs can activate T cells better.

Also within the lymph node IFN-I plays an important role, by allowing T cells and cDC1 cells to form the platform in which $CD4^+$ T-cell help is delivered through the cDC1 to the $CD8^+$ T cell. Thus, IFN-I is crucial for $CD4^+$ T-cell help. However, in tumors there is often a lack of IFN-I production. This could be an important underlying mechanism why $CD4^+$ T-cell help to tumor-specific $CD8^+$ T cells can not be delivered well, and the $CD8^+$ T cells become dysfunctional.

Chapter 7

In this chapter we reviewed the scientific literature about the mechanisms of action of PD-1 blockade, currently the most used and successful immune therapy in cancer patients. We also explain how our theory about $CD4^+$ T-cell help can explain certain mechanisms of action of PD-1 blockade in cancer.

PD-1 is a receptor, that means a molecule on the outside of a cell that can transmit signals into the cell, on certain types of T cells and other immune cells. When PD-1 binds to its binding partner PD-L1, a negative signal is sent into the cell, which inhibits it function. Thus, PD-1 is an inhibitory receptor. By blocking this receptor, the brake is released and this would enhance the function of for example a $CD8^+$ T cell. PD-1 is not present on all $CD8^+$ T cells, but especially on exhausted cells and on stem-like cells. PD-L1 can be on tumor cells or on immune cells.

Previously, it was thought that PD-1 blocking immune therapy, which is used in cancer, acts on exhausted $CD8^+$ T cells in the tumor. The exhausted cells would thereby lose their brake and become active again. However, more recent studies show that it is not the exhausted cells, but the stem-like cells that respond to PD-1 blockade. According to our 'helpless dysfunction model', stem-like cells equal helpless cells, and this means that it is the helpless $CD8^+$ T cells that benefit from PD-1 blocking therapy. In this chapter we suggest that PD-1 blockade can partly mimic the $CD4^+$ T-cell help signals. However, since the publication of this work in 2021, novel studies indicate that PD-1 blockade does not only mimic $CD4^+$ T-cell help signals but also activates $CD4^+$ T cells themselves, allowing for $CD4^+$ T-cell help delivery to take place.

Chapter 8

In this chapter I place the findings and concepts from this thesis in the broader context of the existing scientific literature.

I expand on the ' helpless dysfunction model' and discuss where this theory aligns, and where it opposes findings from other scientists. I discuss which questions are now answered, an which are still open. Also I describe the clinical relevance of my work: What does this mean for treatment of cancer patients with immune therapies?

In conclusion, in this thesis I propose a new theory about $CD8^+$ T-cell differentiation, and I support this theory with experimental evidence. I show that $CD4^+$ T-cell help shifts the differentiation spectrum of $CD8^+$ T cells in the direction of more effector-like. Moreover, I identify a lack of $CD4^+$ T-cell help as important underlying mechanism for the formation of stem-like, and eventually exhausted $CD8^+$ T cells in cancer. These findings offer a new perspective to re-interpret previous studies about $CD8^+$ T cells in cancer. They also highlight $CD4^+$ T-cell help as an important aspect to understand and consider during the development of new immune therapies against cancer.